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Effects of apoptosis by 20α-hydroxysteroid dehydrogenase activity on corpus luteum formation during early pregnancy in cattle

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Abstract: 20α-hydroxysteroid dehydrogenase (*20α-HSD*) is a member of the aldo-keto reductase family. These enzymes play a pivotal role in regulating steroid hormones, such as androgens, oestrogen, and progesterone, and are, therefore, considered vital targets for determining whether a pregnancy is maintained. In this study, we investigated the association between *20α-HSD* and apoptosis-related genes in luteal tissues (at 30, 60, and 90 days of gestation) and cells from early gestation in cattle. The corpus luteum altered the number of large lutein cells from 30–90 days. The change in the junction of the connective tissue appeared to affect the density of the corpus luteum. In addition, *20α-HSD* was detected in the corpus luteum and cultured cells during early pregnancy, in contrast to the results of previous studies. The overall expression pattern of the *20α-HSD* and *Casp-3* proteins was lower on day 50 of gestation than on days 30 and 90. However, the *20α-HSD* expression gradually increased from 30 to 90 days of gestation. When the *20α-HSD* protein was increased to 0.5 μg/ml, 1 μg/ml, and 1.5 μg/ml in the luteal cells collected on day 30 of pregnancy, apoptosis was analysed after 48 hours. *20α-HSD* generated in the cells was confirmed, and the concentration gradually decreased as the concentration increased. However, the expression of *Casp-3* showed an overall similar expression pattern. Notably, the *20α-HSD* and *Casp-3* proteins were lowest at 1.5 μg/ml supplemented with *20α-HSD*, with higher levels in the cytosol than in the cytoplasm. These results suggest that *20α-HSD* plays a role in maintaining normal pregnancy, particularly by regulating the progesterone concentrations during luteal cell development.

Keywords: Casp-3; 20α-HSD; early pregnancy; lutein cells; bovine

After successful ovulation, the corpus luteum is reconstituted into corpus luteum cells from theca and granulosa cells, and progesterone secretion is promoted (McCracken et al. 1999). The corpus luteum is maintained for approximately 19 days regardless of conception, and if pregnancy does not occur, it undergoes a regression process to prepare for the next ovulation. However, in the case of the

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pregnant corpus luteum, the action of interferon tau maintains the normal corpus luteum function during maternal recognition (Oliveira et al. 2008; Yang et al. 2010). Many studies have characterised the differences in the maintenance and reconstitution of the corpus luteum between pregnant and non-pregnant animals. The functional mechanism of the corpus luteum was also investigated by utilising the morphological evaluation of the corpus luteum, the action of steroid-producing cells, stainable collagen, and alterations in the cell diameter (Plendl et al. 1996; Nitta et al. 2011; Hughes and Pate 2019). Particularly, post-pregnancy luteal tissue relies on the smooth secretion of interferon tau to maximise the function of the luteal tissue using the reconstruction pathways of the extracellular matrix, chemokine signalling, insulin growth factor-binding proteins, and cytoskeleton as upregulation of the interferon-stimulated genes (Romero et al. 2013; Basavaraja et al. 2019). The activated luteum is then regenerated by exposure to the placental growth factor (*PGF*) in the uterus, wherein the action of several genes is altered, the luteal tissue is reconstructed, and large luteal cells become apoptotic according to various challenges (Mezera et al. 2019). Several factors are involved in the apoptosis of the corpus luteum. Among them, the generation of apoptotic factors in response to hormonal action can be considered a representative apoptosis process. Particularly, 20α-hydroxysteroid dehydrogenase (*20α-HSD*; *AKR1B5*), which is activated in the uninucleated trophoblast cells of the endometrium stimulated by the aldose reductase gene, interacts with the placental growth hormone to form *20α-HSD*/*PGF*-synthetase (Schuler et al. 2006). By controlling progesterone, it can be seen that the concentration of progesterone in the body is rapidly lowered, thereby controlling the function of the corpus luteum (Sudeshna et al. 2013). Successful corpus luteum degeneration in oestrus and late pregnancy leads to recurrent oestrus and normalises the uterus to induce a new pregnancy. The degeneration of the corpus luteum caused by exposure to placental growth hormone occurs through a fairly complex mechanism. The apoptosis process caused by the decrease in progesterone can be seen as the most representative exaggeration. Apoptosis induced by hypoxic partial pressure can also cause cell apoptosis (Bruick 2000; Li et al. 2003). This action activates hypoxia-inducible factor 1 (*HIF1*) by dimerizing *HIF1A* with *ARNT* (*HIF1B*). From the mitochondria, HIF1 induces the overexpression of B-cell lymphoma 2 integrating protein 3 (*BNIP-3*) through cyto C, and the cell collapse is mediated by the activation of caspases (Nishimura et al. 2006). Finally, the regressive action of the luteum can be seen as inhibiting the expression of the vascular endothelial growth factor, which is involved in the angiogenesis caused by a decrease in *P4* because of hypoxia (Bacci et al. 1996), thereby reducing blood flow to the luteum and causing luteal dissolution (Bruick 2000). 20α-hydroxysteroid dehydrogenase regulates the progesterone in corpus luteum cells or is found during corpus luteum death (Kim et al. 2014). The uterus and corpus luteum combine to maintain a normal pregnancy or control changes in the body for a new pregnancy. It also suggests that Prostaglandin F2 alpha (*PGF2*), expressed in the uterine placenta, plays a selective role in uterine activity during pregnancy (Arosh et al. 2002). This indicates that prostaglandin transporter gene expression in uterine and intrauterine tissues plays a role in the prostaglandin (*PG*) exchange between the mother and fetus and regulates the timing of the corpus luteum collapse (Ealy and Yang 2009; Ezashi and Imakawa 2017). We hypothesised that the regulatory mechanism underlying the persistence of the corpus luteum could be highly dynamic during early pregnancy and that *20α-HSD* could be actively activated during pregnancy. Therefore, the role of *20α-HSD* in the reorganisation and collapse of the corpus luteum and tissue reconstruction through apoptosis was analysed in this study.

