Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.elsevier.com/locate/repbio

Original Research Article

mRNA expression pattern of gonadotropin receptors in bovine follicular cysts

Belkis E. Marelli [a,b], Pablo U. Diaz [a,b], Natalia R. Salvetti [a,b], Florencia Rey [a,b], Hugo H. Ortega [a,b,*]

[a] Laboratorio de Biología Celular y Molecular Aplicada, Facultad de Ciencias Veterinarias, Universidad Nacional del Litoral, Argentina

[b] Instituto de Ciencias Veterinarias del Litoral (ICiVet-Litoral), Consejo Nacional de Investigaciones Científicas y Tecnológicas, (CONICET), Argentina

ARTICLE INFO

Article history:
Received 20 November 2013
Received in revised form
9 August 2014
Accepted 21 August 2014
Available online 30 August 2014

Keywords:
Cystic ovary
Gonadotropin receptors
Cow
LH
FSH

ABSTRACT

Follicular growth and steroidogenesis are dependent on gonadotropin binding to their receptors in granulosa and theca cells of ovarian follicles. The aim of the present study was to evaluate the expression patterns of follicle-stimulating hormone receptor (FSHR) and luteinizing hormone receptor (LHCGR) in ovarian follicular structures from cows with cystic ovarian disease (COD) as compared with those of regularly cycling cows. Relative real-time RT-PCR analysis showed that the expression of FSHR mRNA in granulosa cells was highest in small antral follicles, then decreased significantly as follicles increased in size, and was lowest in cysts. FSHR mRNA was not detected in the theca cells of any follicular category, including cysts. LHCGR mRNA expression in granulosa cells was significantly higher in large antral follicles than in cysts, and not detected in granulosa cells of small and medium antral follicles. In theca cells, the expression level of LHCGR mRNA in medium antral follicles was higher than in small and large antral follicles, whereas that in follicular cysts it was similar to those in small and medium antral follicles, but higher than that in large antral follicles. Our findings provide evidence that there is an altered gonadotropin receptor expression in bovine cystic follicles, and suggest that in conditions characterized by altered ovulation, such as COD, changes in the signaling system of gonadotropins may play a fundamental role in their pathogenesis.

* *Corresponding author at*: Laboratorio de Biología Celular y Molecular Aplicada, Facultad de Ciencias Veterinarias, Universidad Nacional del Litoral, R.P. Kreder 2805, 3080 Esperanza, Santa Fe, Argentina. Tel.: +54 3496 420639; fax: +54 3496 426304.
E-mail addresses: hhortega@fcv.unl.edu.ar, hhortega@arnet.com.ar (H.H. Ortega).

http://dx.doi.org/10.1016/j.repbio.2014.08.002

1. Introduction

The cellular mechanisms regulating the selection, growth, ovulation and regression of follicles are not completely defined. However, a primary role of gonadotropins in the regulation of follicular development has been well established [1,2] and granulosa and theca cells are involved in the synthesis and secretion of steroid hormones through the two-cell/two gonadotropin model [3]. In this model, granulosa cells contain follicle-stimulating hormone (FSH) receptor (FSHR) and theca cells contain luteinizing hormone (LH) receptor (LHCGR) during earlier stages of follicular development [4]. Both receptors belong to the large family of GTP-binding protein (G-protein)-coupled receptors [5]. As follicular development progresses, the acquisition of LHCGR by the granulosa cells of the dominant follicle and increased aromatization of androgens provided by the theca cells in response to LH and FSH are among the changes occurring within the dominant follicle [6].

Cystic ovarian disease (COD) in cows is characterized by persistent anovulatory follicular structures in the absence of corpora lutea (CL) and interrupted or abnormal estrous cycles [7,8]. Ovarian cysts are a major reproductive disorder responsible for economic losses in the dairy industry [9]. In dairy cows, the incidence of COD varies between 10 and 13% [9], but in some herds it reaches 30–40% [10], or even higher, because 60% of the cows that develop ovarian cysts before the first postpartum ovulation re-establish ovarian cycles spontaneously [11].

