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THE ROLE OF ENDOCANNABINOID SYSTEM BASED ON mRNA EXPRESSION DURING THE LATE LUTEAL PHASE AND ESTRUS IN THE BOVINE ENDOMETRIUM

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Abstract

There are several findings indicating that the endocannabinoid system (ECS) is an important factor, acting in multiple ways in regulating reproductive function but changes of this system in the bovine endometrium have rarely been investigated; therefore, this study was designed to consider an association between endometrial ECS expression and different stages of the estrous cycles. MRNA expressions of the ECS were investigated during the late luteal phase and estrus using real-time PCR. Following estrous synchronization of sixteen Holstein dairy cows (34±1.3 kg/day of milk production), using two PGF2α injections given 14 days apart, at 30 and 44 days in milk (DIM), blood samples and ultrasonography (US) were performed every other day from the day of second PGF2α injection (44 DIM) until the start of the next estrous cycle (67±2 DIM) to verify CL development and ovulation. Based on blood and US results endometrial tissue was collected on days 16 (late luteal phase) and 21 (estrus) of the synchronized estrous cycle (ovulation = d 0). Real-time PCR analysis of ECS mRNA expression revealed endocannabinoid receptor (CNR2), diacylglycerol lipase (DAGL), cyclooxygenase-2 (COX-2), fatty acid amide hydrolase (FAAH) and monoglyceride lipase (MGLL) had significant fold differences when comparing two different stages of the estrous cycle (late luteal phase vs. estrus). CNR2 and DAGL showed 2.01 and 2.57 fold increase, respectively (P=0.04 and P=0.02), in estrous cows. Among the analyzed genes *FAAH* (P=0.01) and *MGLL* (P=0.02) were significantly down-regulated in estrous cows, with a 5.01- and 2.44-fold difference in mRNA expression, respectively. Overall, this study highlights an association between the expression of the ECS in the bovine endometrium and stage of the estrous cycle.

Key words: endocannabinoid system (ECS), estradiol, progesterone, real-time PCR

The endometrium plays a pivotal role in coordinating the events that lead to fertilization, implantation and pregnancy. Throughout the pregnancy and estrous cycle, the endometrium is subjected to a host of functional and morphological changes, regulated by the hormones progesterone, estradiol and oxytocin (Spencer et al., 2004). The molecular mechanisms underlying endometrial function may contribute

to reproductive performance in dairy cows (Killeen et al., 2014). Using a global gene profiling approach, evidence identified numerous differentially expressed genes and related functional pathways in bovine endometrium under various conditions: during the different phases of the estrous cycle (Wolf and Bauersachs, 2010), in pregnant and cycling animals with artificially induced high, and normal systemic progesterone concentrations (Forde et al., 2010; McCarthy et al., 2012) and during early pregnancy in animals that produced viable and non-viable embryos (Beltman et al., 2010).

Endocannabinoids (EC, endogenous cannabinoids) are an emerging class of lipid mediators which includes fatty acid amides (FAA; oleoylethanolamide, anandamide) (Maccarrone et al., 2002). Anandamide (*N*-arachidonoylethanolamine, AEA) and 2-arachidonoylglycerol (2-AG) are the best studied ligands for the cannabinoid receptors (CNRs) (De Petrocellis and Di Marzo, 2009). They bind to endocannabinoid receptors CNR1 and CNR2, which are G protein-coupled receptors mainly found in central nervous system, but also in peripheral tissues (Gorzalka and Dang, 2012). The enzymes responsible for the synthesis and degradation of AEA (N-acyl transferase (NAT) and N-arachidonoyl phosphatidylethanolamine phospholipase D (NAPE-PLD) and fatty acid amide hydrolase (FAAH) and of 2-AG (Di-acyl glycerol lipase (DAGL) and monoacyl glycerol lipase (MGLL)), convert these two endocannabinoids into arachidonic acid (AA) and ethanolamine or AA and glycerol, respectively (Di Marzo, 2008). Cyclooxygenase 2 (COX-2) converts AEA into prostaglandin ethanolamines. The endocannabinoid system (ECS) has been well-studied in non-ruminants which have orexigenic, anorexigenic, or anti-inflammatory properties (Lazzarin et al., 2004; Habayeb et al., 2004). There are few studies about the role of this system in ruminant reproduction (Weems et al., 2009; Abolghasemi et al., 2016; Dirandeh and Ghaffari, 2018). Bonsale et al. (2018) reported ECS can be considered as an endometrial inflammatory marker in dairy cows. There was a negative relationship between stimulation of either CNR1 or CNR2 receptors, luteal function and progesterone concentrations in sheep (Weems et al., 2009; Tsutahara et al., 2011). Indeed, it has been reported that low plasma AEA levels are essential for implantation and early pregnancy success in human (Demuth and Molleman, 2006); moreover, it has been shown that high AEA concentrations are embryotoxic in rats (Beltramo et al., 1997), and elevated plasma levels are associated with miscarriage in women (Dinh et al., 2002). Habayeb et al. (2004) reported plasma AEA levels in the early follicular phase (days 2–7) were higher than those of the late luteal phase (days 20–25) of the menstrual cycle.