MATERIAL AND METHODS

Preparation and certification of animals

The corpus luteum of cattle was collected from a local slaughterhouse in Pyeongnong, Pyeongtaek, from six Korean cattle at 30, 60, and 90 days of pregnancy. The harvested tissue was placed in warm phosphate buffered saline (pH 7.2) and transferred to the laboratory within 2 h, dissected into small pieces, and the tissues for the cell culture were prepared by washing twice or more with Dulbecco's phosphate buffered saline (Gibco, Carlsbad, CA, USA), and tissues for analysis were stored at −80 °C and used for the experiments.

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This study was conducted per the guidelines of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, USA. The study protocol was approved by the Committee on the Ethics of Animal Experiments of Hankyong National University, South Korea (IACUC approval HK-2018-1).

Corpus luteum cell isolation and culture

The corpus luteum cells were isolated and cultured according to the method described by Kim et al. (2018). The isolated corpus luteum tissue (30 days) was washed twice with Dulbecco's phosphate buffered saline (Gibco, Carlsbad, CA, USA) containing 300 IU/ml penicillin (Gibco, Carlsbad, CA, USA) and 300 µg/ml streptomycin (Gibco, Carlsbad, CA, USA), and then cut finely with scissors. Dulbecco's phosphate buffered saline containing 0.2% collagenase type I (Gibco, Carlsbad, CA, USA) and 1% bovine serum albumin (Bioworld, Irving, TX, USA) was added and reacted at 37 °C for 2 h, and the large luteal cells were extracted by centrifugation (1 500 *g*, 5 min). After culturing for 24 h in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS; Thermo Fisher Scientific, Waltham, MA, USA) and 5% antibiotics (Sigma-Aldrich, St. Louis, MO, USA), the cells were cultured for the first two days. The treatment group was cultured in a medium (DMEM + 5% antibiotic + 10% FBS) supplemented with *20α-HSD* (AKR1C1; Sigma-Aldrich, St. Louis, MO, USA, 0.5, 1, and 1.5 µg/ml) for 48 h, and the cells were subjected to immunofluorescence and real-time polymerase chain reaction (PCR) analyses.

Real-time polymerase chain reaction

The total RNA, extracted from each corpus luteum tissue (30, 60 and 90 days) scraping using the TRIzol reagent (Cat No. 10296028; Invitrogen, Waltham, MA, USA), was treated with DNAse (Ambion, Austin, TX, USA), as per the manufacturer's instructions, and quantified by ultraviolet (UV) spectrophotometry. The first-strand cDNA was synthesiwed by reverse transcription of mRNA using Oligo (dT) primer and SuperScript II Reverse Transcriptase (Cat No. 18064022; Invitrogen, Waltham, MA, USA). The real-time PCR (Rotor-Gene Q; QIAGEN, Hilden, Germany) was performed according to the experimental method of the One-Step SYBR RT-PCR Kit (TaKaRa, Kasatsu, Japan). Initially, 1 mg of the total RNA was isolated from the tissues at each stage of the cattle gestation period. The primers used for the PCR are listed in Table 1. The real-time PCR reaction was performed once at 42 °C for 15 min, 95 °C for 2 min, and subsequently at 95 °C for 40 s, 58 °C for 15 s, and 60 °C for 32 s for 40 cycles. A final annealing step was performed once at 72 °C for 5 minutes. The gene amplification was quantified based on the cycle threshold (Ct) value from a semi-log amplification plot in the geometric region. Rotor-Gene Real-Time Software v6.0 (Qiagen, Hilden, Germany) was used to analyse the Ct and to obtain a semi-log amplification plot. Finally, the relative expression levels of each gene were calculated using the $2^{-\Delta\Delta Ct}$ method by normalisation with bovine-glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) mRNA levels.

Western blot analysis

For the western blot, the total protein was extracted from the tissues using the Pro-prep solution (Intron, Seoul, Korea) according to the manufacturer's instructions. The total protein was quantified using the Bradford protein assay (Bio-Rad, Hercules, CA, USA), and the final protein samples were stored at –80 °C. To compare and analyse the expression patterns of *20α-HSD* (Kim et al. 2014) and *Casp-3* (ab4051; Abcam, Cambridge, United Kingdom) using proteins (30 µg) in the corpus luteum, which are tissues in early pregnancy of cows, they were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then transferred to a polyvinylidene difluoride membrane (0.2 µm) using a semi-dry electroblotting apparatus. After blotting, the membrane was blotted with a 5% skim milk blocking reagent for 1 h and incubated with primary antibodies against *20α-HSD*, *Casp-3*, and β-*actin* (sc-47778; all from Santa Cruz Biotechnology, Santa Cruz, CA, USA) for overnight at 4 °C. Next, washing was repeated three times for 15 min using Tris-buffered saline-T (1× Tris + 1× Nacl + 0.05% Tween 20) to remove the unbound antibody. For the secondary antibody reaction, a secondary antibody linked to anti-rabbit

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Table 1. Primers used for the real-time PCR analysis of the apoptosis-associated genes