The pathogenesis of COD is still not completely understood, mainly because the etiology is multifactorial, and genetic, phenotypic and environmental factors are involved [12]. The endocrine environment associated with the formation and maintenance of ovarian follicular cysts has been extensively studied and the most widely accepted hypothesis is based on neuroendocrinological dysfunction of the hypothalamic–pituitary–gonadal axis [12]. However, intraovarian components are also involved in the pathogenesis of COD [13–17]. Although dynamics of follicular growth has been characterized, the cellular and molecular changes that occur within the ovarian follicle prior to anovulation are still unclear and inconclusive. In this process, gonadotropins play a fundamental role and it has been suggested that alterations in the expression levels of their receptors may be a relevant factor in the development of cysts [6]. The distribution pattern and expression levels of gonadotropin receptors in healthy ovaries have been evaluated with the use of several molecular techniques. However, real-time PCR – one of the most consistent and effective methods to quantify variations in mRNA expression – has been scarcely used to study the pathogenesis of COD. In an attempt to elucidate the mechanisms underlying COD, in the current study we evaluated the changes in the expression pattern of gonadotropin receptors in bovine cystic ovaries by using real-time RT-PCR.

2. Materials and methods

All procedures were performed with the approval of the Institutional Ethics and Security Committee (Facultad de Ciencias Veterinarias – Universidad Nacional del Litoral, Argentina) and are consistent with the ''Guide for the Care and Use of Agricultural Animals in Research and Teaching'' (Federation of Animal Science Societies, 2010).

2.1. Collection and preparation of tissues

Normal ovaries ($n = 40$) were collected at a local abattoir, within 20 min of slaughter, from Argentinean Holstein cows assessed visually as being non-pregnant and without macroscopic abnormalities in the reproductive system and in the absence of CL in the two ovaries. The complete ovaries were washed, refrigerated and immediately transported to the laboratory. During dissection of the ovaries, the follicular diameter was measured, and follicular fluid and granulosa cell from each follicle were aspirated and centrifuged at 400 × *g* for 10 min. The granulosa cell pellets were resuspended in Trizol LS reagent (Invitrogen, Life Technology, Carlsbad, CA, USA) and follicular fluid stored at −20 °C for steroid determination to confirm the follicular health status. The remaining follicular walls were further washed several times with phosphate buffered saline (PBS) to remove residual granulosa cells. Then, the theca cells were excised from the surrounding stroma and washed several times with diethylpyrocarbonate (DEPC)-water [13,14,17]. The tertiary follicles were classified into three categories according to their diameters: small (<5 mm), medium (5–10 mm) or large (>10 mm) [18]. All samples were aliquoted, snap-frozen in liquid nitrogen and stored at −80 °C until RNA extraction.

Ovaries with spontaneous cystic follicles were collected at local dairy farms. Following initial diagnosis of COD by the veterinary responsible of dairy herd health management, the ovaries were examined daily using transrectal ultrasonography (5 MHz linear transducer, Honda HS101V, Tokyo, Japan). Ten multiparous Argentinean Holstein cows at least 45 days after calving (3.3 ± 1.5 lactations, range 2–7), high yielding (mean 29.7 ± 6.2 kg of milk per day at diagnosis) affected by COD were selected (65.9 ± 27.8 days in milk) for the study. A COD case was defined when a cow had a single cyst of 20 mm or more in diameter that persisted for at least 10 days in the absence of CL [12], and then the ovaries were removed by transvaginal ovariectomy. Briefly, animals were sedated with 150 mg of ketamine hydrochloride (10% ketamine, Alfasan, Santa Fe, Argentina) and low epidural anesthesia was performed with 100 mg of lidocaine hydrochloride (2% lidocaine, Vetue, Santa Fe, Argentina). The perineal area and the vaginal canal were cleaned and disinfected performed using a solution of 5% chloroxylenol. Then, using a retractor, the vaginal vault was tensioned and moved away from the rectum (cranio-ventral direction) to avoid contact. A hidden dismountable blade scalpel was introduced and a 5 cm longitudinal incision was performed on the vaginal vault, beginning the cut from cranial to caudal at about 7 cm from cervix. The next step was to introduce three fingers into the pelvic cavity, locate and retract the uterus along with the ovaries into the vagina. At this place, the blood vessels that supply both ovaries were ligated by transfixion and then the ovaries were removed. To prevent the breakage of a cyst and loss of follicular fluid, follicles were aspirated before ovariectomy using a digital ultrasound system 8300vet Chison equipped with a micro-convex transducer of 5.0 MHz mounted on a transvaginal probe

