

Journal of the Hellenic Veterinary Medical Society

Vol 71, No 3 (2020)



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S. BENBIA, Y. BELKHIRI, M. YAHIA

doi: [10.12681/jhvms.25083](https://doi.org/10.12681/jhvms.25083)

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To cite this article:

BENBIA, S., BELKHIRI, Y., & YAHIA, M. (2020). Apoptotic Cell Death in Ewe Endometrium during the Oestrous Cycle. *Journal of the Hellenic Veterinary Medical Society*, 71(3), 2323–2330. <https://doi.org/10.12681/jhvms.25083>

Apoptotic Cell Death in Ewe Endometrium during the Oestrous Cycle

S. Benbia^{1*}, Y. Belkhiri¹, M. Yahia²

¹Department of Biology of organism, Faculty of Life and Natural Sciences, University Batna 2, Algeria.

^{1,2}Laboratory of Biotechnology of the Bioactive Molecules and the Cellular Physiopathology, Department of Biology of organism, Faculty of Life and Natural Sciences, University Batna 2, Algeria

²Department of cellular and molecular biology, Faculty of Life and Natural Sciences, University Abass Laghrour , Khenchela, Algeria.

ABSTRACT: We hypothesized that endometrial tissues from ewes undergo spatial and temporal changes. Thus, two regulatory events were investigated in this study: cell death (apoptosis) and cell proliferation. Uteri were obtained from healthy ewes at Batna abattoir (Algeria). Based on macroscopic observation of the ovaries and plasma progesterone, uteri were assigned to follicular, early and active luteal phases. Apoptosis and proliferation were assessed by detection of cleaved caspase-3 and Ki-67, respectively. Ki-67 and cleaved caspase-3 (CCP-3) were expressed in both phases of the oestrous cycle and all endometrium cells types [luminal epithelia (LE), superficial gland epithelia (SG) and deep gland epithelia (DG)]. Immunohistochemistry for cleaved caspase-3 revealed few or no apoptotic stained cells in all endometrium locations during the entire oestrous cycle. However, Ki-67 was significantly higher in the follicular phase than in the early and active luteal phase. Besides, expression of CCP-3 in LE was higher than in SG and DG at the follicular phase and early luteal phase. However, Ki -67 and CCP-3 levels in all endometrium cells types did not significantly change at active luteal phase. Therefore, it is concluded that apoptosis and proliferation were occurred in ewe endometrium in a cyclic pattern and under the influence of the endocrine profile.

Keywords: ewes; endometrium; apoptosis; proliferation; oestrous cycle.

Corresponding Author:

Souheyla Benbia, Laboratory of Biotechnology of the Bioactive Molecules and the Cellular Physiopathology, Department of Biology of organism, Faculty of Life and Natural Sciences, University Batna 2, P.O. Box 05000, Algeria
E-mail address: s.benbia@univ-batna2.dz

Date of initial submission: 13-11-2019
Date of revised submission: 08-02-2020
Date of acceptance: 07-02-2020

INTRODUCTION

It is well known that the major reason for the failure of the embryo implantation is abnormal endometrial receptivity. During this period, morphological and biochemical changes occur in mammalian endometrium. These modifications are regulated by a plethora of factors, including oestrogen, progesterone, and their receptors, transcriptional factors and many others (Lonergan and Forde, 2014; Simmons et al. Recent investigations have demonstrated the importance of apoptosis in the processes of endometrial tissue remodeling during the oestrous cycle (Arai et al., 2013; Simmons et al., 2009). The balance between cell proliferation and apoptosis regulates the periodic repair and shedding of endometrial cells and leads to the menstruation (in the woman) or prepare the mucosal layer of endometrium for the implantation of the embryo (Stewart et al., 1999; Antsiferova and Sotnikova, 2016).