Primer name	Sequence	Gene ID
Glyceraldehyde-3-phosphate dehydrogenase		
Bos GAPDH FW	5'-GGTCACCAGGGCTGCTTTTA-3'	NM_001034034
Bos GAPDH RV	5'-CCAGCATCACCCCACTTGAT-3'	
20 alpha-hydroxysteroid dehydrogenase		
Bos 20α-HSD FW	5'-GGA AAG CGG ATA GTC AGG GTG ATC-3'	NM_001167660.1
Bos 20α-HSD RV	5'-GCC ATT GCC AAA AAG CAC AAG-3'	
Caspase-3		
Bos Casp-3 FW	5'-AGC CAT GGT GAA GAA GGA ATC A-3'	NM_001077840.1
Bos Casp-3 RV	5'-GGT ACT TTG AGT TTC GCC AGG A-3'	
B-cell lymphoma 2		
Bos BCL-2 FW	5'-GAGTTCGGAGGGGTCATGTG-3'	NM_001166486.1
Bos BCL-2 RV	5'-GGGCCATACAGCTCCACAAA-3'	
BCL-2-associated X		
Bos Bax FW	5'-GCCCTTTTGCTTCAGGGTTT-3'	NM_173894.1
Bos Bax RV	5'-ACAGCTGCGATCATCCTCTG-3'	
Progesterone-receptor		
Bos P4-r FW	5'-TGG TTT GAG GCA AAA AGG AG-3'	NM_001205356.1
Bos P4-r RV	5'-CCC GGG ACT GGA TAA ATG T-3'	
Vascular endothelial growth factor		
Bos VEGF FW	5'-TGT AAT GAC GAA AGT CTG CAG-3'	NM_174216.1
Bos VEGF RV	5'-TCA CCG CCT CGG CTT GTC ACA-3'	
Apoptosis-related genes encoding BCL-2 interacting protein 3		
Bos BNIP-3 FW	5'-CCCTGCGAGTCGTTTCTTCT-3'	XM_027528566.1
Bos BNIP-3 RV	5'-CCTCAGGCAGTCCTCCTAGT-3'	

20α-HSD = 20α-hydroxysteroid dehydrogenase; BCL = B-cell lymphoma; BNIP-3 = B-cell lymphoma 2 interacting protein 3; GAPDH = glyceraldehyde-3-phosphate dehydrogenase; P4-r = progesterone-receptor; VEGF = vascular endothelial growth factor

IgG-peroxidase (37.5 µl/15 ml of blocking solution) was reacted at room temperature for 2 h and washed three times for 15 min using Tris-buffered saline with Tween® 20. Subsequently, the membrane was fluoresced with 2 ml Lumi-Light substrate solution (ECL HRP substrate; Sigma-Aldrich, St. Louis, MO, USA) and exposed to the X-ray film for 1–10 minutes.

Haematoxylin & eosin stain

The luteal tissue of each pregnancy was fixed at 70% ethanol alcohol (EtOH) for 24 h, dehydrated sequentially at room temperature for 30 min from 70% to 100% EtOH, and cleared using a xylene substrate solution, and then embedded in a paraffin mould dissolved at about 60 °C.

In preparing for staining, the tissue was first dehydrated by removing the paraffin penetration, then stained with haematoxylin and eosin (Sigma-Aldrich, St. Louis, MO, USA). After dehydration and clearing, Immunomount (Thermo Fisher Scientific, Waltham, MA, USA) was used for encapsulation, and the shape of the corpus luteum was observed under a microscope (AX70; Olympus, Tokyo, Japan) after 48 hours.

Immunohistochemistry

For the immunohistochemistry experiments, paraffin slides of each pregnant tissue were used. Deparaffinisation/hydration was repeated twice for 10 min in xylene, 100% ethanol, and 95% ethanol, washed in distilled water for 5 min, and boiled

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in 10 mM sodium citrate for 10 minutes. This was repeated three times for 20 min on ice, 5 min in distilled water, and 10 min in 3% hydrogen peroxide, washed in 1× phosphate buffered saline (PBS) for 5 min, and blocked at room temperature for 1 hour. The slides were incubated with the primary antibodies against *Casp-3* overnight at 4 °C. After washing with PBS for 5 min, the secondary antibody incubation was performed at 4 °C for 1 hour. After washing three times with 1× PBS for 5 min, the ABC solution was prepared at room temperature for 30 min and then washed five times with 1× PBS for 3 min each. 3,3'-diaminobenzidine (DAB) staining was performed by incubating in 300 µl of a DAB solution for 1–10 min of reaction, distilled water for 5 min, 400 µl of haematoxylin for 10 s, followed by two stages of dehydration in xylene, 95% ethanol, and 100% ethanol for 10 min each. After fixing with 50 µl of Permount, the upper side of the slide was covered with a cover slip and observed under an Olympus AX70 microscope (Olympus, Tokyo, Japan).

Immunofluorescence

The experiment was conducted using the method described by Kim et al. (2014). In the case of the tissue, after making a paraffin slide, deparaffinization/hydration was performed, and the experiment was performed. The cells were cultured on poly D-lysine-coated chamber slides. After reacting the primary antibody (anti-*20α-HSD* and *Casp-3*) at 4 °C overnight, it was washed with 1× PBS for 5 min, the secondary antibody was reacted with Alexa 594-conjugated anti-rabbit secondary antibodies (ab150080; Abcam, Cambridge, UK) and Alexa 488-conjugated anti-mouse secondary antibodies (ab150113; Abcam, Cambridge, UK) at 4 °C for 30 min, and washed three times with 1× PBS for 5 min each. After 4',6-diamidino-2-phenylindole (DAPI) staining, the cells were sealed with a fluorescent mounting medium (Dako, Carpinteria, CA, USA) and analysed using an Olympus AX70 fluorescence microscope (Olympus, Tokyo, Japan).

Statistical analyses

The real-time RT-PCR results were analysed for statistical significance using the SAS package v9.4 (Statistical Analysis System Institute, Cary, NC, USA). The data were analysed using Welch's *t*-test, fold change calculations, and with general linear models in SAS. The data are presented as mean ± SD, and the differences between the groups were considered significant at $P < 0.05$.

RESULTS

Morphological analysis of the corpus luteum tissue in early pregnancy

Figure 1 shows the results of analysing the changes in the luteal tissue from 30–90 days of early pregnancy. On day 30 of pregnancy, the luteal tissue increased in the luteal zone compared to that in the oestrus lutein, and large lutein cells were increased in the tissue. Particularly, the boundary with the theca lutein zone was evident, although the intercellular density was not high. After 60 days, the aortic follicle changed, and a defect was observed in the connective tissue. The luteal tissue density was higher than 30 days, and the number of lutein cells increased.

