PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
MESTRADO EM CIÊNCIA ANIMAL

EXPRESSÃO DIFERENCIAL DO RECEPTOR DE LH, DA PROTEÍNA DE LIGAÇÃO DE MRNA DO LHR, BTA-MIR-222 E ENZIMAS ESTEROIDOGÊNICAS NO OVÁRIO BOVINO EM DESENVOLVIMENTO

MARINA PLATZECK CHAVES

Presidente Prudente – SP
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Defesa apresentada a Pró-Reitoria de Pesquisa e Pós-Graduação, Universidade do Oeste Paulista, como parte dos requisitos para obtenção do título de Mestre em Ciência Animal - Área de Concentração: Fisiopatologia Animal.

Orientador: Prof. Dr. Anthony César de Souza Castilho

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Presidente Prudente, 30 de maio 2018.

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Agradecimentos ao programa de Mestrado em Ciência Animal e a Pró-Reitoria de Pesquisa e Pós Graduação da Universidade do Oeste Paulista.

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“Antes da descoberta da Austrália, as pessoas do Mundo Antigo estavam convencidas de que todos os cisnes eram brancos. Uma crença inquestionável por evidências empíricas. Deparar-se com o primeiro cisne negro pode ter sido uma surpresa interessante para alguns ornitólogos. Mas não é essa a importância da história. Ela ilustra a limitação do aprendizado por meio de observações ou experiências e a fragilidade do nosso conhecimento. Uma única observação pode invalidar uma afirmação originada pela existência de milhões de cisnes brancos. Tudo o que se precisa é de um único pássaro negro (que também, pelo que sei, é muito feio).” – A Lógica do Cisne Negro (Nassim Nicholas Taleb)

RESUMO

EXPRESSÃO DIFERENCIAL DO RECEPTOR DE LH, DA PROTEÍNA DE LIGAÇÃO DE MRNA DO LHR, BTA-MIR-222 E ENZIMAS ESTEROIDOGÊNICAS NO OVÁRIO BOVINO EM DESENVOLVIMENTO

Esteroides e gonadotrofinas são essenciais para a regulação do desenvolvimento folicular antral e os estágios finais do desenvolvimento pré-antral. Embora o receptor do hormônio luteinizante (LHR) tenha sido detectado nos folículos pré-antrais de ratos, coelhos e porcos, a expressão deste receptor no ovário fetal bovino não foi demonstrada. O presente estudo teve como objetivo quantificar a expressão do LHR e a abundância de mRNA da proteína de ligação LHR (LRBP), STAR, HSD3B1, CYP17A1 e CYP19A1 durante o desenvolvimento do ovário fetal bovino. Além disso, objetivamos identificar e quantificar a expressão de bta-miR-222 (microRNA regulador do gene LHCGR). Em resumo, a expressão de LHR foi observada no folículo pré-antral no ovário fetal de bovino e a abundância de mRNA foi menor no dia 150 do que no dia 60. No entanto, a abundância de mRNA da LRBP seguiu o padrão oposto. Semelhante a LRBP, a abundância de bta-miR-222 foi maior no dia 150 do que no dia 60 ou 90. Com relação à expressão gênica de enzimas esteroidogênicas; apenas a abundância de mRNA de STAR foi maior no dia 150 do que no dia 60. A proteína LHR foi detectada em oogônia, folículos primordiais, primários e secundários. Além disso, ambos os oócitos e células da granulosa apresentaram imunolocalização positiva para LHR. Em conclusão, estes resultados sugeriram o envolvimento da regulação do LHCGR / LBPB com mecanismos relacionados ao desenvolvimento de folículos pré-antrais, especialmente durante o estabelecimento de folículos secundários. Além disso, os presentes dados reforçaram que a expressão reduzida de mRNA de LHR em ovários fetais bovinos no dia 150 estava relacionada à maior expressão de LRBP e bta-miR-222.