Taken together, there are several findings indicating that endocannabinoids are important factors, acting in multiple ways in regulating reproductive function. Since no data concerning the expression of ECS in different stages of the estrous cycle are available for the bovine endometrium, the current study was conducted to determine and compare different components of the ECS in the bovine endometrium throughout the estrous cycle, which may open new perspectives for understanding the role of the ECS in the bovine reproduction.

Material and methods

All animal experimental procedures were approved by the Iranian Ministry of Agriculture (experimental permission No. 2018-03-01). This study was conducted from March to September 2018 on a commercial, 5,600-cow dairy herd located in Iran.

Animals and housing

This study was carried out using sixteen Holstein dairy cows (milk production: 34.5±1.4 kg/day, parity: 3.3±0.8 and body condition score (BCS): 3.2±0.07) in the north part of Iran. All cows were housed in a free stall barn equipped with fans and sprinklers that were automatically turned on when ambient temperature reached 26.7°C. Cows were fed a TMR diet three times daily with *ad libitum* access to feed and water. The diet was formulated to meet or exceed National Research Council (NRC) requirements (NRC, 2001) for high-producing lactating dairy cows. Throughout the experiment, cows were milked 3 times daily at approximately 8-h intervals. All healthy cows based on veterinarian check and confirmation were enrolled in the study.

Blood samples

Blood samples were collected every other day from the day of the second PGF2α injection until the next estrus (67±2 DIM) via jugular venipuncture into commercial blood collection tubes (Vacutainer, 5 mL; Ava Pezeshk, Arak, Iran) containing sodium heparin to verify ovulation (estradiol ≥ 2 pg/mL, Sina et al., 2018) and CL development (progesterone ≥1 ng/mL, Heidari et al., 2017). After collection, the blood samples were placed immediately on ice, centrifuged (2500 ×g for 30 minutes, 4°C) for plasma harvest, and stored at -20°C on the same day of collection for further analysis of estradiol 17β and progesterone concentrations using an ELISA procedure according to manufacturer's guidelines (Diaplus, North York, Ontario, Canada). Intra- and interassay coefficients of variation were less than 5%. Sensitivity of the assay was 0.2 ng/mL and 0.4 pg/mL for progesterone and estradiol respectively.

Estrous cycle synchronization and ultrasound examinations

The estrous cycles of 16 cows were synchronized with two intramuscular injections of PGF2α (Synchromate®, 150 g cloprostenol sodium, Aburaihan Co., Tehran, Iran) given 14 days apart (30 and 44 DIM). Transrectal ultrasonography (7.5-MHz transducer, 500 V; Aloka, Wallingford, CT, USA) was performed every other day from the day of the second PGF2α injection until the next estrus (67±2 DIM) to verify ovulation and CL development.

Tissue collection

Endometrial tissue was collected from sixteen cows on days 16 (late luteal phase) and 21 (estrus) of the synchronized estrous cycle (ovulation = d 0, 45±1 DIM) by passing biopsy forceps as previously described (Abolghasemi et al., 2016). Endometrial samples (weight 100 mg) were collected, washed in sterile PBS, and immediately snap frozen in liquid nitrogen.