However, after 90 days, the cells surrounding the atretic follicles were condensed and removed. The corpus luteum tissue at 90 days was denser than at 30 and 60 days. However, the number of large lutein cells was deficient, and the number of small lutein cells was evident. Overall, the corpus luteum shows a change in the number of large lutein cells from 30–90 days, and the difference in the junction of the connective tissue appears to affect the density of the corpus luteum.

Action of 20α-hydroxysteroid dehydrogenase in the luteal tissue

The association between the *20α-HSD* activity and *Casp-3* according to the early gestation period (Figure 2) showed that the *20α-HSD* and *Casp-3* expressed in the luteal tissue from 30–90 days of gestation have similar activities. The expression pattern of *20α-HSD* was similar in the close section of the large lutein cells, where *Casp-3* was mainly expressed. The expression of *20α-HSD* gradually increased from days 30–90 of gestation (Figure 2A). The expression pattern of the *20α-HSD* and *Casp-3* proteins was lower at 60 days of gestation than on days 30 and 90. However, the expression of the

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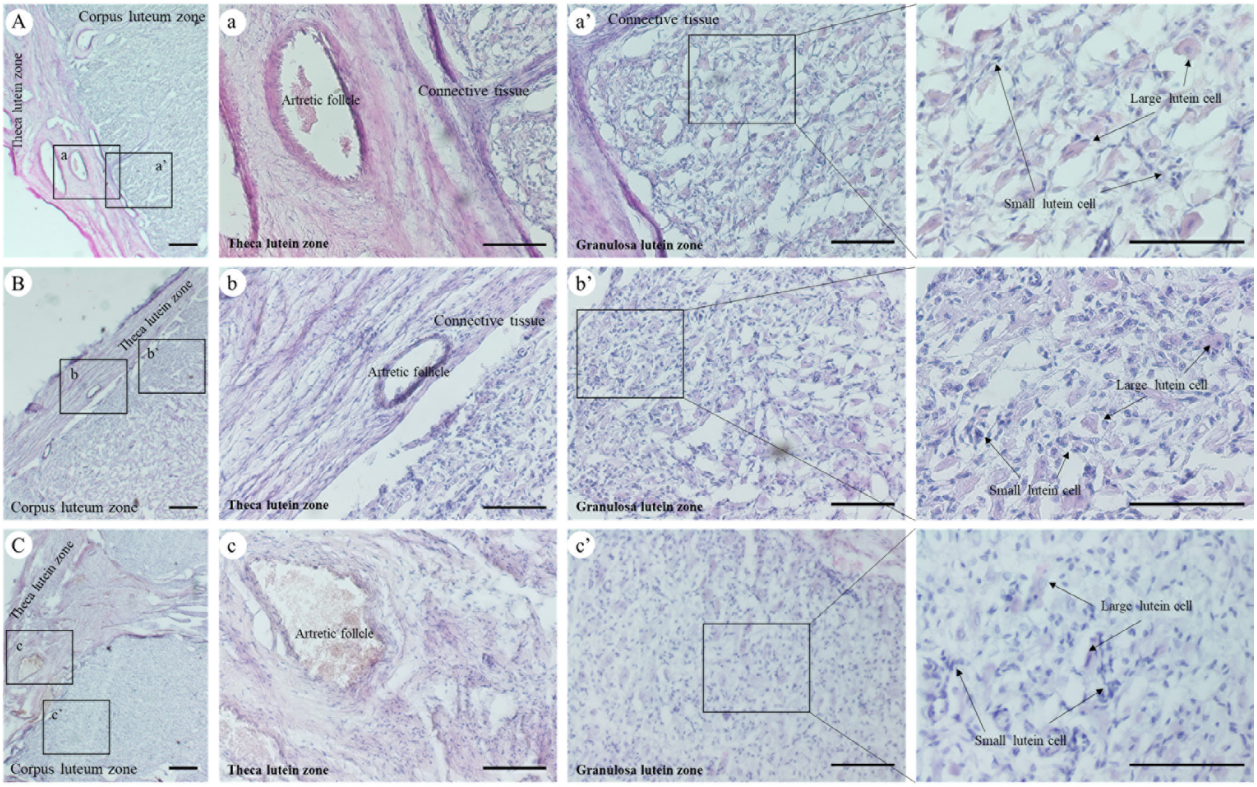

Figure 1. Morphological analysis of the corpus luteum during early pregnancy in bovines. Analysis of the morphological and cellular compositional differences between the theca and granulosa lutein zones of the luteal tissue from 30–90 days of early pregnancy

(A, a, a') Corpus luteum of pregnancy on day 30; (B, b, b') corpus luteum of pregnancy on day 60; (C, c, c') corpus luteum of pregnancy on day 90. Size bar = 100 μm. The magnification in panels A, B, and C is 100×; a, b, and c is 200×; and 400× in the other panels

expressed *20α-HSD* protein gradually increased from 30–90 days of gestation (Figure 2B). The results of the apoptosis-related genes encoding Bax were similar to those of *20α-HSD* and *Casp-3*, but the *BNIP-3* gene was high at 30 days and low at 60 days. However, in the case of vascular endothelial growth factor, B-cell lymphoma 2 (*BCL-2*), and progesterone receptor (*P4-r*), which are associated with cell survival and apoptosis, the expression patterns of *20α-HSD* and *Casp-3* exhibited different results. The overall apoptosis increased rapidly in the large lutein cells at 90 days of pregnancy (Figure 2C).