Palavras-chave: LHCGR, mevalonato quinase, folículo pré-antral, esteroidogênese, microRNAs

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SUMÁRIO

O presente manuscrito está formatado conforme as normas do periódico *Animal Reproduction Science*.

Differential expression of LH receptor, LHR mRNA binding protein, bta-miR-222 and steroidogenic enzymes in the developing bovine ovary

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ABSTRACT

Steroids and gonadotrophins are essential for the regulation of antral follicular development and the late stages of preantral development. Although the luteinizing hormone receptor (LHR) has been detected in the preantral follicles of rats, rabbits, and pigs, the expression of this receptor in bovine fetal ovary has not been demonstrated. The present study aimed to quantify the expression of the LHR and the mRNA abundance of the genes LHR binding protein (LRBP), STAR, HSD3B1, CYP17A1, and CYP19A1 during the development of bovine fetal ovary. In addition, we aimed to identify and quantify the expression of bta-miR-222 (a regulatory microRNA of the LHCGR gene). In summary, LHR expression was observed in the preantral follicle in bovine fetal ovary, from oogonias to primordial, primary and secondary stages, and the mRNA abundance was lower on day 150 than day 60. However, the mRNA abundance of LRBP followed the opposite pattern. The LHR protein was detected in oogonia, primordial, primary, and secondary follicles. Moreover, both oocytes and granulosa cells showed positive immunostaining for LHR. Similar to LRBP, the abundance of bta-miR-222 was higher on day 150 than day 60 or 90 of gestation. With regard to the gene expression of steroidogenic enzymes; only the mRNA abundance of STAR was higher on day 150 than on day 60. In conclusion, these results suggested the involvement of LHCGR/LRBP regulation with mechanisms related to the development of preantral follicles, especially during the establishment of secondary follicles. Furthermore, the present data reinforced that the reduced expression of LHR mRNA in bovine fetal ovaries on day 150 was related to the higher expression of LRBP and bta-miR-222.

Key words: LHCGR, mevalonate kinase, preantral follicle, steroidogenesis, microRNAs.

1. Introduction

In cattle, all preantral follicular stages occur in the fetal ovary during pregnancy (Erickson, 1966). After formation, the quiescent primordial follicles begin their growth; subsequently, the granulosa cells multiply and change to a cuboidal shape, setting the transition from the primordial to the primary follicle (Wandji et al., 1996). In the bovine fetus, (Castilho et al., 2014) demonstrated that the preantral follicular dynamics in zebu were similar to those described in taurines (Tanaka et al., 2001): primordial follicles appeared at 75 days of gestation, primary at 90 days, secondary at 150 days, and early antral follicles at 210 days of gestation.

Although fetal ovarian development occurs independently of gonadotrophins, these hormones do influence this process (Fortune and Eppig, 1979). As follicle stimulating hormone (FSH) receptors (FSHR) have been detected in bovine primary follicles (Wandji et al., 1996), the primary role of FSH at this stage of development cannot be excluded (Gutiérrez et al., 1997; McNatty et al., 1999; Gutierrez et al., 2000; Webb et al., 2003). Unlike FSHR, there are no studies that report the expression and regulation of LHR in different preantral follicular types during fetal ovarian development in bovine species.

Luteinizing hormone (LH) plays a key role in the control of physiological processes in the ovary, such as the development of antral follicles and ovulation (Xu et al., 1995). In antral follicles, the LH receptors have been detected in theca cells of healthy follicles and, subsequently, in granulosa cells (Xu et al., 1995; Bao et al., 1997; Fortune, 2001; Fortune et al., 2001; Garverick et al., 2002; Nogueira et al., 2007; Ereno et al., 2015). The possible mechanisms involved in the LHR regulation of granulosa cells include the LH receptor binding protein (LRBP) and certain microRNAs. LRBP is a mRNA binding protein that binds to the LHCGR (LHR gene) coding region and represses its translation (Nair et al., 2002). In cattle, Ereno et al. (2015) first demonstrated the inverse correlation between LRBP expression