RNA extraction

The RNA from endometrial tissues was extracted using TRIzol reagent (Invitrogen Corp., Carlsbad, CA) according to instructions provided by the manufacturer and on-column DNase treatment and cleanup was performed (RNeasy mini-kit, Qiagen). RNA quality and quantity were tested in the Bioanalyzer (2100, Agilent Technologies Inc., Santa Clara, CA) and a Nano Drop 1000 (Thermo Fisher Scientific, Inc.), respectively.

Reverse transcription synthesis of cDNA

Total RNA (1000 ng) was reverse transcribed into cDNA using the High-Capacity cDNA Reverse Transcription kit (Applied Biosystems) as per the manufacturer's instructions.

Real-time PCR

All real-time PCR reactions were carried out in duplicate on the Corbett Rotor-GeneTM 3000 quantitative PCR system (Corbett Life Sciences, Sydney, Australia) using 50 ng of cDNA, 300 nM specific primers (Table 1, Abolghasemi et al., 2016) and 7.5 µl of FAST SYBR Green Master Mix (Applied Biosystems), with a final reaction volume of 15 µl. Cycling conditions were as follows: an initial denaturation step 95°C for 15 min and a cycling step (40 cycles of 95°C for 30 s, 60°C for 30 s, and 30 s extension at 72°C), followed by amplicon dissociation.

Table 1. Gene ID, GenBank accession number and sequence of primers for *Bos taurus* used to analyze mRNA expression by Real-time PCR

Symbol	Accession #	Primers	Primers (5'-3')2
CNR2	NM_001192303.1	F	TCTTCGCCGGCATCATCTAC
		R	CATCCGGGCTATTCCAGACA
FAAH	NM_001099102.1	F	TTCCTGCCAAGCAACATACCT
		R	CACGAAATCACCTTTGAAGTTCTG
MGLL	XM_581556.5	F	GCAACCAGCTGCTCAACAC
		R	AGCGTCTTGTCCTGGCTCTT
NAPEPLD	NM_001015680.1	F	AGAGATCACAGCAGCGTTCCAT
		R	ACTCCAGCTTCTTCAGGGTCATC
NAAA	NM_001100369.1	F	CAGCACTACGACCGGGACTT
		R	CCGGGACGACTTTTCTGATC
COX-2	AF004944	F	TTT TGGTAGGTC TTC TGGTG
		R	GCATGG CCT GTA CAA CCT CAA
UBQ	NM_174133	F	AGATCCAGGATAAGGAAGGCA
		R	GCTCCACCTCCAGGGTGAT

CNR2: cannabinoid receptor type 2. FAAH: fatty acid amide hydrolase. MGLL: monoacylglycerol lipase. NAPEPLD: N-acyl phosphatidylethanolamine phospholipase D. CB1: cannabinoid receptor type 1. NAAA: 5-nitroanthranilic acid aminohydrolase. COX-2: prostaglandin-endoperoxide synthase 2. UBQ: ubiquitin.

Statistical analysis

Samples were run in duplicate, and were expressed relative to UBQ as housekeeping gene, which was stable under the culture conditions used. mRNA expression results were calculated using the $2^{-\Delta\Delta CT}$ method. Fold changes in mRNA expression between late luteal phase and estrus were determined.

All data were analyzed using the Statistical Analysis Systems (SAS) software version 9.1 (SAS Institute, Cary, NC). mRNA expression data were examined to determine whether they were normally distributed (PROC UNIVARIATE, SAS). Differences in mean relative mRNA expression values between the two groups (late luteal phase and estrus) were analyzed by ANOVA (PROC GLM, SAS). The Tukey critical difference test was used to determine statistical differences between late luteal phase and estrus mean values. Mean differences of $P \leq 0.05$ were considered to be significant.