Analysis of the apoptosis effect according to the concentration of the *20α-HSD* protein treatment in the corpus luteum cells

Figure 3 shows whether an increase in the *20α-HSD* concentration can promote apoptosis during the corpus luteum cell culture. As a result of increasing the *20α-HSD* protein from 0.5 μg/ml to 1.5 μg/ml and analysing the cell death after 48 h, as the concentration of the *20α-HSD* protein in the medium increased, the synthesis of the *20α-HSD* protein detected in the cells decreased and gradually lowered. However, the detection of the *Casp-3* protein, which has an apoptotic effect, increased, but relatively gradually, and was detected at the highest level in the cytoplasmic section of the 1 μg/ml and 1.5 μg/ml groups (Figure 3A). As a result of analysing the expression patterns of the apoptosis-related genes and progesterone-receptor mRNA in the corpus luteum cells cultured according to the *20α-HSD* concentrations, as *20α-HSD* mRNA decreased, *Casp-3* and *Bax* gradually increased. However, *P4-r* and *BCL-2* gradually increased from 0.5 μg/ml to 1 μg/ml, but were very low at 1.5 μg/ml (Figure 3B). Therefore, although the addition of the *20α-HSD* protein was not proportional to the *Casp-3* expression, adding a small amount of *20α-HSD* promotes the expression of *20α-HSD* in the luteal cells.

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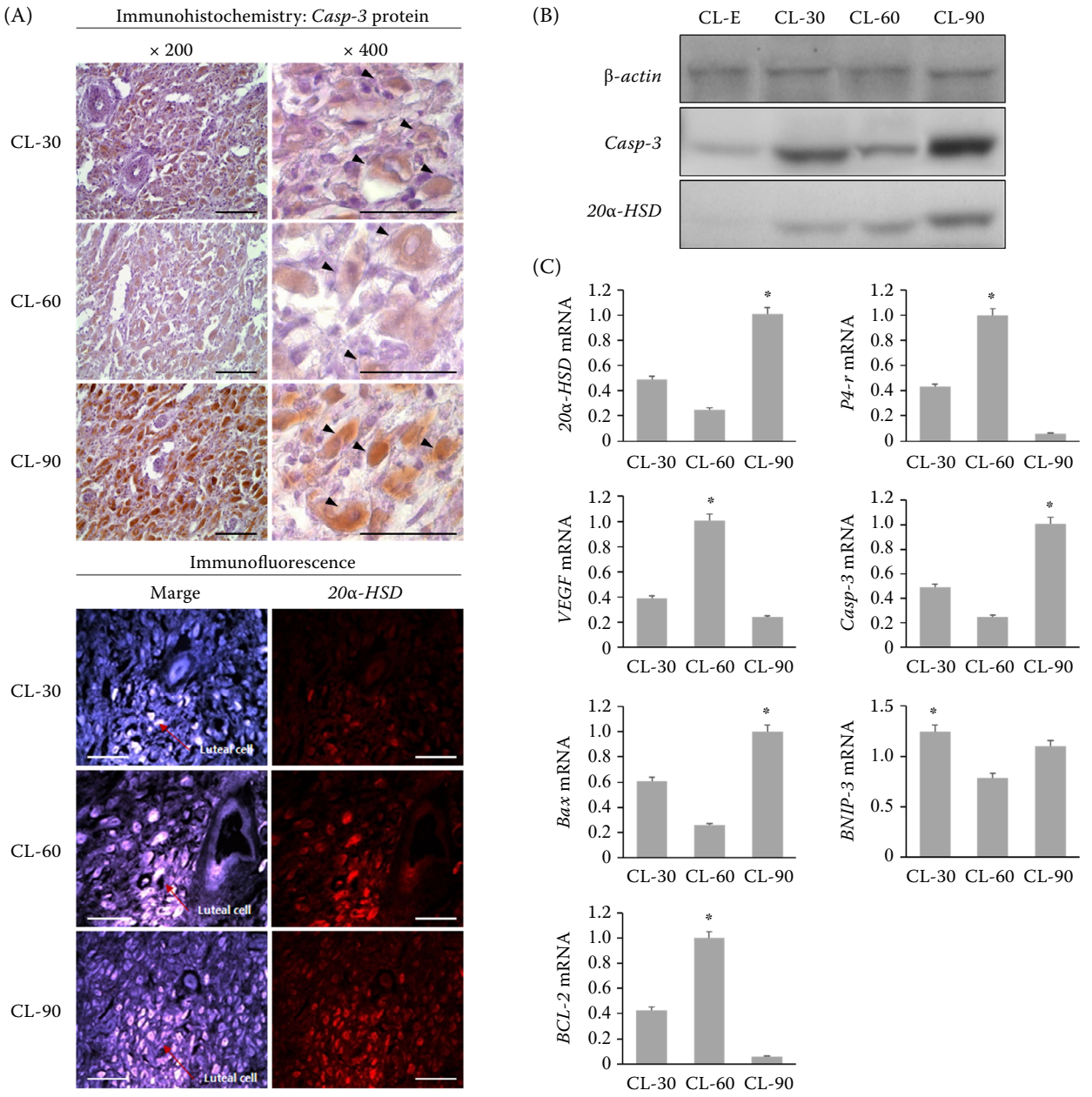

Figure 2. Evaluation of the cell survival- and death-related gene expression patterns in the luteal tissue during early pregnancy

The protein and mRNA expression patterns of the target genes were analysed in the corpus luteum cells obtained from the corpus luteum tissue. (A) Immunohistochemistry analysis of the *Casp-3* localisation and immunofluorescence analysis of the *20α-HSD* protein; (B) western blotting analysis of the *Casp-3* and *20α-HSD* protein; (C) real-time PCR analysis of the cell survival and death-related gene expression. The protein and mRNA expression patterns were quantified using housekeeping proteins and genes, respectively. Size bar = 100 μm

*Significant differences between the groups ($P < 0.05$)

DISCUSSION

Few studies have been conducted on changes in the corpus luteum tissue during pregnancy, and most suggest that *20α-HSD*-mediated apoptosis during early pregnancy may indicate abortion (Naidansuren et al. 2012). However, the corpus luteum undergoes active tissue reorganisation during pregnancy and the functional activity of the corpus luteum changes accordingly (Kim et al.