for follicular aspiration (Watanabe Applied Technology Limited, Sao Paulo, Brazil). Finally, the animals were treated with antibiotics (penicillin–streptomycin 20,000 IU/kg/day for 5 days, Río de Janeiro, Santa Fe, Argentina) and anti-inflammatory drugs (Flunixin meglumine 2.2 mg/kg/day for 3 days; Algimine 5%, Zoovet, Santa Fe, Argentina). After excision, the ovaries and follicular fluid were refrigerated and immediately transported to the laboratory for processing as described above.

2.2. *Real-time RT-PCR*

Total RNA was isolated from granulosa and theca cells using Trizol LS reagent (Invitrogen). Samples of each follicle were processed individually. Briefly, 50–100 mg of tissue or granulosa cell pellets were homogenized in 750 μL of Trizol reagent and incubated for 5 min on ice. The homogenized samples were mixed with chloroform, shaken vigorously and incubated for 15 min at 4 °C. After centrifugation at 12,000 × *g*, the aqueous phase was transferred to a clean tube and the RNA was precipitated by mixing with isopropyl alcohol. The samples were incubated overnight at −20 °C and centrifuged at 12,000 × *g*. RNA pellets were washed once with 75% ethanol and then allowed to dry. RNA was dissolved in DEPC-water pre-warmed to 55–60 °C. Finally, the purified RNA was assessed for quality and quantity by Qubit RNA HS Assay Kit with Qubit fluorometer (Invitrogen), aliquoted and stored at −80 °C.

The primers for LHCGR and FSHR have been previously described [19]. These primers are designed to partially amplify exon 8 of the LHCGR gene and exon 10 of the FSHR gene, which are present in all functional transcripts of both receptors. The primer sequences for cytochrome P450 aromatase (CYP19a1) and cytochrome P450 17α-hydroxylase/17,20-lyase (CYP17a1) were used to confirm the mRNA purity of bovine granulosa and theca cells (no cross-contamination) [20]. The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control of reverse transcription and reaction efficiency by using primers previously described [21]. All primers were purchased from Invitrogen and the sequences are summarized in Table 1.

Reverse transcription was performed using 1 μg of DNAse I-treated RNA in a 20 μL volume of RT master mix that contained 4 μL 5× RT reaction buffer, 0.25 μL random hexamers (1 μg/μL), 0.4 μL dNTPs (25 mM), 2 μL DTT (0.1 M), 0.5 μL ribonuclease inhibitor (40 U/μL) and 1 μL MMLV reverse transcriptase (200 U/μL) (all reagents from Invitrogen). The RT reactions were carried out under the following conditions: 10 min of annealing at 25 °C, 50 min of cDNA synthesis at 37 °C and 15 min of inactivation at 70 °C.

A total of 15 follicles of each category (small, medium, large) and 10 follicular cyst samples that provided useful granulosa and theca cell RNA samples were selected for analysis. StepOne Real-time PCR System (Applied Biosystems, Life Technology, Carlsbad, CA, USA) and SYBR Green I (Invitrogen) were used for real-time PCR. cDNA diluted in 4 μL of each sample was combined with a PCR reaction mix containing 1× PCR buffer (Invitrogen) with 1:20,000 dilution of SYBR Green I, 1.5 mM $MgCl_2$, 200 μM dNTP, 400 nM forward primer, 400 nM reverse primer, and 1 U of Taq polymerase (Invitrogen) in a final volume of 20 μL. Thermal cycling conditions included 45 cycles of denaturation at 95 °C for 30 s, annealing at 52 and 57 °C (Table 1) for 30 s and extension at 72 °C for 30 s. A single initial denaturation step at 95 °C for 5 min and a final extension step at 72 °C for 10 min were performed. GAPDH was included as the housekeeping gene. Controls containing no template DNA were included in all assays.