Results

The peak of plasma progesterone concentration occurred on day 15 of the estrous cycle (14.20±0.56 ng/mL, Figure 1). The diameter of the corpus luteum (CL) at d 15 of estrous cycle was greatest, 19.12±1.35 mm. The peak of plasma estradiol 17β concentration occurred at d 21 of the estrous cycle (13.50±0.70 pg/mL, Figure 2). The diameter of the largest follicle at d 21 of the estrous cycle was 14.52±1.80 mm.

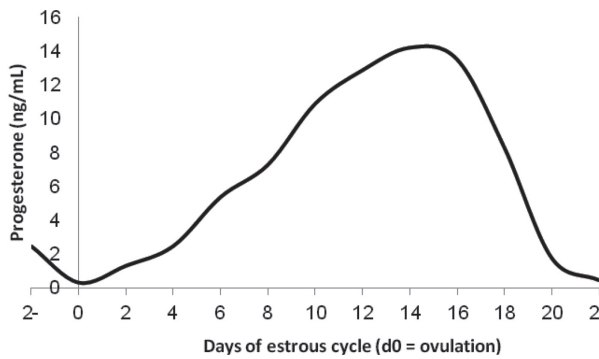

Figure 1. Plasma progesterone concentrations (ng/mL) during estrous cycle of dairy cows. Blood samples were collected from the day of the second PGF2α injection (day -2) until the start of the next estrous cycle (day 22), ovulation = day 0. Mean plasma P_4 concentration during estrous cycle was 6.26±0.32 ng/mL

The results from endometrial gene transcription analysis showed that genes related to the endocannabinoid system, *CNR2*, *DAGL*, *COX-2*, *FAAH* and *MGLL* had significant fold differences when comparing two different stages of the estrous cycle (late luteal phase vs. estrus).

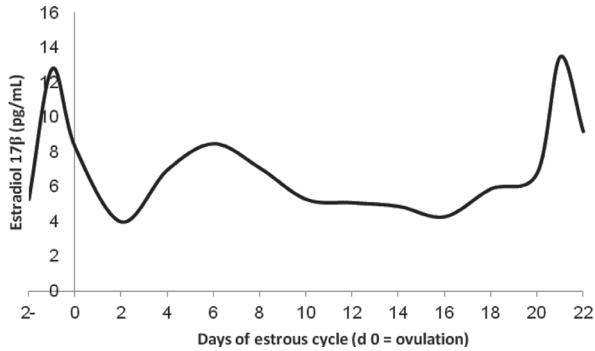

Figure 2. Plasma estradiol concentrations (pg/mL) during estrous cycle of dairy cows. Blood samples were collected from the day of the second PGF2α injection (day –2) until the start of the next estrous cycle (day 22), ovulation = day 0. Mean plasma E_2 concentration during estrous cycle was 7.26±0.51 ng/mL