Apoptosis is a form of programmed cell death in which cells condense and fragment their nuclear material, condense their cytoplasmic material and then release their contents in membrane-bound apoptotic bodies (Mcilwain et al., 2013). Cells are induced to undergo apoptosis through either extrinsic or intrinsic pathways, which are controlled by the expression of a number of regulatory apoptosis-related genes, such as bcl-2 family (Li et al., 1998; Mcilwain et al., 2013), death receptor and caspases (Li et al., 1997; Mcilwain et al., 2013). Caspases, a family of aspartic acid-specific cysteine proteases are considered a key mediator of apoptosis in various cells (Brenner and Mak, 2009). Caspase-3 is the best-identified protein among the 14 caspase family members (Brenner and Mak, 2009; Mcilwain et al., 2013).

Ki-67 is a non-histone nuclear protein observed in proliferating cells and found in all phases of the cell cycle, except of G0 phase (Gap 0), and comprises two molecules of 345- and 395-kD weight; its gene is located on human chromosome 10. This marker is expressed during G1(Gap 1), S (Synthesis), G2, and M (Mitosis) phases of cell cycle. In the first phase, Ki-67 is frequently revealed near the nucleus. During S and G2 phases, this protein is detected throughout the nucleus (Verheijen et al., 1989a). Generally, Ki-67 expression was observed during mitosis phase (Verheijen et al., 1989b).

Although cell proliferation has already been verified in the sheep endometrium (Zheng et al., 1996; Johnson et al., 1997a, 1997b), to our knowledge no

study data on apoptotic cells are available. The aim of this investigation was to examine the occurrence of endometrial apoptosis and proliferation at defined stages of the oestrous cycle of the ewe, through the assessment of active caspase-3 and Ki-67, respectively.

MATERIAL AND METHODS

Collection of endometrium and blood samples

Twenty five normal uteri without a visible conceptus were collected from ewes at commercial abattoir, Batna (Algeria). Based on the methodology described previously by Benbia et al. (2017), ten of the ewes were classified in follicular phase and fifteen were in luteal phases (six at ELP and nine at ALP). Endometrial biopsies were obtained from the uterine horns, and then fixed in formol 10% before embedding. Jugular blood samples were collected into EDTA tubes from all animals before slaughter; plasma was stored at -20°C until assay.

Immunohistochemistry and scoring

Sections of 3-4 µm were placed on Super Frost slides (Menzel-Glaser, Freiburg, Germany). Slides were deparaffinized and subjected to antigen-retrieval at 95°C for 20 minutes on a Dako PT Link Envision Flex-Target retrieval solution at low pH (pH 6.0). Thereafter, slides were cooled and incubated for 10 min in Envision Flex Washing buffer.

Sections were stained in an automatic immunohistochemical staining device (Dako Autostainer Link 48) with mouse monoclonal anti Ki-67 antibody, clone MIB-1 (M7240; Dako-Cytomation, Glostrup, Denmark) as the proliferation marker and rabbit polyclonal anti cleaved caspase 3 (Asp175) antibody (#9661; Cell Signalling Technology, Boston, USA) as the apoptotic cell marker. Normal mouse or rabbit serum was used instead of the primary antibody (for negative control). Sections were incubated with secondary antibodies (EnVision FLEX; Dako) for 20 minutes. Staining was performed with 3,3-Diaminobenzidine (DAB) as chromogen for 20 minutes. Finally, sections were counterstained with Mayer's hematoxylin, in a graded series of ethanol, cleared in xylene, and covered with a cover slip using a monted medium.

Staining was assessed at: luminal epithelium (LE), superficial gland epithelium (SG) and deep gland epithelium (DG). Immunoreactivity of both markers was calculated using Quick Score (QS). The following formula was used for the evaluation of QS as

described by Sağsöz et al. (2011) stromal cells and smooth muscle cells. Generally, in the cervix, ER α immunoreactivity was more intense in the epithelial and smooth muscle cells during the follicular phase and in the epithelial cells during the luteal phase ($p < 0.05$).