The threshold cycle (C_T) for each sample was calculated using the StepOne Software v2.2 (Applied Biosystems) with a manual fluorescence threshold setting. The relative expression levels of each target were calculated based on the $2^{-\Delta\Delta CT}$ method with fold difference from cystic follicles [16,22]. The efficiency of PCRs was assessed for each target using the standard-curve method by amplification of serial dilutions (over five orders of magnitude) of pooled cDNAs of the transcripts under analysis. Melting-curve analyses were performed after real-time PCR to confirm PCR product purity. Also, all PCR products were initially run in agarose gels to verify the product sizes. The specificity of the PCR products was confirmed by DNA sequencing by using the Macrogen Sequencing Service (Macrogen, Seoul, Korea). Oligonucleotide primers and amplification products were tested using BLAST (http://www.ncbi.nlm.nih.gov/BLAST) to confirm gene specificity and to determine nucleotide locations, making sure that they were not designed from any homologous regions coding for other genes.

2.3. *Hormone assays*

Estradiol (E_2), progesterone (P_4) and testosterone (T) levels in undiluted follicular fluid of large antral follicles and cystic

Table 1 – Primer sequences, regions of the target genes and conditions used for semi-quantitative RT-PCR.

Name		Sequence (5′–3′)	Gene accession no.	Amplicon position	Amplicon size (bp)	Annealing temperature (°C)	Reference
FSHR	Forward	GCCCCTTGTCACAACTCTATGTC	BOVFSHR	1635–1738	104	57	[19]
	Reverse	GTTCCTCACCGTGAGGTAGATGT					
LHCGR	Forward	TTGCCACATCATCCTATTCTC	BTU20504	780–901	122	57	[19]
	Reverse	CTCGTTTGTTGGCAAGTTTCT					
GAPDH	Forward	CACCCTCAAGATTGTCAGCA	BC102589	492–594	103	52	[21]
	Reverse	GGTCATAAGTCCCTCCACGA					
CYP17a1	Forward	GGAGGCGACCATCAGAGAAGTGC	NM_174304	1105–1423	319	60.8	[20]
	Reverse	CAGCCGGGACATGAAGAGGAAGAG					
CYP19a1	Forward	TAAAACAAAGCGCCAATCTCTACG	BTCYP19	8–348	341	55.4	[20]
	Reverse	GGAACCTGCAGTGGGAAATGA					

Table 2 – Steroid concentration (mean ± SEM) in the follicular fluid of large antral follicles of normal ovaries and spontaneous follicular cysts.

	Estradiol (pg/mL)	Progesterone (ng/mL)	Testosterone (pg/mL)
Large antral follicles	247.3 ± 27.7^{a}	61.1 ± 6.6^{a}	21.1 ± 5.2^{a}
Follicular cysts	181.9 ± 19.6^{a}	31.8 ± 7.1^{b}	62.1 ± 13.1^{b}

Means with no common letters are significantly different ($p < 0.05$).

follicles were measured by ELISA kits (Diagnostic Systems Laboratories, Webster, TX, USA). The follicular fluid of small and medium follicles could not be analyzed due to an insufficient sample volume. The assay sensitivity was 7 pg/mL for E_2, 0.13 ng/mL for P_4 and 0.04 pg/mL for T. The intra-assay coefficients of variation for E_2, P_4 and T were 9.3%, 8.2% and 11.4%, respectively and the inter-assay coefficients of variation were 7.5%, 6.8% and 11.9%, respectively.

2.4. *Statistical analysis*

A statistical software package (SPSS 11.0 for Windows, SPSS Inc., Chicago, IL, USA) was used to perform the statistical tests. Gene expression data were compared by one-way analysis of variance followed by Duncan's multiple range test. The hormonal data were compared using an unpaired two-tailed Student's *t*-test. The relation between cyst size and mRNA expression was analyzed by Pearson correlation. A $p < 0.05$ value was considered significant. Results are expressed as mean ± SEM.