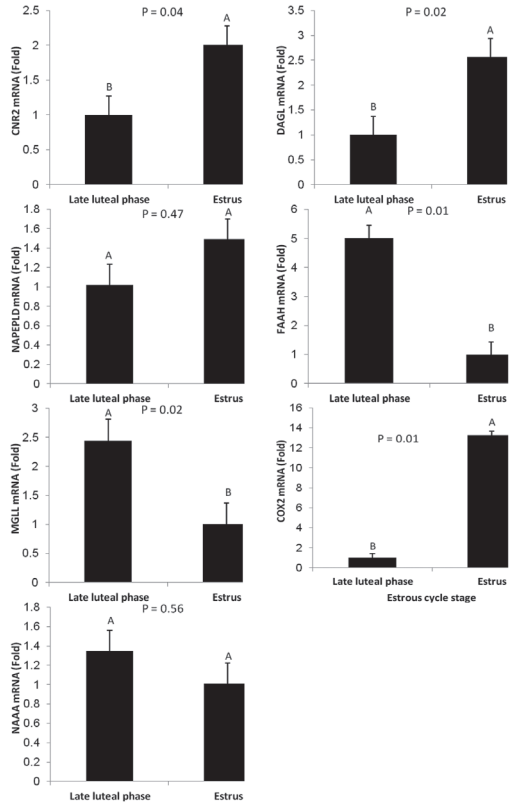

Figure 3. Effect of estrus expression on endometrial mRNA expression of endocannabinoids network. Significant fold difference based on estrus expression as a referent has been shown for genes with significant pattern of expression in endometrial tissue. Endocannabinoid receptor-2 [CNR2], diacylglycerol lipase [DAGL], N-acyl phosphatidylethanolamine phospholipase D [NAPEPLD], fatty acid amide hydrolase [FAAH], monoglyceride lipase [MGLL], N-acylethanolamine acid amidase [NAAA] and cyclooxygenase-2 [COX2]. For this graph, the (A, B) refers to P<0.05

CNR2 and *DAGL* showed 2.01 and 2.57 fold increase, respectively (P=0.04 and P=0.02), in estrus compared to late luteal phase. Among the analyzed genes, *FAAH* (P=0.01) and *MGLL* (P=0.02) were significantly down-regulated in estrous cows, with a 5.01- and 2.44-fold difference in mRNA expression, respectively. Mean expression of *COX2*, which belongs to the prostaglandin biosynthesis group, showed a 13.25 fold up-regulation of mRNA expression in estrus, when compared with the late luteal phase. *NAAA* and *NAPEPLD* showed no significant difference between estrus and late luteal phase in their mRNA levels (P>0.4). The fold change differences of the genes in the endometrium are shown in Figure 3.

Discussion

This research was the first comprehensive study that simultaneously demonstrated details of ECS expression profiling in the bovine endometrium during the late luteal phase and estrus. There were limited data describing ECS in the endometrium of other species during different phases of the estrous cycle.

Results of the present study showed *CNR2* (ECS receptor), *DAGL* (ECS synthesizing enzyme) and *COX2* mRNA expression increased at estrus compared to late luteal phase, whereas *FAAH* (degradation enzyme) and *MGLL* (degradation enzyme) decreased at estrus compared to late luteal phase. In agreement with our results previous studies reported AEA and 2-AG levels appeared to be highest during diestrus and lowest during estrus in the anterior pituitary of mice (Gonzalez et al., 2000; Bradshaw et al., 2006). A similar pattern was shown in humans, circulating AEA levels were higher during the follicular phase and highest during ovulation and lower during the luteal phase (Habayeb et al., 2004; El-Talatini et al., 2010).

Maximal expression of COX-2 mRNA and protein occurs on d 7–17 and 10–21 of the bovine estrous cycle, respectively (Arosh et al., 2004). Moreover, it has been demonstrated that PGF2α treatment increases COX-2 expression and luteal production of PGF2α in ruminants (Dirandeh et al., 2015).

FAAH activity was highest, and serum AEA was lowest, during the implantation in humans (El-Talatini et al., 2009). This led to the suggestion that low AEA levels are required to allow successful implantation and carrying offspring to term. This is supported by reducing levels of circulating AEA during pregnancy (Habayeb et al., 2004). Additionally, increased AEA or treatment with cannabinoid agonists has been associated with miscarriages in humans (Habayeb et al., 2008) and disruptions to implantation and embryonic development in rodents (Schmid et al., 1997). El-Talatini et al. (2010) reported there was a statistically significant positive correlation between AEA, estradiol, LH, FSH levels but not progesterone.

The release of progesterone from the corpus luteum can be attenuated by endocannabinoid activity. Chronic administration of AEA in pregnant rats decreased serum progesterone and LH content (Habayeb et al., 2002). Treatment with either CNR1 or CNR2 receptor agonists reduced levels of serum progesterone, corpus luteum weights, corpus luteum LH receptor mRNA content, and corpus luteum LH

receptor density in sheep (Tsutahara et al., 2011). This suggests that the release of progesterone is at least partially regulated by central endocannabinoid control over LH release, but is also controlled by direct endocannabinoid binding onto receptor sites on the corpus luteum.