QS = Intensity score (IS) + Proportional score (PS).

The IS was defined as the intensity of brown colour of nuclei. The intensity of staining for each protein was quantified using Image-Pro Plus 6.3 image processing and analysis software according to the manufacturer's instructions (Media Cybernetics, Inc., Bethesda, MD, USA). The PS was evaluated by manually counting the number of cells showing nuclear staining in 100 epithelial cells. The Immunoreactivity was categorized as described in table 1.

Hormone assessment in blood

Plasma progesterone concentration was determined using a radioimmunoassay kit (ImmuChem™ Double Antibody Progesterone I¹²⁵ RIA Kit, MP Bio-medicals, USA). The sensitivity of this assay was 0.18 ng/ml. Plasma concentrations of oestradiol 17 β was assessed using E2 Diasorin RIA kit (Sorin Diagnostic, Antony, France). The sensitivity of the assay was 0.07 pg/ml.

Statistical analysis

Statistical analyses were performed using Graph Pad Prism 6 (ver. 5.02, GraphPad Software, Inc., CA, USA). Initially, data were tested for normality and homogeneity (Bartlett test) of variances. The factors in

the procedure were: stage of oestrous cycle (FP, ELP and ALP), and endometrium cells types (LE, SG and DG).

Effects of stage of the oestrous cycle on cell-specific expression of Ki 67 and CCP-3 were analyzed using two-way ANOVA. Comparison of means was performed by Bonferroni post hoc test. Possible correlations among cell apoptosis (CCP-3), cell proliferation (Ki-67) and plasma oestradiol 17 β concentration, in the three endometrium cell types were tested using spearman correlation. Statistical significance was considered as $P < 0.05$.

RESULTS

The plasma concentration of P4 was 3.2 ± 0.06 ng/ml in the active luteal phase and decreased ($P < 0.05$) to 1.1 ± 0.49 ng/ml in the next phase. The lowest P4 concentration was observed in the early luteal phase (0.5 ng/ml).

Localization and expression of Ki-67-positive cells throughout the oestrous cycle

As shown in Figure 1, endometrial cell proliferation and apoptosis were detected during the oestrous cycle of the ewes. Even though Ki-67 and CCP-3 were expressed in both follicular and luteal phases of the oestrous cycle, the Quick score of cells expressing Ki-67 was significantly higher than CCP-3 at the FP and ELP ($P < 0.05$) (Fig1), while CCP-3 was significantly higher in FP compared to ALP. Furthermore, Ki-67 marker was higher at the early luteal phases compared to the active luteal phase ($P < 0.05$) (Fig 1). Regarding cleaved caspase 3, no difference was observed among luteal phases ($P = 0.95$) (Fig1).

Table 1: Immunosensitivity categorization - Grading of Ki-67 and CCP-3.

IS		PS	
0	Absent staining	1	<1%
1	Weak staining	2	1–10%
2	Moderate staining	3	11–33%
3	Strong staining	4	34–66%
		5	67–100%

IS: intensity score.; PS: proportional score (%)