3. Results

All large antral follicles were categorized as estrogen-active (E_2:$P_4 > 1$) and non-atretic (E_2:$T > 1$), and there were no differences in E_2 concentration between cystic and large antral follicles (Table 2). The mean P_4 concentration was significantly higher ($p < 0.05$) in the follicular fluid of large antral follicles, whereas T was higher ($p < 0.05$) in follicular cysts. All follicles were categorized as estrogen-active and non-luteinized.

All granulosa cell samples analyzed were positive for CYP19a1 mRNA and negative for CYP17a1 mRNA, whereas all theca cell samples analyzed were positive for CYP17a1 mRNA and negative for CYP19a1 mRNA. The identity of the PCR products was confirmed by sequencing (range 97–99% homology with bovine reference sequences). Relative real-time RT-PCR analysis showed that the expression of FSHR mRNA in granulosa cells was highest in small antral follicles, then decreased significantly as follicles increased in size ($p < 0.05$), and was lowest in cysts ($p < 0.05$). FSHR mRNA was not detected in theca cells of any follicular category, including cysts (Fig. 1). On the other hand, LHCGR mRNA expression in granulosa cells was significantly higher in large antral follicles than in cysts ($p < 0.05$), but was not detected in granulosa cells of small and medium antral follicles. In theca cells, the expression of LHCGR mRNA in medium antral follicles was higher than that in small and large antral follicles ($p < 0.05$). Also, the expression level of LHCGR mRNA in theca cells of follicular cysts was similar to those in small and medium antral follicles, but higher than that in large antral follicles ($p < 0.05$) (Fig. 1). No correlation ($p > 0.05$) was observed between the size of follicular cyst and mRNA expression of gonadotropin receptors.

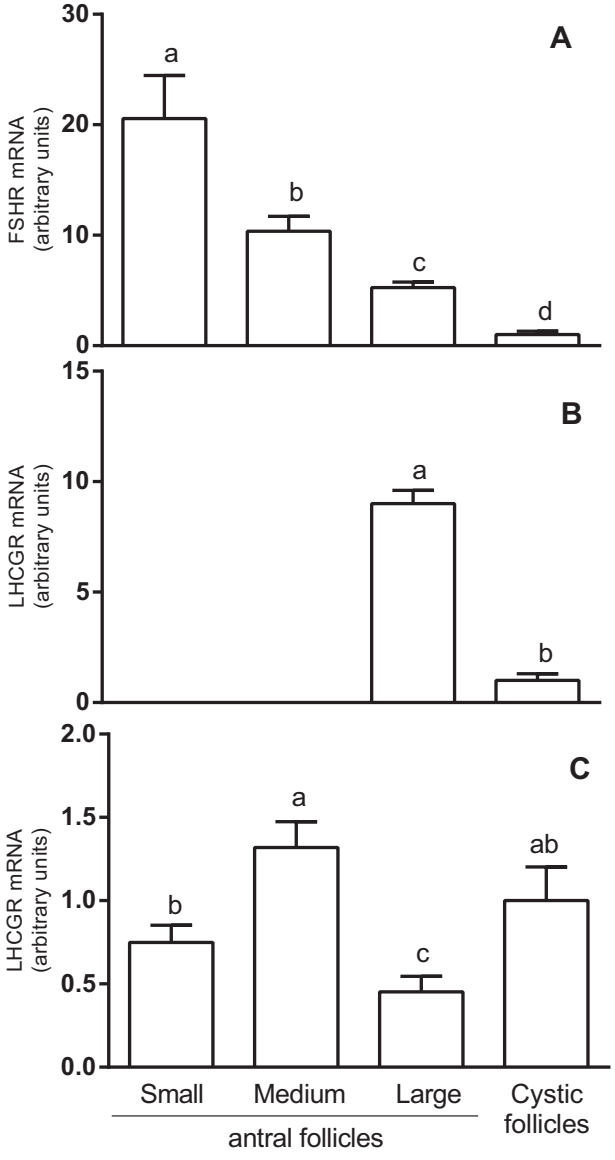

Fig. 1 – Relative mRNA expression (mean ± SEM) of (A) FSHR and (B and C) LHCGR in granulosa (A and B) and theca (C) cells of antral and cystic follicles. The expression of FSHR or LHCGR genes is related to the expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Different letters show significant differences ($p < 0.05$).