Progesterone regulates functioning of FAAH, the principal catabolic enzyme for the endocannabinoid AEA at the transcriptional and translational level. Progesterone up-regulated the FAAH expression interacting with a transcription factor in the promoter region of the FAAH gene (Maccarrone et al., 2001, 2003). Lazzarin et al. (2004) demonstrated once ovulation occurs, the increase in progesterone induced FAAH expression and activity leads to a decrease in plasma AEA levels.

EL-Talatini et al. (2007) demonstrated a direct correlation between AEA levels in follicular fluid and follicle size, supporting the suggestion that AEA may be involved in folliculogenesis. The fact that serum estradiol levels and plasma AEA levels are significantly correlated suggests a closer association between these two molecules. Maccarrone et al. (2001, 2003) showed that AEA release from endothelial cells was stimulated by estradiol, supporting the possibility that estradiol and AEA levels are closely linked. Habayeb et al. (2008) reported that plasma AEA levels below 2nM were predictive of live birth in women with a threatened miscarriage, suggesting that plasma AEA levels above this value were not conducive to successful pregnancy. The FAAH enzyme also appears to be a major site of interaction between the endocannabinoid system and estrogens. Estrogens appear to decrease FAAH activity in the mouse uterus (Maccarrone et al., 2000). The FAAH gene contains an estrogen response element; translocation of the estrogen receptor caused a down-regulation of FAAH transcription (Waleh et al., 2002). This is consistent with the finding that a CNR1 receptor antagonist reversed the anxiolytic effect of estradiol in rats and that the FAAH inhibitor URB 597 produced an anxiolytic effect similar to that produced by estradiol (Hill et al., 2007). However, estradiol administration in ovariectomized female rats also increased the levels of synthesized AEA in the medial basal hypothalamus, suggesting that estradiol may also directly interact with endocannabinoid synthesis (Scorticati et al., 2004). Estrogen modulates endocannabinoid signaling via CNR1 expression in the CNS, as well as by up-regulating AEA content by decreasing FAAH transcription in both peripheral and central regions.

Conclusion

The results of the present study showed enzymes that synthesize and degrade ECS are affected by stage of estrous cycle and showed a different pattern during the late luteal phase and estrus. Changes of ECS network might result in high ECS levels during estrus and low levels during the late luteal phase. Further studies are needed to improve our understanding of the role of the ECS in bovine fertility.

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References

Abolghasemi A., Dirandeh E., Ansari Z., Shohreh B. (2016). Dietary conjugated linoleic acid supplementation alters the expression of genes involved in the endocannabinoid system in the bovine endometrium and increases plasma progesterone concentrations. Theriogenology, 86: 1453–1459.

Arosh J.A., Banu S.K., Chapdelaine P., Madore E., Sirois J., Fortier M.A. (2004). Prostaglandin biosynthesis, transport, and signaling in corpus luteum: a basis for autoregulation of luteal function. Endocrinology, 145: 2551–2560.

Beltman M.E., Forde N., Furney P., Carter F., Roche J.F., Lonergan P., Crowe M.A. (2010). Characterisation of endometrium mRNA expression and metabolic parameters in beef heifers yielding viable or non-viable embryos on day 7 after insemination. Reprod. Fert. Develop., 22: 987–999.

Beltramo M., Stella N., Calignano A., Lin S.Y., Makriyannis A., Piomelli D. (1997). Functional role of high-affinity anandamide transport, as revealed by selective inhibition. Science, 277: 1094–1097.

Bonsale R., Seyed Sharifi R., Dirandeh E., Hedayat N., Mojtahedin A., Ghorbanalinia M., Abolghasemi A. (2018). Endocannabinoids as endometrium inflammatory markers in lactating Holstein cows. Reprod. Domest. Anim., 53: 769–775.

Bradshaw H.B., Rimmerman N., Krey J.F., Walker J.M. (2006). Sex and hormonal cycle differences in rat brain levels of pain-related cannabimimetic lipid mediators. Am. J. Physiol. - Reg. I., 291: 349–358.

Demuth D.G., Molleman A. (2006). Cannabinoid signalling. Life Sci., 78: 549–563.

De Petrocellis L., Di Marzo V. (2009). An introduction to the endocannabinoid system, from the early to the latest concepts. Best Pract. Res. Cl. En., 23: 1–15.