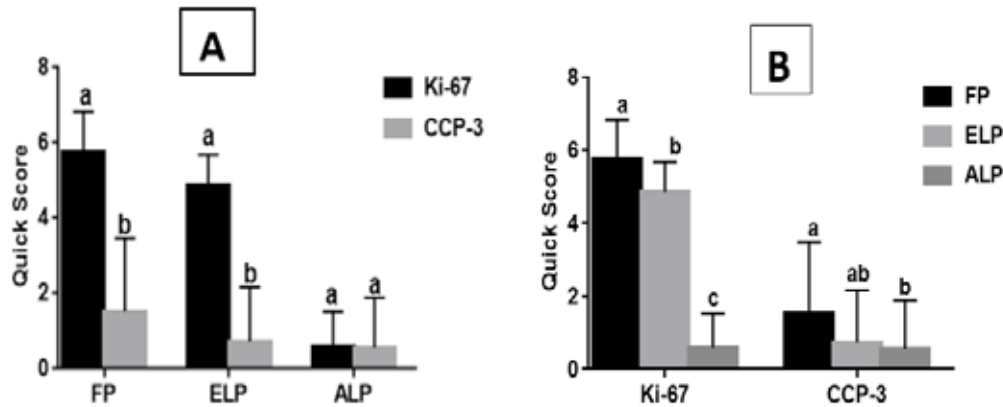


Figure 1. Comparison between total Quick score of CCP-3 (mean±SEM) and Ki-67 (mean±SEM) expression in sheep endometrium during the oestrous cycle. Values with different superscript letters differ significantly ($P < 0.05$), between Ki-67 and CCP-3 (figure 1.A) and among the FP, ELP and ALP (figure 1.B).

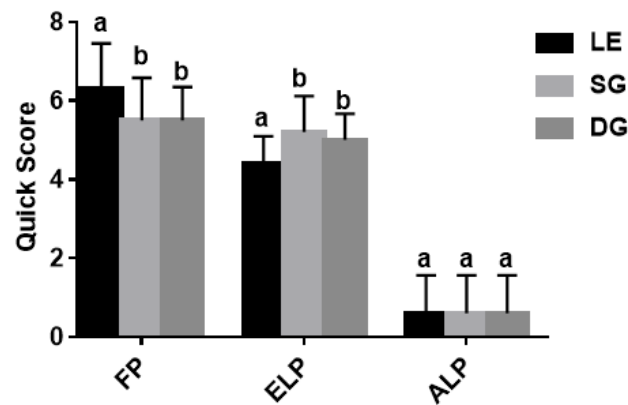


Figure 2. Quick score of Ki-67 (mean±SEM) in different sheep endometrial compartments (luminal epithelium, superficial and deeper glands) at the follicular phase and at different stages of the luteal phase. LE: luminal Epithelium; SG: Superficial Glands; DG: Deeper Glands; FP: Follicular Phase; ELP: Early Luteal Phase; ALP: Active Luteal Phase. Values with different superscript letters differ significantly, among the endometrium cell types (LE, SG, DG).

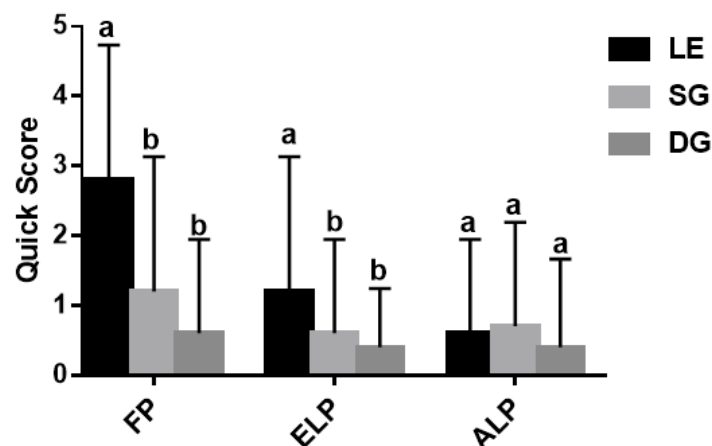


Figure 3. Quick score of CCP-3 (mean±SEM) in different sheep endometrial compartments (luminal epithelium, superficial and deeper glands) at the follicular phase and at different stages of the luteal phase. LE: luminal Epithelium; SG: Superficial Glands; DG: Deeper Glands; FP: Follicular Phase; ELP: Early Luteal Phase; ALP: Active Luteal Phase. Values with different superscript letters differ significantly, among the endometrium cell types (LE, SG, DG).