and LHCGR mRNA regulation at the time of follicular deviation in granulosa cells. Similarly, recent studies demonstrated that the post-transcriptional regulation of LHCGR by miRNA occurs in the ovary of several species (Menon et al., 2013; Troppmann et al., 2014; Menon et al., 2015; Gilchrist et al., 2016); this includes miR-222, which was suggested by Hossain et al. (2009) as a possible regulator of LHCGR expression. Salilew-Wondim et al. (2014) investigated the expression of miR-222 in the theca and granulosa cells of bovine antral follicles and reported lower expression in the granulosa cells from dominant follicles. In addition, Santos et al. (2018) reported lower miR-222 expression in granulosa cells from superstimulated cows submitted to ovarian superstimulation than control animals and inverse proportionality to the abundance of LHCGR mRNA.

The steroid hormones and their actions are directly dependent on the expression of gonadotrophic receptors and steroidogenic enzymes in follicular somatic cells (Fortune et al., 2001). Although there is evidence that the developing gonads produce steroids throughout gestation (Shemesh et al., 1978; Tanaka et al., 2001), probably by using the same sequential enzymatic processing of the adult ovary (Conley and Bird, 1997), the role of steroids during the development of bovine ovaries has not been sufficiently clarified.

Thus, the objectives of the present study were to localize and quantify the LHR expression in the fetal ovary and to measure the mRNA abundance of LRBP and expression of bta-miR-222 in the developing bovine fetal ovary. In addition, we sought to quantify the mRNA abundance of genes encoding steroidogenic enzymes STAR (StAR protein), HSD3B1 (3 Beta-Hydroxysteroid Dehydrogenase), CYP17A1 (17 Steroid - Alpha Monooxygenase) and CYP19A1 (Aromatase).

2. Materials and methods

2.1. Tissues

In accordance with the methods of Tanaka et al. (2001) and Castilho et al. (2014), 20 female fetuses at 60, 90, 120, and 150 days of gestational (n = 5/group), predominantly from Nellore cattle (*Bos Taurus indicus*), were obtained from a local abattoir near the University of São Paulo State campus at Assis city and were classified according to specific crown-rump lengths intervals. Subsequently, one fetal ovary of each fetus was transported to the laboratory in TRIzol® Reagent for RNA extraction and the other was transported in methacarn solution (60% methanol, 30% chloroform, 10% acetic acid) for histology and immunohistochemistry.

2.1.2. Gene expression

For total RNA extraction, the whole fetal ovaries were homogenized (Precellys®, Bertin Technologies) and later subjected to the TRIzol® protocol (Invitrogen®, São Paulo, Brazil) to obtain total RNA in accordance with the manufacturer's instructions. The total RNA concentration was measured by spectrophotometry using a Nanodrop (ND-2000®). The total RNA from samples (1μg) was incubated with DNAse (1 U/μg; Invitrogen, Carlsbad, CA, USA) and then reverse transcribed by using a random primer in accordance with protocol provided by High Capacity Kit instructions (Applied Biosystems, Foster City, CA, USA).

The RT-qPCR analysis for each gene (LHCGR, LRBP, STAR, HSD3B1, CYP17A1, and CYP19A1) were performed by QuantStudio™ 7 Flex using Power Sybr® Green PCR Master Mix system (Applied Biosystems). The mRNA abundance of target genes was assessed in a total reaction volume of 25 μL, with 1.0 μL of each sample and 24 μL of probe plus primers in accordance with the methods of Machado et al. (2009) for endogenous genes, and Castilho et al. (2015) and Ereno et al. (2015) for the target genes (Table1). The thermal

cycling conditions comprised 95°C for 10 min, followed by 40 cycles of denaturing at 95°C for 10 s, annealing, and extension for 1 min, with different temperatures used for different genes. There actions were optimized to provide the maximum amplification efficiency for each gene. The specificity of each PCR product was determined by melting curve analysis. Each sample was analyzed in duplicate and negative controls were run for each plate.

Table 1
Primers used in RT-qPCR.