Di Marzo V. (2008). Endocannabinoids: synthesis and degradation. Rev. Physiol. Biochem. Pharmacol., 160: 1–24.

Dinh T.P., Freund T.F., Piomelli D. (2002). A role for monoglyceride lipase in 2-arachidonoylglycerol inactivation. Chem. Physics Lipids, 121: 149–158.

Dirandeh E., Towhidi A., Ansari Z., Saberifar T., Akhlaghi A., Rodbari A.R. (2015). The endometrial expression of prostaglandin cascade components in lactating dairy cows fed different polyunsaturated fatty acids. Theriogenology, 83: 206–212.

Dirandeh E., Ghaffari J.A. (2018). Effects of dietary omega-3 fatty acid sources on reproduction might be through a mechanism involving the endocannabinoid system in the bovine endometrium. Theriogenology, 121: 141–146.

El-Talatini M.R., Lam P.M.W., Elson J.C., Taylor A.H., Konje J.C. (2007). The endocannabinoid, anandamide, is involved in human folliculogenesis and oocyte maturation during IVF treatment. Proc. 2nd SGI International Summit in Reproductive Medicine – from embryo and endometrium to implantation, the translational research. Valencia, Spain, Abstract book, 59, 2.

El-Talatini M.R., Taylor A.H., Elson J.C., Brown L., Davidson A.C., Konje J.C. (2009). Localisation and function of the endocannabinoid system in the human ovary. PLoS One, 4, 4579.

El-Talatini M.R., Taylor A.H., Konje J.C. (2010). The relationship between plasma levels of the endocannabinoid, anandamide, sex steroids, and gonadotrophins during the menstrual cycle. Fertil. Steril., 93: 1989–1996.

Forde N., Spencer T.E., Bazer F.W., Song G., Roche J.F., Lonergan P. (2010). Effect of pregnancy and progesterone concentration on expression of genes encoding for transporters or secreted proteins in the bovine endometrium. Physiol. Genom., 41: 53–62.

Gonzalez S., Bisogno T., Wenger T., Manzanares J., Milone A., Berrendero F., Di Marzo V., Ramos J.A., Fernandez-Ruiz J.J. (2000). Sex steroid influence on cannabinoid CB (1) receptor mRNA and endocannabinoid levels in the anterior pituitary gland. Biochem. Biophys. Res. Commun., 270: 260–266.

Gorzalka B.B., Dang S. (2012). Minireview: Endocannabinoids and gonadal hormones, bidirectional interactions in physiology and behavior. Endocrinology, 153: 1016–1024.

Habayeb O.M., Bell S.C., Konje J.C. (2002). Endogenous cannabinoids, metabolism and their role in reproduction. Life Sci., 70: 1963–1977.

Habayeb O.M., Taylor A.H., Evans M.D., Cooke M.S., Taylor D.J., Bell S.C., et al. (2004). Plasma levels of the endocannabinoid anandamide in women, a potential role in pregnancy maintenance and labor? J. Clin. Endocrinol. Metab., 89: 5482–5487.

Habayeb O.M., Taylor A.H., Finney M., Evans M.D., Konje J.C. (2008). Plasma anandamide concentration and pregnancy outcome in women with threatened miscarriage. JAMA, 299: 1135–1136.

Heidari F., Dirandeh E., Ansari Pirsaraei Z., Colazo M.G. (2017). Modifications of the G6G timed-AI protocol improved pregnancy per AI and reduced pregnancy loss in lactating dairy cows. Animal, 11: 2002–2009.

Hill M.N., Karacabeyli E.S., Gorzalka B.B. (2007). Estrogen recruits the endocannabinoid system to modulate emotionality. Psychoneuroendocrinology, 32: 350–357.

Killeen A.P., Morris D.G., Kenny D.A., Mullen M.P., Diskin M.G., Waters S.M. (2014). Global mRNA expression in endometrium of high and low fertility heifers during the mid-luteal phase of the estrous cycle. BMC Genomics, 15: 234.