4. Discussion

Although many factors have been linked to the predisposition of cattle to COD, the causes of this disease have not been well defined. However, the most widely accepted hypothesis is that COD results from a neuroendocrine imbalance involving the hypothalamic–pituitary–gonadal-axis, which generates a disruption along the pathway from the hypothalamus to the ovaries [12,23]. While this general hypothesis may be accepted, to fully understand how follicular cysts develop and how they can be treated and prevented, it is vital to determine the specific disruption and the specific site along the pathway where this disruption occurs.

In the present study, we evaluated the possible alterations in the expression pattern of gonadotropin receptors as a cause of abnormal follicular development and anovulation. There is currently a controversy concerning this issue. Hormonal binding assays suggest that the number of FSH and LH receptors in granulosa cells of cysts are lower when compared to those in normal antral follicles [24]. However, studies using a similar binding assay, but with a different sample preparation without a clear separation of the follicular layers, have shown that LHCGR and FSHR concentrations measured in the follicular cysts are similar to those of control follicles [25]. In the present study, the results of real-time RT-PCR assay indicated that the expression of LHCGR and FSHR mRNAs in granulosa cells of cysts is significantly lower than that found in all the stages of normal follicular development. In contrast, the expression level in theca cells of follicular cysts was similar to those of small and medium antral follicles, but lower than that of large antral follicles. Contrary to our results, using *in situ* hybridization, Calder et al. [26] found that the expression of LHCGR mRNA was higher in granulosa cells of dominant cysts than in dominant follicles, but not different in theca cells, and that the expression of FSHR mRNA in granulosa cells did not differ among various follicular categories. However, the sequences analyzed by these authors encoded a portion of the extracellular domain of the bovine FSHR and ovine LHCGR [27], while in our study, we amplified a sequence located in exon 9 of the LHCGR gene and exon 10 of the FSHR gene, which are common to all isoforms of both receptors. Also, discrepancies between these two studies may result from methodological differences, such as division of cysts into estrogen-active and estrogen-inactive [12]. Also, these differences may be caused by the fact that other authors considered the follicular wall as a whole and did not analyze the granulosa and theca cells separately.

Previous studies have shown that growing and cystic follicles from COD animals show a decrease in cell proliferation and apoptosis detected *in situ*, as well as in the expression of pro-apoptotic genes in relation to the anti-apoptotic ones [13,28]. It is known that alterations in the proliferation of granulosa cells and the fate of the follicles (*i.e.*, atresia or formation of cysts) are related specifically to steroid hormone and gonadotropin receptors. Also, intrafollicular steroid concentrations are consistent with those previously described, indicating that follicular cysts, similar to preovulatory follicles, produce low P_4 and E_2 as well as high concentrations of T, possibly due to deficient aromatization in granulosa [3,4,8,12,14,17,29]. The intrafollicular role of steroids in the regulation of follicular development have been postulated and directly tested in several previous studies. Clear evidence exists for a direct role of androgen receptor (AR) in the stimulation of FSHR expression in granulosa cells [30]. The androgen produced in theca cells acts as an intercellular regulator of granulosa cell function by direct stimulation of FSHR expression. However, a clear decrease in AR mRNA expression in tertiary follicles and follicular cysts has been previously observed in animals with COD [31], which may partially explain the changes in FSHR observed in this study.

In summary, the ovaries from animals with COD exhibited an altered gonadotropin receptor expression compared with ovaries from control animals. Although our findings provide evidence that an altered gonadotropin signaling system may be present in bovine cystic follicles, additional studies are necessary to understand the potential associations with other follicular regulators. Also, considering that the efficaciousness of the treatment of COD with GnRH and gonadotropins with LH activity depends on adequate LHCGR expression, our results suggest that COD cows are unresponsive to hormone treatment because the decreased concentration of follicular LHCGR does not allow luteinization of the cystic structure. In conclusion, taking into account the importance of the expression of hormonal receptors in the function of the reproductive system and in the regulation of many aspects of ovarian function, we postulate that small changes in the receptor expression cause important alterations in the follicular dynamics. Therefore, it is reasonable to suggest that in conditions characterized by impaired ovulation, such as COD, changes in the expression of ovarian gonadotropin receptors could play a fundamental role in the pathogenesis of this disease.