Gene	Forward	Reverse	Final concentration	Temp. annealing (Cº)
LHR	5'GCATCCACAAGC TTCCAGATGTTACG A 3'	5'GGGAAATCAGCGTT GTCCCATTGA 3'	300	60
LRBP	5'TGTTGTCAGAAG TCCTGCTGGTGT 3'	5'TAAGTTGAGGCCCA CTCTCCCATT 3'	300	56
CYC A	5'TATCTGCACTGC CAAGACTGAGTG3'	5'CTTCTTGCTGGTCTTGC CATTCC3'	125	60
STAR	5'CCCAGCAGAAGG GTGTCATC 3'	5'TGCGAGAGGACCTG GTTGAT 3'	400	62
CYP19A1	5'CTGAAGCAACAG GAGTCCTAAATGTA CA 3'	5'AATGAGGGGCCCA ATTCCCAGA 3'	400	62
CYP17 A1	5'GTGGAGACCACC ACCTCTGT3'	5'GCTGAAACCCACA TTCTGGT3'	108	67
HSD3B1	**5'TCCTTGGCCTC TCTACTCCA3'**	**5'AGACAGCATATGG GGTCAGC3'**	**125**	**62**

To choose the most stable reference gene for the detailed analyses of fetal ovaries, the amplification profiles of peptidylprolyl isomerase A (PPIA), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and histone H2AFZ (H2AFZ) were compared by using the geNorm applet for Microsoft Excel (medgen.ugent.be/genorm; (Ramakers et al., 2003)). The most stable housekeeping gene was PPIA. The ΔΔCt method with efficiency correction was used to calculate relative expression values (target genes/PPIA) for each target gene; the mean value for the day 60 group was used as a calibrator (Pfaffl, 2001).

2.1.3. miRNA expression

The miRNA extraction was performed from 50 µg of total RNA by using a mirVana™ miRNA Isolation Kit (Life Technologies®, Carlsbad, USA) in accordance with the manufacturer's instruction and subsequently stored at -80°C. To reverse transcribe target miRNAs (bta-miR-222, Has-miR-191, and RNU-43), we used TaqMan® Reverse Transcription Reagents (Applied Biosystems, Foster City, CA, USA) in accordance with the manufacturer's protocols. The qPCR analyses were performed by QuantStudio™ 7 Flex using TaqMan® Universal PCR Master Mix. The final volume of PCR mix was 20 µL and the cycling conditions were 95°C for 10 min, for enzyme activation, followed for 40 cycles of denaturation (95ºC for 15 s) and annealing/extension (60°C for 60 s). All samples were run in duplicate.

Table 2
Details of miRNA assays used in RT-qPCR.

MiRNA	Mature miRNA Sequence	Code	MiRBase accession number
Has-miR-222	AGCUACAUCUGGCUACUGGGU	002276	MIMAT0000279
Has-miR-191	CAACGGAAUCCCAAAAGCAGCUG	002299	MIMAT0000440
RNU43	**GAACTTATTGACGGGCGGACAGAAACTGT GTGCTGATTGTCACGTTCTGATT**	**001095**	

To quantify the relative abundance of bta-miR-222, we used the geometric mean of the expression of RNU43 and has-miR-191 as a reference (Table 2). The ΔΔCt method with efficiency correction was used to calculate the relative expression value (bta-miR-222/RNU43_has-miR-191 geometric mean) with the mean value on day 60 used as the calibrator (Pfaffl, 2001).

2.1.4 Immunohistochemistry

The tissues from all groups were fixed in methacarn for 6h and stored in 70% ethanol. Tissue dehydration was performed by using a series of graded ethanol solutions and the dehydrated tissues were embedded in Paraplast® (Oxford Labware, St. Louis, MO, USA). The blocks were sectioned into 4 μm thick slices and the sections were placed on poly-L-lysine–coated slides, which were deparaffinized and washed. One ovary at each estimated fetal age was subjected to immunohistochemical staining to localize LHR (ab96603, Abcam, Cambridge, UK).