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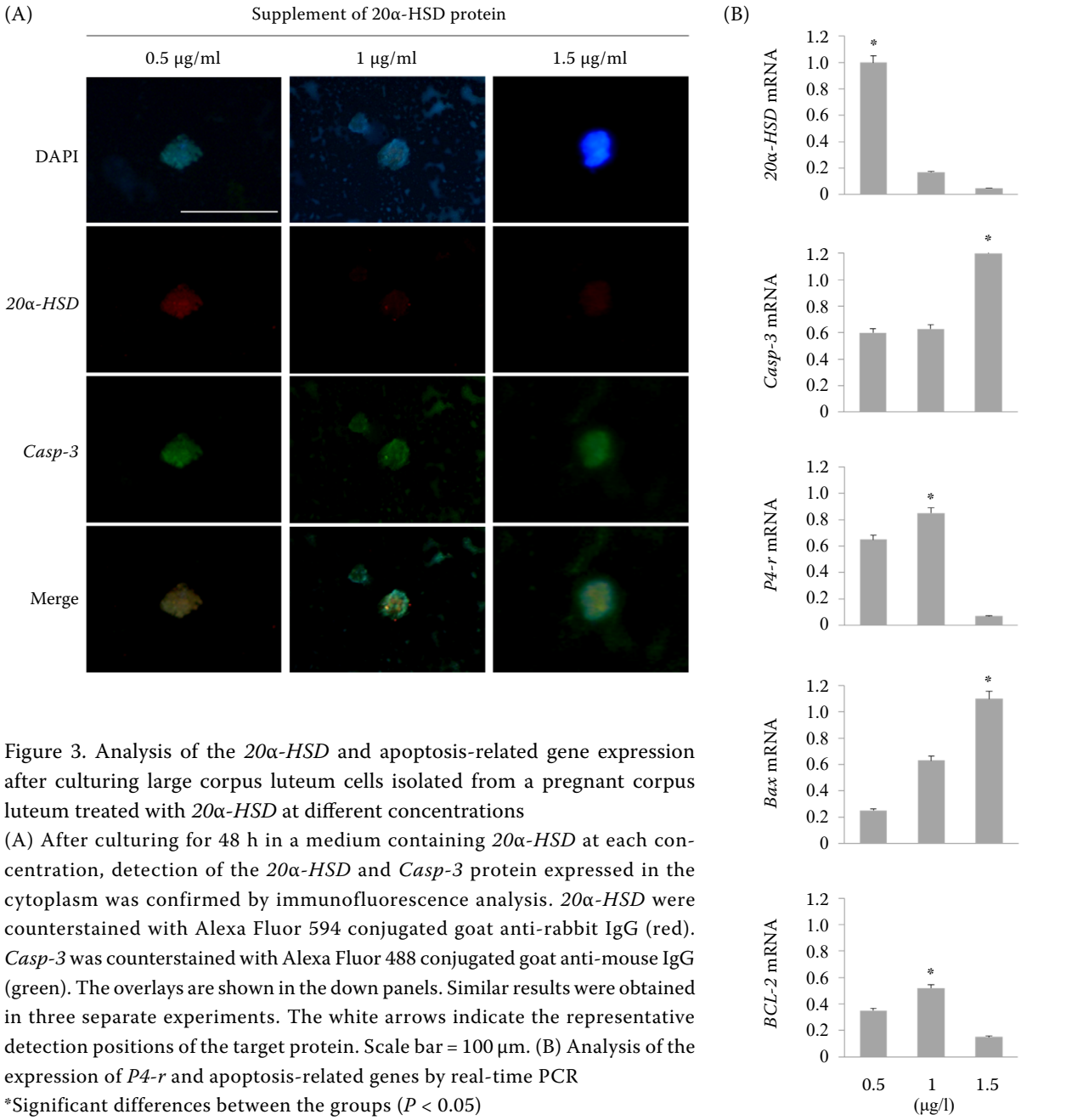

Figure 3. Analysis of the *20α-HSD* and apoptosis-related gene expression after culturing large corpus luteum cells isolated from a pregnant corpus luteum treated with *20α-HSD* at different concentrations

(A) After culturing for 48 h in a medium containing *20α-HSD* at each concentration, detection of the *20α-HSD* and *Casp-3* protein expressed in the cytoplasm was confirmed by immunofluorescence analysis. *20α-HSD* were counterstained with Alexa Fluor 594 conjugated goat anti-rabbit IgG (red). *Casp-3* was counterstained with Alexa Fluor 488 conjugated goat anti-mouse IgG (green). The overlays are shown in the down panels. Similar results were obtained in three separate experiments. The white arrows indicate the representative detection positions of the target protein. Scale bar = 100 μm. (B) Analysis of the expression of *P4-r* and apoptosis-related genes by real-time PCR

*Significant differences between the groups ($P < 0.05$)

2014; Dolezel et al. 2017). The process of reconstitution of the corpus luteum is accomplished by transforming growth factor-beta (*TGF-β*) signalling, T cell receptor signalling/Ilyushin-2 (*IL2*) signalling, which is a luteinising enzyme, and the activity of matrix metalloproteinases by extracellular matrix collagen (ADAMTS1) affects the extracellular matrix, resulting in the overall degeneration of the corpus luteum tissue (Kim et al. 2014; Kim and Yoon 2019; Kim et al. 2019). In other words, the corpus luteum cells are degenerated or controlled by some specific upper-level signal transduction action (Sichtar et al. 2013; Pate 2020; Mezera et al. 2021), but it is not considered as an upper-level action of the actual functional and morphological changes. The histological changes observed in this study suggest that the functional change of the corpus luteum appears to increase the functional performance, whereas the corpus luteum appears to include large lutein cells in the first 30 days of pregnancy (Mezera et al. 2021). On the other hand, the large luteal cell section increased overall as the pregnancy period increased, and the size of the corpus luteal body also appeared