Ki-67 nuclear expression in different endometrial cells (LE, SG, DG) throughout the follicular and luteal phase are presented in Figure 2. Ki-67 stains all endometrium cells with high intensity, so intensity score was always as grade 3. The proliferative activity of luminal epithelium (LE) was significantly higher compared with endometrial glands (SG and DG) at follicular phase ($P<0.01$) (Fig 2). The immunoreactivity of cells expressing Ki-67 protein was significantly increased in the follicular phase and early luteal phase than in the active luteal phase. Besides, expression of this marker in LE, SG and DG was higher during the early luteal stage than in the active luteal phase ($P<0.05$) (Fig 2). Furthermore, Ki-67 nuclear expression in endometrial glands (SG, DG) did not significantly change during the follicular and early luteal stage transition (Fig 2).

Expression Ki-67 in uterine epithelium (LE, SG and DG) did not significantly change at active luteal phase.

Localization and expression of CCP-3-positive cells in ewe endometrium

Active CCP-3 expression was detected in both lu-

minal and glandular epithelial cells in the sheep endometrium during all phases of the oestrous cycle (Fig 3). CCP-3 was more expressed in the luminal epithelium than in the glandular epithelial cells at the follicular phase and early luteal phase ($P<0.05$); however, the active luteal phase endometrium cells types did not affect the expression of this protein (Fig 3).

Relationship between cell apoptosis (CCP-3) and cell proliferation (Ki-67)

Table 2 shows that a negative correlation was revealed between CCP-3 and Ki-67 expression; this correlation was significant during the ELP ($P=0.02$) and approached significance during FP ($P=0.05$).

Relationship between cell proliferation and plasma oestradiol 17β concentration

As shown in a Table 3, positive approached significant correlation was found between Ki-67 expression and plasma oestradiol 17β concentration in ewe endometrium in LE (ELP, $P=0.06$; ALP, $P=0.05$) and SG (ELP, $P=0.08$) during oestrous cycle phases ($P>0.05$) (this taken from the limited number of samples in this work).

Table 2. Correlation between cell apoptosis (CCP-3) and cell proliferation (Ki-67), during the oestrous cycle.

CCP-3 Quick score	Ki-67 Quick score					
	PF		ELP		ALP	
	r		r	P	r	P
	-0.62	0.05	-0.751	0.02	0.334	0.2

FP: Follicular Phase; ELP: Early Luteal Phase; ALP: Active Luteal Phase; r: correlation coefficient; P: Probabilities significant ($P<0.05$)

Table 3. Correlation between cell proliferation and plasma oestradiol 17β concentration, in the three endometrium cell types during the oestrous cycle.

Ki-67 Quick score		Plasma oestradiol 17β concentration					
		FP		ELP		ALP	
		r	P	r	P	r	P
	LE	0.691	0.059	0.501	0.06	0.689	0.05
	SG	0.61	0.063	0.761	0.08	0.565	0.084
	DG	0.40	0.19	0.39	0.49	0.32	0.33

FP: Follicular Phase; ELP: Early Luteal Phase; ALP: Active Luteal Phase; LE: luminal epithelium; SG: superficial gland; DG: deeper gland; r: correlation coefficient; P: Probabilities significant ($P<0.05$).

DISCUSSION

The present study examined, for the first time, the occurrence of apoptosis (CCP-3) in the various cells types of sheep endometrium, during the stages of the oestrous cycle. In many mammals, endometrium function and homeostasis is an important physiological phenomenon that ensures a dynamic balance between cell proliferation and cell death (apoptosis) throughout the oestrous cycle (Johnson et al., 1997b, 1997a) on cow (Arai et al., 2014, 2013), goat (Zhang et al., 2018, 2019) and equine (Costa et al., 2007), but, to our best knowledge, not in sheep.

According to our results Ki-67 and cleaved caspase-3 were differentially expressed in endometrium of sheep, and this expression exhibited spatial and temporal changes. It was noteworthy that there were few or no apoptotic stained cells in all endometrium locations during the entire oestrous cycle.