The antigens were retrieved at high temperature (100 °C) for 30 min in 10 mM citrate buffer (pH 6.0). Endogenous peroxidase activity was quenched through incubation with 3% H_2O_2 diluted in methanol for 15 min and nonspecific protein binding was blocked by the incubation of the slides in bovine serum albumin (BSA), diluted to 3% in PBS, plus 0.1% NP-40. The primary antibody anti-hCG receptor (ab96603, Abcam, 1/100) was diluted in 1% BSA in PBS plus 0.1% NP-40 and the slides were incubated overnight in this solution at 4 °C. For the immunoperoxidase assay, the slides were rinsed in PBS, incubated with secondary antibody biotinylated Goat Anti-Rabbit IgG H&L (ab97049, Abcam), followed by a VECTASTIN ABC Kit (Vector Laboratories Ltd), and visualized with by using diaminobenzidine. The protocol used was standardized by Mendes et al. (2015). The sections were counterstained with Harris hematoxylin. LHR immunostaining was performed in the corpus luteum of adult bovine ovary (positive control) to confirm the quality of the primary antibody. The negative control was performed in the absence of the primary antibody.

2.2. Statistical analysis

Data were transformed to logarithms if not normally distributed. ANOVA was used to test for effects of gestational days on the relative abundance of miRNA and target genes. The differences between means were determined by the Tukey test. The analyses were computed

by using JMP software (SAS Institute Cary, NC). The data are presented as the mean ± SEM; and differences were considered significant for $P \leq 0.05$.

3. Results

The expression of the target genes and bta-miR-222 was observed in bovine fetal ovary during gestation. When the effect of gestational day was investigated, the abundance of LHCGR mRNA was lower on day 150 of gestation than on day 60 and 90 (P=0.04; Fig. 1A); in contrast, the mRNA abundance of LRBP was higher on day 150 than on day 60 and 90 (P=0.03; Fig. 1B). Similar to LRBP, the expression of bta-miR-222 was also higher on day 150 than days 60 and 90 (P=0.02; Fig.1C).

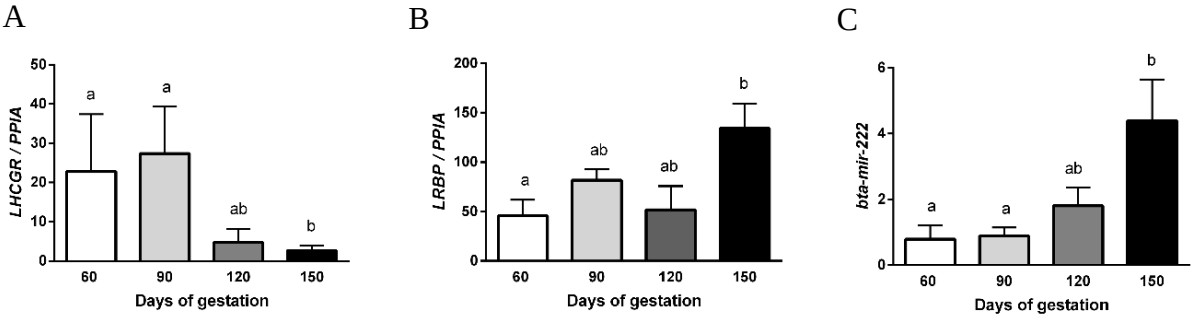

Fig.1. Relative mRNA abundance of LHCGR (A), LRBP (B), and bta-miR222 (C) in the days 60, 90, 120 and 150 of gestation. The data are presented as the mean (± S.E.M.). Bars with different letters are significantly different ($P \leq 0.05$).

The immunolocalization of LHR was demonstrated in the fetal ovary. The LHR protein was found in oogonia, primordial, primary, and secondary follicles (Fig. 2A). Moreover, both oocyte and granulosa cells showed positive results for LHR immunostaining (Fig.2B). Another important finding was the stronger immunoreactivity of LHR in the ovarian cortex (Fig. 2A) than in the medullar region, which also showed positive immunostaining for stromal cells and blood vessels (Fig. 2B).