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to increase. In our study, *20α-HSD* was mainly detected in the large corpus luteum cells as the gestation period increased, and the *Casp-3* protein was also detected in the same section. However, the protein expression level of *Casp-3* increased at 30 and 90 days and was relatively low at 60 days, unlike the immunolocalisation analysis. These results may mean that the overall composition of the corpus luteum changes due to the increase in the small luteum cells and intercellular proximity from 90 days after pregnancy. In other words, the expression of *20α-HSD* in the lute during pregnancy is thought to be expressed in the large luteal cells at a specific time to suppress the cell expression (Hirabayashi et al. 2004). As a result of the study by Kim et al. (2014), the corpus luteum appears to function through reconstitution. During the progression of pregnancy, an increase in the concentration of PGF2 transported from the placenta to the uterus increases the transcriptional stimulation of *20α-HSD*. It is believed that the inflow of *20α-HSD* during the culture of luteal cells affects the function of the cells. In particular, as the concentration of *20α-HSD* gradually increases, the cell seems to reduce the synthesis of *20α-HSD* and increase the expression of *Casp-3*. These results indicate that PGF2 induces the *20α-HSD* expression by activating JunD through the nuclear receptor 4A1 (NUR77)-mediated calmodulin-dependent activation of ERK1/2 (Stocco et al. 2002) and, indirectly regulating the expression of *20α-HSD* in the gestational corpus luteum drives the cells to undergo *Casp-3*-mediated apoptosis, which is contrary to the results of previous studies (Naidansuren et al. 2011; Kim et al. 2014). According to the action of the PGF2 expressed in the placenta, the *20α-HSD*/PGFS synthesising enzyme is associated with the modification of the progesterone metabolism in the luteum. In addition, the action of *20α-HSD* is inferred to regulate the activity of progesterone to a certain level, as in previous studies, and induce the deformation of the progesterone metabolism in the luteum (Schuler et al. 2006; Basavaraja et al. 2019; Pate 2020). In this study, the histological changes in the corpus luteum appear to utilise the *20α-HSD* system to control the progesterone in the corpus luteum, which is introduced from the corpus luteum and converted into placental hormones during pregnancy. Determining whether continuous changes in the corpus luteum during early pregnancy promote the survival of the corpus luteum and maintain a successful pregnancy based on the results alone is challenging. However, at least in the early stages of pregnancy, the functional change of the corpus luteum could determine the success or failure of the pregnancy maintenance by controlling the *20α-HSD*-mediated progesterone synthesis.

CONCLUSION

Judging the reproductive efficiency through the current normal pregnancy might affect the maintenance of the early pregnancy governed by the corpus luteum-mediated progesterone feedback loop. This is due to the successful dissolution of the corpus luteum, which was formed at the end of the oestrous cycle in the uterus, or the functional action of the corpus luteum in early pregnancy. According to the results of this study, the corpus luteum in early pregnancy activates progesterone and induces embryo implantation, thereby maintaining a successful pregnancy. According to previous studies, the uterine secretion of PGF2A decreases during early pregnancy. However, the corpus luteum utilises *20α-HSD* to control progesterone for a certain period and reconstructs the luteum tissue by itself using the *Casp-3* apoptosis signalling mechanism. As the gestation period increases from days 30–90, the primary cells of the corpus luteum increase the number of small lutein cells to convert the corpus luteum into new cells, and the existing cells appear to undergo apoptosis. Therefore, the corpus luteum itself is thought to express *Casp-3* through *20α-HSD* regulation during its reconstitution in early pregnancy, thereby inducing functional changes according to the time and optimising the functionality of the pregnancy.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

Arosh JA, Parent J, Chapdelaine P, Sirois J, Fortier MA. Expression of cyclooxygenases 1 and 2 and prostaglandin E synthesis in bovine endometrial tissue during the estrus cycle. Biol Reprod. 2002 Jul 1;67(1):161-9.

https://doi.org/10.17221/116/2022-CJAS

Bacci ML, Barazzoni AM, Forni M, Lalatta Costerbosa G. In situ detection of apoptosis in regressing corpus luteum of pregnant sow; Evidence of an early presence of DNA fragmentation. Dom Anim Endocrinol. 1996 Jul 1; 13(4):361-72.

Basavaraja R, Madusanka ST, Drum JN, Shrestha K, Farberov S, Wiltbank MC, Sartori R, Meidan R. Interferon-tau exerts direct prosurvival and antiapoptotic actions in luteinized bovine granulosa cells. Sci Rep. 2019 Oct 11; 9(1): 11 p.

Bruick RK. Expression of the gene encoding the proapoptotic Nip3 protein is induced by hypoxia. Proc Natl Acad Sci USA. 2000 Aug 1;97(16):9082-7.

Dolezel R, Chambert C, Musilova D, Cech S, Palenik T. Induction of accessory corpus luteum by gonadorelin in relation to the time of treatment and the follicle size in inseminated cows. Czech J Anim Sci. 2017 Apr 28; 62(5):195-200.

Ealy AD, Yang QE. Control of interferon-tau expression during early pregnancy in ruminants. Am J Reprod Immunol. 2009 Feb;61(2):95-106.

Ezashi T, Imakawa K. Transcriptional control of IFNT expression. Reproduction. 2017 Nov;154(5):F21-31.

Hirabayashi K, Ishida M, Suzuki M, Yamanouchi K, Nishihara M. Characterization and functional analysis of the 5'-flanking region of the mouse 20α-hydroxysteroid dehydrogenase gene. Biochem J. 2004 Sep 15; 382(3):975-80.

Hughes C, Pate JL. The ovary. New Amsterdam, The Netherlands: Elsevier; 2019. p. 269-92.

Kim SH, Yoon JT. The role of programmed cell death in the reorganization of bovine corpus luteal cells. J Anim Health Prod. 2019 Sep 15;7(3):119-25.