Lazzarin N., Valensise H., Bari M., Ubaldi F., Battista N., Finazzi-Agro A., et al. (2004). Fluctuations of fatty acid amide hydrolase and anandamide levels during the human ovulatory cycle. Gynecol. Endocrinol., 18: 212–218.

Maccarrone M., DeFelici M., Bari M., Klinger F., Siracusa G., Finazz-Agro A. (2000). Down-regulation of anandamide hydrolase in mouse uterus by sex hormones. Europ. J. Biochem., 267: 2991–2997.

Maccarrone M., Valensise H., Bari M., Lazzarin N., Romanini C., Finazzi-Agro A. (2001). Progesterone up-regulates anandamide hydrolase in human lymphocytes, role of cytokines and implications for fertility. J. Immunol., 166: 7183–7189.

Maccarrone M., Falciglia K., Di Rienzo M., Finazzi-Agro A. (2002). Endocannabinoids, hormone-cytokine networks and human fertility. Prostag. Leukot Ess., 66: 309–317.

Maccarrone M., Bari M., Di Rienzo M., Finazzi-Agro A., Rossi A. (2003). Progesterone activates fatty acid amide hydrolase (FAAH) promoter in human T lymphocytes through the transcription factor Ikaros. Evidence for a synergistic effect of leptin. J. Biol. Chem., 278: 32726–32732.

McCarthy S.D., Roche J.F., Forde N. (2012). Temporal changes in endometrium mRNA expression and protein localization of members of the IGF family in cattle. Effects of progesterone and pregnancy. Physiol. Genomics, 44: 130–140.

NRC-National Research Council (2001). Nutrient requirements of dairy cattle. Natl. Acad. Sci., 7th rev. ed., Washington, DC, USA.

Schmid P.C., Paria B.C., Krebsbach R.J., Schmid H.H., Dey S.K. (1997). Changes in anandamide levels in mouse uterus are associated with uterine receptivity for embryo implantation. Proc. Natl. Acad. Sci. USA, 94: 4188–4192.

Scorticati C., Fernandez-Solari J., De Laurentiis A., Mohn C., Prestifilippo J.P., Lasaga M., Seilicovich A., Billi S., Franchi A., McCann S.M., Rettori V. (2004). The inhibitory effect of anandamide on luteinizing hormone-releasing hormone secretion is reversed by estrogen. Proc. Natl. Acad. Sci. USA, 101: 11891–11896.

Sina M., Dirandeh E., Deldar H., Shohreh B. (2018). Inflammatory status and its relationships with different patterns of postpartum luteal activity and reproductive performance in early lactating Holstein cows. Theriogenology, 108: 262–268.

Spencer T.E., Johnson G.A., Burghardt R.C., Bazer F.W. (2004). Progesterone and placental hormone actions on the uterus, insights from domestic animals. Biol. Reprod., 71: 2–10.

Tsutahara N.M., Weems Y.S., Arreguin-Arevalo J.A., Nett T.M., LaPorte M.E., Uchida J., Pang J., McBride T., Randel R.D., Weems C.W. (2011). Effects of endocannabinoids 1 and 2 (CNR1, CNR2) receptor agonists on luteal weight, circulating progesterone, luteal mRNA for luteinizing hormone (LH) receptors, and luteal unoccupied and occupied receptors for LH *in vivo* in ewes. Prostag. Oth. Lipid M., 94: 17–24.

Waleh N.S., Cravatt B.F., Apte-Deshpande A., Terao A., Kilduff T.S. (2002). Transcriptional regulation of the mouse fatty acid amide hydrolase gene. Gene, 291: 203–210.

Weems Y.S., Lewis A.W., Neuendorff D.A., Randel R.D., Weems C.W. (2009). Endocannabinoid 1 and 2 (CNR1, CNR2) receptor agonists affect negatively cow luteal function *in vitro*. Prostag. Oth. Lipid M., 90: 89–93.

Wolf E., Bauersachs S. (2010). Functional genome research in reproductive biology and biotechnology – a mini review. Anim. Sci. Pap. Rep., 28: 123–130.

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