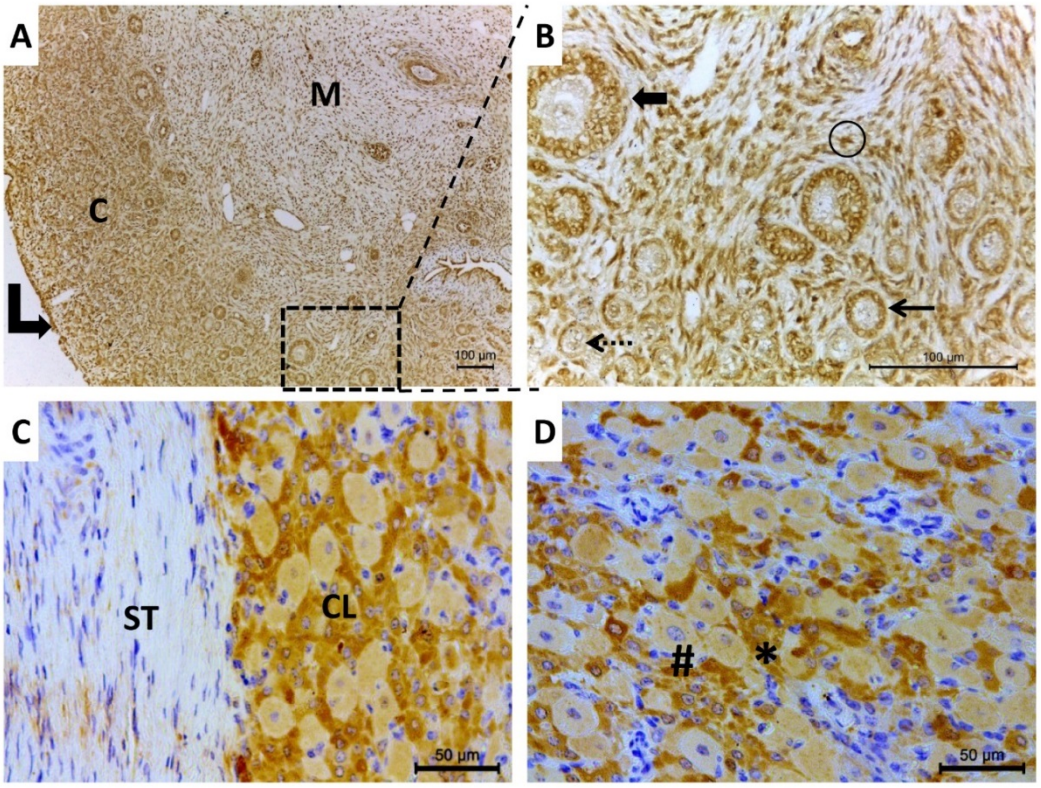

Fig. 2. Representative photomicrographs showing immunolocalization of LHR protein in day 150 bovine fetal ovary (A and B). Overview LHR staining in cortex (C) and medulla (M) with immunostaining in the ovarian surface (curve arrow) (A). The ovarian cortex showed granulosa LHR positive cells in secondary (thick arrow), primary (thin arrow) and primordial (dashed arrow) follicles as well as stromal positive cells (circle) (B). Corpus luteus (CL) was used as positive control and the adult ovary (C and D) stroma are not stained (ST). Strong immunoreactivity in cytoplasm of small luteal cells (asterisk) and weak immunoreactivity in large luteal cells (hashtag) are evident (D). Bars: A and B: 100 µm; C – D: 50 µm.

With regarding to the mRNA expression of steroidogenic enzymes, only the abundance of STAR mRNA was differentially expressed during the development of the fetal ovary, with a higher expression observed on day 150 than day 60 (P=0.01; Fig. 3).