Conflict of interest

None declared.

Acknowledgment

This study was supported by a grant from the Argentine National Agency for the Promotion of Science and Technology (ANPCyT) (PICT 2012-2638/PICT 2011-1274).

REFERENCES

[1] Nogueira MFG, Buratini J, Price CA, Castilho ACS, Pinto MGL, Barros CM. Expression of LH receptor mRNA splice variants in bovine granulosa cells: changes with follicle size and regulation by FSH in vitro. Mol Reprod Dev 2007;74(6):680–6.

[2] Nimz M, Spitschak M, Schneider F, Fürbass R, Vanselow J. Down-regulation of genes encoding steroidogenic enzymes and hormone receptors in late preovulatory follicles of the cow coincides with an accumulation of intrafollicular steroids. Domest Anim Endocrinol 2009;37(1):45–54.

[3] Fortune JE, Quirk SM. Regulation of steroidogenesis in bovine preovulatory follicles. J Anim Sci 1988;66(2):1–8.

[4] Bao B, Garverick HA. Expression of steroidogenic enzyme and gonadotropin receptor genes in bovine follicles during ovarian follicular waves: a review. J Anim Sci 1998;76 (7):1903–21.
[5] Kraaij R, Verhoef-Post M, Grootegoed JA, Themmen AP. Alternative splicing of follicle-stimulating hormone receptor pre-mRNA: cloning and characterization of two alternatively spliced mRNA transcripts. J Endocrinol 1998;158(1):127–36.
[6] Wiltbank MC, Gümen A, Sartori R. Physiological classification of anovulatory conditions in cattle. Theriogenology 2002;57(1):21–52.
[7] López-Díaz MC, Bosu TK. A review and update of cystic ovarian degeneration in ruminants. Theriogenology 1992;37 (6):1163–83.
[8] Silvia WJ, Hatler TB, Nugent AM, Laranja da Fonseca LF. Ovarian follicular cysts in dairy cows: an abnormality in folliculogenesis. Domest Anim Endocrinol 2002;23(1–2): 167–77.
[9] Bartlett PC, Kirk JH, Mather EC. Repeated insemination in Michigan Holstein-Friesian cattle: incidence, descriptive epidemiology and estimated economic impact. Theriogenology 1986;26(3):309–22.
[10] Archibald LF, Thatcher WW. Ovarian follicular dynamics and management of ovarian cysts. In: Van Horn HH, Wilcox CJ, editors. Large Dairy Herd Management. Champaign: American Dairy Science Association; 1992.
[11] Ijaz A, Fahning ML, Zemjanis R. Treatment and control of cystic ovarian disease in dairy cattle: a review. Br Vet J 1987;143(3):226–37.
[12] Vanholder T, Opsomer G, de Kruif A. Aetiology and pathogenesis of cystic ovarian follicles in dairy cattle: a review. Reprod Nutr Dev 2006;46(2):105–19.
[13] Salvetti NR, Stangaferro ML, Palomar MM, Alfaro NS, Rey F, Gimeno EJ, et al. Cell proliferation and survival mechanisms underlying the abnormal persistence of follicular cysts in bovines with cystic ovarian disease induced by ACTH. Anim Reprod Sci 2010;122(1–2):98–110.
[14] Salvetti NR, Alfaro NS, Velázquez MML, Amweg AN, Matiller V, Díaz PU, et al. Alteration in localization of steroid hormone receptors and coregulatory proteins in follicles from cows with induced ovarian follicular cysts. Reproduction 2012;144(6):723–35.
[15] Rodríguez FM, Salvetti NR, Panzani CG, Barbeito CG, Ortega HH, Rey F. Influence of insulin-like growth factor-binding proteins-2 and -3 in the pathogenesis of cystic ovarian disease in cattle. Anim Reprod Sci 2011;128(1–4):1–10.