In this research, we found higher levels of Ki-67 in the follicular and early luteal phase, compared with active luteal phase endometrium when low expression of this protein was observed in the various endometrium cell types. These Ki-67 patterns during luteal phase are in agreement with the results of García-Palencia et al. (2007), who demonstrated that intra-vaginal progestogen treatment had a great effect on the immunoexpression of cell proliferation marker in sheep endometrium.

In the present study, cleaved caspase-3 expression increased significantly in the follicular phase and decrease in early and active luteal phase. Additionally, we revealed that CCP-3 is differentially expressed in the various cell types of sheep endometrium. It was well-known that the main executioner involved in the cell death pathway is caspase-3 (CPP3), which is activated during the early stage of apoptosis in rat (Alan and Liman, 2016). The assessment of the active form of this protein is one of the most exploited diagnostic methods for revealing apoptosis in various cell types (Jin and El-Deiry, 2005). Likewise, it has been suggested that endometrial apoptosis was regulated by the expression of active caspase-3 during the oestrous cycle in goat and cow (Zhang et al., 2018; Arai et al., 2013) and during early pregnancy in the rat (Öner et al., 2010).

Recently, it was indicated that, several regulators of programmed cell death and cell proliferation, such

as PTEN, PTEN/PI3K/AKT, Bcl-2 family, FAS, p53 and Caspase-3 are implicated in the apoptosis and proliferation of the endometrium cells of dairy goats in vitro, (Zhang et al., 2019). For example, Zhang et al. (2018) reported that miR-26a mimic increased the Caspase-3 protein levels in endometrium epithelial cells, but it decreased the expression of this protein in endometrium stromal cells of dairy goats (Zhang et al., 2018). However, no such research has been performed in the endometrium of sheep. These in vitro studies suggested that there were enormous and complicated regulatory networks expressed differentially in cell types and functions in the endometrium of ruminants. Except the oestrogen and progesterone, a variety of factors contributed in this regulation, such as growth factors (Stevenson et al., 1994; Md. Rashedul Islam et al., 2016) and integrins (Park et al., 2017).

In this study, we found that the expression of CCP-3 and Ki-67 were highly regulated temporally and among cell types, mainly in uterine luminal epithelium. Surface epithelium is the first contact point of the embryo during the early stage of implantation (Bazer, 2013). Our result suggest that apoptosis may be indispensable for the establishment of endometrial function in sheep.

Previous investigations in several mammalian species have revealed an inverse correlation between cell apoptosis and cell proliferation in the uterus (Harada et al., 2004; Costa et al., 2007; Okano et al., 2007) which was consistent with the results of the present study in the follicular phase and early luteal phase endometrium of sheep.

In the present study, a positive correlation was suggested between oestrogen concentration and cell proliferation score (approach significant) and was significant during early luteal phase in LE and SG cells. Thus, Thus, most proliferation occurred during the follicular and early luteal phases, when follicular growth and maturation result in the highest production of E2, followed by the second E2 peak during metestrus (Robinson et al., 2001).

Endometrial epithelial cells proliferation decreased during the active luteal phase when circulating P4 levels were high.. Shiozawa et al. (2001) hypothesized that in the luteal stage, P4 can raise the levels of some negative regulators, such as p27 (Shiozawa et al., 2001) and cyclin G1 (Yuan et al., 2014) required for the proliferation inhibition as it has been observed in human endometrial epithelial cells. Recently Arm-

strong et al. (2017) revealed that apoptosis was rapidly induced after the progesterone-withdrawal in mouse and human endometrium (Armstrong et al., 2017). We hypothesized, probably, that a similar mechanism might also apply in the case of sheep.

CONCLUSION

It is concluded in the present report that both Ki-67((proliferation) and cleaved caspase-3 (apoptosis) were expressed in the endometrium of sheep, and this expression exhibited spatial and temporal variations. Tissue remodeling was mainly observed in luminal epithelium cells of endometrium.