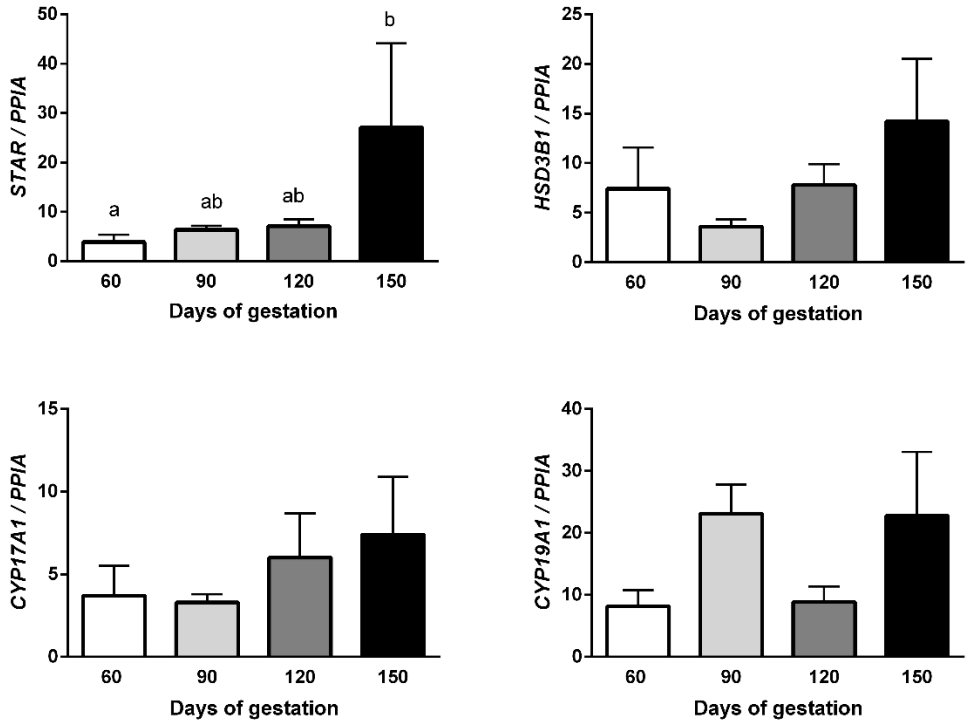

Fig. 3. Relative mRNA abundance of STAR, HSD3B1, CYP17A1, and CYP19A1 between 60 and 150 days of gestation. The data were presented as the mean (± S.E.M.). Bars with different letters (a and b) are significantly different ($P \leq 0.05$).

4. Discussion

This is the first study to describe the time-dependent expression patterns of LHR, LRBP, bta-miR-222, and some steroidogenic enzymes in bovine fetal ovary, which reinforce their steroidogenic capacity. LH plays a key role in the control of the physiological processes in the ovary, such as the development of antral follicles and ovulation, and acts via the LH receptor (Xu et al., 1995; Bao et al., 1997; Fortune, 2001; Garverick et al., 2002; Nogueira et al., 2007). LHCGR mRNA has been detected in the preantral follicles of rats, rabbits, and pigs (Eppig, 2001), however, the location of LHR in fetal bovine ovaries was not previously demonstrated, only in adult ovaries (Braw-Tal and Roth, 2005).

It has been proposed that steroids play a regulatory role on preantral folliculogenesis. The appearance of the preantral stages in the ovary has a species-specific temporal pattern. In

442 cattle, all pre-antral stages are manifested in the fetal ovary during pregnancy (Erickson,
443 1966). The data regarding the specific moment of appearance of each preantral category are
444 conflicting. Although Rüsse (1983) reported the appearance of primordial, primary, and
445 secondary follicles at approximately 90, 140, and 210 days of gestation, respectively, Tanaka
446 et al. (2001) observed the same stages of development at approximately 74, 91, and 150 days
447 of gestation. This process involves qualitative and quantitative changes, mainly in the
448 metabolism, follicular structure (Gosden et al., 1997) and steroidogenic production (Yang and
449 Fortune, 2008). Therefore, our results reinforced that the steroidogenic capacity of the fetal
450 ovary maybe related to preantral follicle assembly to guarantee further stages of follicle
451 development.