[16] Rodríguez FM, Salvetti NR, Colombero M, Stangaferro ML, Barbeito CG, Ortega HH, et al. Interaction between IGF1 and IGFBPs in bovine cystic ovarian disease. Anim Reprod Sci 2013;140(1–2):14–25.
[17] Amweg AN, Salvetti NR, Stangaferro ML, Paredes AH, Lara HH, Rodríguez FM, et al. Ovarian localization of 11β-hydroxysteroid dehydrogenase (11βHSD): effects of ACTH stimulation and its relationship with bovine cystic ovarian disease. Domest Anim Endocrinol 2013;45(3):126–40.
[18] Parrott JA, Skinner MK. Thecal cell-granulosa cell interactions involve a positive feedback loop among keratinocyte growth factor, hepatocyte growth factor, and Kit ligand during ovarian follicular development. Endocrinology 1998;139(5):2240–5.
[19] Luo W, Gumen A, Haughian JM, Wiltbank MC. The role of luteinizing hormone in regulating gene expression during selection of a dominant follicle in cattle. Biol Reprod 2011;84(2):369–78.
[20] Lagaly DV, Aad PY, Grado-Ahuir JA, Hulsey LB, Spicer LJ. Role of adiponectin in regulating ovarian theca and granulosa cell function. Mol Cell Endocrinol 2008;284(1–2):38–45.
[21] Shibaya M, Matsuda A, Hojo T, Acosta TJ, Okuda K. Expressions of estrogen receptors in the bovine corpus luteum: cyclic changes and effects of prostaglandin F2alpha and cytokines. J Reprod Dev 2007;53(5):1059–68.
[22] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. Methods 2001;25(4):402–8.
[23] Kesler DJ, Garverick HA. Ovarian cysts in dairy cattle: a review. J Anim Sci 1982;55(5):1147–59.
[24] Kawate N, Inaba T, Mori J. A quantitative comparison in the bovine of steroids and gonadotropin receptors in normally developing follicles and in follicular and luteinized cysts. Anim Reprod Sci 1990;23(4):273–81.
[25] Odore R, Re G, Badino P, Donn A, Vigo D, Biolatti B, et al. Modifications of receptor concentrations for adrenaline, steroid hormones, prostaglandin F2alpha and gonadotropins in hypophysis and ovary of dairy cows with ovarian cysts. Pharmacol Res 1999;39(4):297–304.
[26] Calder MD, Manikkam M, Salfen BE, Youngquist RS, Lubahn DB, Lamberson WR, et al. Dominant bovine ovarian follicular cysts express increased levels of messenger RNAs for luteinizing hormone receptor and 3 betahydroxysteroid dehydrogenase delta(4), delta(5) isomerase compared to normal dominant follicles. Biol Reprod 2001;65(2):471–6.
[27] Xu Z, Garverick HA, Smith GW, Smith MF, Hamilton SA, Youngquist RS. Expression of follicle-stimulating hormone and luteinizing hormone receptor messenger ribonucleic acids in bovine follicles during the first follicular wave. Biol Reprod 1995;53(4):951–7.
[28] Salvetti NR, Panzani CG, Gimeno EJ, Neme LG, Alfaro NS, Ortega HH. An imbalance between apoptosis and proliferation contributes to follicular persistence in polycystic ovaries in rats. Reprod Biol Endocrinol 2009;7:68.
[29] Amweg AN, Paredes A, Salvetti NR, Lara HE, Ortega HH. Expression of melanocortin receptors mRNA, and direct effects of ACTH on steroid secretion in the bovine ovary. Theriogenology 2011;75(4):628–37.
[30] Luo W, Wiltbank MC. Distinct regulation by steroids of messenger RNAs for FSHR and CYP19A1 in bovine granulosa cells. Biol Reprod 2006;75(2):217–25.
[31] Alfaro NS, Salvetti NR, Velazquez MM, Stangaferro ML, Rey F, Ortega HH. Steroid receptor mRNA expression in the ovarian follicles of cows with cystic ovarian disease. Res Vet Sci 2012;92(3):478–85.