ACKNOWLEDGEMENTS

This work was supported by Laboratory of Bioactive Molecules and Cellular Physiopathology, Faculty of Natural and Life Sciences , Batna -2 University, 05000, Batna, Algeria and Directorate-General For Scientific Research And Technological Development (DGSRTD), Algeria.

CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

REFERENCES

- Alan E and Liman N (2016) Involution Dependent Changes in Distribution and Localization of Bax , Survivin , Caspase-3 , and Calpain-1 in the Rat Endometrium. *Microscopy Research And Technique*. 297(79):285–297.
- Antsiferova YS and Sotnikova NY (2016) Apoptosis and endometrial receptivity: Relationship with in vitro fertilization treatment outcome. *World Journal of Obstetrics and Gynecology*. 5(1):87.
- Arai M, Yoshioka S, Nishimura R and Okuda K (2014) FAS / FASL-mediated cell death in the bovine endometrium. *Animal Reproduction Science*. 151(3–4):97–104, Elsevier B.V.
- Arai M, Yoshioka S, Tasaki Y and Okuda K (2013) Remodeling of bovine endometrium throughout the estrous cycle. *Animal Reproduction Science*. 1–9, Elsevier B.V.
- Armstrong GM et al. (2017) Endometrial apoptosis and neutrophil infiltration during menstruation exhibits spatial and temporal dynamics that are recapitulated in a mouse model. *Scientific Reports*. 7:17416.
- Bazer FW (2013) Pregnancy recognition signaling mechanisms in ruminants and pigs. *Journal of animal science and biotechnology*. 4(1):.
- Benbia S, Yahia M, Letron IR and Benounne O (2017) Endometrial Cells Morphology Depending on Estrous Cycle and Histologic Layers in Cows: Morphometric Study Biotechnology's Laboratory of the Bioactive Molecules and the. *Global Veterinaria*. 18(1):68–73.
- Brenner D and Mak TW (2009) Mitochondrial cell death effectors. *Current Opinion in Cell Biology*. 21:871–877.
- Costa RPR da et al. (2007) Caspase-3 mediated apoptosis and cell proliferation in the equine endometrium during the estrous cycle. *Reproduction, Fertility and Development*. 19:925–932.
- Garcia-Palencia P et al. (2007) Sex steroid receptor expression in the oviduct and uterus of sheep with estrus synchronized with progestagen or prostaglandin analogues. *Animal Reproduction Science*. 97(1–2):25–35.
- Harada T et al. (2004) Apoptosis in human endometrium and endometriosis. *Human Reproduction Update*. 10(1):29–38.
- Jin Z and El-Deiry WS (2005) Overview of Cell Death Signaling Pathways ND ES SC. *Cancer Biology & Therapy*. 4(2):147–171.
- Johnson ML, Redmer D a and Reynolds LP (1997a) Uterine growth, cell proliferation, and c-fos proto-oncogene expression throughout the estrous cycle in ewes. *Biology of reproduction*. 56(2):393–401.
- Johnson ML, Redmer D a and Reynolds LP (1997b) Effects of ovarian steroids on uterine growth, morphology, and cell proliferation in ovariectomized, steroid-treated ewes. *Biology of reproduction*. 57(January 1997):588–596.
- Li H, Zhu H, Xu CJ and Yuan J (1998) Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis. *Cell*. 94(4):491–501.
- Li P et al. (1997) Cytochrome c and dATP-Dependent Formation of Apaf-1 / Caspase-9 Complex Initiates an Apoptotic Protease Cascade. 91479–489.
- Loneragan P and Forde N (2014) Maternal-embryo interaction leading up to the initiation of implantation of pregnancy in cattle. *Animal*. 8(s1):64–69.
- McIlwain DR, Berger T and Mak TW (2013) Caspase Functions in Cell Death and Disease. *Cold Spring Harb Perspect Biol*. 5:1–27.
- Md. Rashedul Islam, Yamagami K, Yoshii Y and Yamauchi N (2016) Growth factor induced proliferation, migration, and lumen formation of rat endometrial epithelial cells. *Journal of Reproduction and Development*. 62(3):271–278.
- Okano A, Ogawa H, Takahashi H and Geshi M (2007) Apoptosis in the porcine uterine endometrium during the estrous cycle, early pregnancy and Post partum. *Journal of Reproduction and Development*. 53(4):923–930.
- Öner H, Öner J and Demir R (2010) Distributions of PCNA and Cas-3 in rat uterus during early pregnancy. *Folia Histochemica Et Cytobiologica*. 48(1):71–77.
- Park HJ et al. (2017) Integrins functioning in uterine endometrial stromal and epithelial cells in estrus. *Reproduction*. 153:351–360 Introduction.
- Robinson RS, Mann GE, Lamming GE and Wathes DC (2001) Expression of oxytocin, oestrogen and progesterone receptors in uterine biopsy samples throughout the oestrous cycle and early pregnancy in cows. *Reproduction*. 122(6):965–979.
- Sağsöz H, Akbalik ME, Saruhan BG and Ketani M a (2011) Localization of estrogen receptor α and progesterone receptor B in bovine cervix