Kim SH, Shin YS, Kang MH, Yoon JT, Min KS. Gene expression and localization of 20α-hydroxysteroid dehydrogenase (HSD) in reproductive tissues during early pregnancy of cattle. Anim Reprod Sci. 2014 Jun;147(1-2):1-9.

Kim SH, Kim KL, Lee JH, Shin DH, Jung NH, Lee HJ, Yoon JT. Detection of matrix metalloproteinases patterns in bovine luteum cell during pregnancy. J Embryo Transf. 2018 Jun 29;33(2):61-8.

Kim SH, Lee JH, Yoon JT. Expression of matrix metalloproteinases to induce the expression of genes associated with apoptosis during corpus luteum development in bovine. PeerJ. 2019 Jan 30;7: 15 p.

Li C, Issa R, Kumar P, Hampson IN, Lopez-Novoa JM, Bernabeu C, Kumar S. CD105 prevents apoptosis in hypoxic endothelial cells. J Cell Sci. 2003 Jul;116(Pt 3):2677-85.

McCracken JA, Custer EE, Lamsa JC. Luteolysis: A neuroendocrine-mediated event. Physiol Rev. 1999 Apr; 79(2):263-323.

Mezera MA, Hamm CS, Gamarra CA, Gennari RS, Prata AB, Sartori R, Wiltbank MC. Profiles of prostaglandin $F_{2\alpha}$ metabolite in dairy cattle during luteal regression and pregnancy: Implications for corpus luteum maintenance. Biol Reprod. 2019 Jul 1;101(1):76-90.

Mezera MA, Li W, Wiltbank MC. Pregnancy-induced changes in the transcriptome of the bovine corpus luteum during and after embryonic interferon-tau secretion. Biol Reprod. 2021 Jul 2;105(1):148-63.

Naidansuren P, Park CW, Kim SH, Nanjidsuren T, Park JJ, Yun SJ, Sim BW, Hwang S, Kang MH, Ryu BR, Hwang SY, Yoon JT, Yamanouchi K, Min KS. Molecular characterization of bovine placental and ovarian 20α-hydroxysteroid dehydrogenase. Reproduction. 2011 Nov;142(5):723-31.

Naidansuren PJ, Park CW, Nanjidsuren TM, Park JJ, Yun SJ, Kang MH, Yamanouchi K, Min KS. Ovarian and placental expressing of 20α-hydroxysteroid dehydrogenase during pregnancy in deer. Anim Reprod Sci. 2012 Jan; 130(1-2):63-73.

Nishimura R, Sakumoto R, Tatsukawa Y, Acosta TJ, Okuda K. Oxygen concentration is an important factor for modulating progesterone synthesis in bovine corpus luteum. Endocrinology. 2006 Sep;147(9):4273-80.

Nitta A, Shirasuna K, Haneda S, Matsui M, Shimizu T, Matsuyama S, Kimura K, Bollwein H, Miyamoto A. Possible involvement of INFT in lymphangiogenesis in the corpus luteum during the maternal recognition period in the cow. Reproduction. 2011 Dec;142(6):879-92.

Oliveira F, Henkes LE, Ashley RL, Purcell SH, Smirnova NP, Veera-machaneni DNR, Anthony RV, Hansen TR. Expression of interferon (IFN)-stimulated genes in extrauterine tissues during early pregnancy in sheep is the consequence of endocrine IFN-τ release from the uterus. Endocrinology. 2008 Mar;149(3):1252-9.

Pate JL. Roadmap to pregnancy during the period of maternal recognition in the cow: Changes within the corpus luteum associated with luteal rescue. Theriogenology. 2020 Jul 1;150:294-301.

Plendl J, Neumuller C, Sinowatz F. Differences of microvascular endothelium in the bovine corpus luteum of pregnancy and the corpus luteum of the estrous cycle. Biol Cell. 1996 Jan 1;87(3):179-88.

Romero JJ, Antoniazzi AQ, Smirnova NP, Webb BT, Yu F, Davis JS, Hansen TR. Pregnancy-associated genes contribute to antiluteolytic mechanisms in ovine corpus luteum. Physiol Genomics. 2013 Nov;45(22):1095-108.

Schuler G, Teichmann U, Kowalewski MP, Hoffmann B, Madore E, Fortier MA, Klisch K. Expression of cyclooxygenase-II (COX-II) and 20α-hydroxysteroid dehydrogenase (20α-HSD)/prostaglandin F-synthase (PGFS) in bovine placentomes: Implications for the initiation

https://doi.org/10.17221/116/2022-CJAS

of parturition in cattle. Placenta. 2006 Sep-Oct;27 (9-10):1022-9.

Sichtar J, Rajmon R, Hoskova K, Rehak D, Vostry L, Hartlova H. The luteal blood flow, area and pixel intensity of corpus luteum, levels of progesterone in pregnant and nonpregnant mares in the period of 16 days after ovulation. Czech J Anim Sci. 2013 Nov;58(11):512-9.

Stocco CO, Lau LF, Gibori G. A calcium/calmodulin-dependent activation of ERK1/2 mediates JunD phosphorylation and induction of nur77 and 20α-hsd genes by prostaglandin $F_{2\alpha}$ in ovarian cells. J Biol Chem. 2002 Feb 1;277(9-10):3293-302.

Sudeshna T, Anand K, Medhamurthy R. Analysis of 20α-hydroxysteroid dehydrogenase expression in the corpus luteum of the buffalo cow: Effect of prostaglandin F2-alpha treatment on circulating 20α-hydroxyprogesterone levels. Reprod Biol Endocrinol. 2013 Dec;11(1): 10 p.

Yang L, Wang XL, Wan PC, Zhang LY, Wu Y, Tang DW, Zeng SM. Up-regulation of expression of interferon-stimulated gene 15 in the bovine corpus luteum during early pregnancy. J Dairy Sci. 2010 Mar;93:1000-11.

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