- and vagina during the follicular and luteal phases of the sexual cycle. *Biotechnic & histochemistry*. 86(4):262–71.
- Shiozawa T et al. (2001) Up-Regulation of p27Kip1 by Progestins Is Involved in the Growth Suppression of the Normal and Malignant Human Endometrial Glandular Cells. *Endocrinology*. 142(4):4182–4188.
- Simmons RM et al. (2009) Insulin-like growth factor binding protein-1 in the ruminant uterus: potential endometrial marker and regulator of conceptus elongation. *Endocrinology*. 150(9):4295–4305.
- Stevenson KR, Gilmour RS and Wathes DC (1994) Localization of insulin-like growth factor-I (IGF-I) and -II messenger ribonucleic acid and type I IGF receptors in the ovine uterus during the estrous cycle and early pregnancy. *Endocrinology*. 134(4):1655–1664.
- Stewart CJR, Critchley HOD and Farquharson MA (1999) Endometrial apoptosis in patients with dysfunctional uterine bleeding. *Histopathology*. 34:99–105.
- Verheijen R, Kuijpers HJH, Driel R Van, et al. (1989a) Ki-67 detects a nuclear matrix-associated proliferation-related antigen II. Localization in mitotic cells and association with chromosomes. *Journal of Cell Science* 92, 92531–540.
- Verheijen R, Kuijpers HJH, Schlingemann RO, et al. (1989b) Ki-67 detects a nuclear matrix-associated proliferation-related antigen I. Intracellular localization during interphase. *J Cell Sci*. 92((Pt 1)):123–30.
- Yuan D -z. et al. (2014) Progesterone-Induced Cyclin G1 Inhibits the Proliferation of Endometrial Epithelial Cell and its Possible Molecular Mechanism. *Endocrine Research*. 46:761–767.
- Zhang L et al. (2018) MiR-26a promoted endometrial epithelium cells (EECs) proliferation and induced stromal cells (ESCs) apoptosis via the PTEN-PI3K/AKT pathway in dairy goats. *Journal of Cellular Physiology*. 233(6):4688–4706.
- Zhang L et al. (2019) Endometrial Epithelial Cell Apoptosis Is Inhibited by a ciR8073-miR181a-Neurotensin Pathway during Embryo Implantation. *Molecular Therapy: Nucleic Acid*. 14(22):262–273, Elsevier Ltd.
- Zheng J, Johnson ML, Redmer DA and Reynolds LP (1996) Estrogen and progesterone receptors, cell proliferation, and c-fos expression in the ovine uterus during early pregnancy. *Endocrinology*. 137(1):340–348.