452 The transcriptional regulation of LHR can be influenced by several factors, particularly
453 in granulosa cells, which are FSH-dependent (Nogueira et al., 2007). The data regarding the
454 time at which the ovarian follicles acquire LHR in the granulosa cells are conflicting. Some
455 authors have demonstrated that the dominant follicle acquires LHR before follicle deviation
456 (Ginther et al., 2001), but other reports show that LHR expression occurs after this step (Xu et
457 al., 1995; Bao et al., 1997; Fortune, 2001). In the present study, we demonstrated that the
458 LHR was present in oogonia, primordial, primary, and secondary follicles and that the
459 expression was not exclusive from granulosa and theca cells, as described in bovine antral
460 follicles (Xu et al., 1995; Bao et al., 1997; Fortune et al., 2001; Garverick et al., 2002;
461 Nogueira et al., 2007). Therefore, we considered that LHR expression may be differentially
462 regulated in the preantral follicle and modified when these follicles become gonadotropin
463 dependent during the later stages of their differentiation.

464 Previous data showed the presence of gonadotropins receptors in the cumulus-oocyte
465 complex (Amsterdam et al., 1976; Bodensteiner et al., 1996; Baltar et al., 2000). In support of
466 these results, Baltar et al. (2000) described the positive and conclusive binding of LH or hCG

467 to LH receptors in the bovine cumulus-oocyte complex. The same findings were described by
468 Teerds and Dorrington (1995), who observed that the cumulus cells of mice possessed LH
469 receptors, although the quantities were smaller than those found in granulosa cells. Our data
470 on the expression of the LH receptor in fetal ovaries agree with Teerds and Dorrington (1995),
471 and Bao et al. (1997), who showed that the receptors were in cumulus cells, granular cells,
472 and preantral follicles but disagree with Eppig (1991) who reported the absence of these
473 receptors in these cells types, and Braw-Tal and Roth (2005) which have detected LHR only
474 in inner Teca cells.

475 The negative correlation between LRBP expression and LHCGR mRNA regulation, at
476 the follicular deviation stage in cattle, was also reported by Ereno et al. (2015). The authors
477 suggested that the lower abundance of LRBP mRNA in dominant follicles was consistent with
478 the involvement of the LHCGR/LRBP system during follicle selection, to ensure the
479 expression of LHCGR mRNA and the acquisition of ovulatory capacity. Similarly, we
480 observed that the decreased abundance of LHCGR was accompanied by an increase in the
481 abundance of LRBP, which suggested that LHCGR in the fetal ovary may be regulated by this
482 protein; therefore, the higher abundance of LRBP on day 150 may contribute to the lower
483 expression of LHCGR in the same period.

484 Post-transcriptional regulation by miRNA in the ovary has been reported in several
485 species (Menon et al., 2013; Troppmann et al., 2014; Menon et al., 2015; Gilchrist et al.,
486 2016). The miRNA expression and their specific roles in bovine ovary were previously
487 reported (Zielak-Steciwko and Evans, 2016) including those of miR-222, which was described
488 by Hossain et al. (2009) as a possible regulator of LHCGR expression .In addition, Salilew-
489 Wondim et al. (2014) demonstrated that the expression of miR-222 in the theca and granulosa
490 cells of bovine antral follicles was lower than that in granulosa cells from bovine dominant
491 follicles. Recently, Santos et al. (2018) demonstrated the expression of bta-miR-222 in adult

and fetal bovine tissues (ovary, testicle, spleen, liver, kidney, heart, and brain). In the same study, the authors showed that the abundance of LHCGR mRNA and the expression of bta-miR-222 followed opposite patterns in superstimulated granulosa cells from Nelore cattle. Here, the lower levels of LHCGR on day 150 of gestation could be supported by the upregulation of bta-miR-222; the higher expression may be required to regulate preantral follicle formation, especially during the secondary follicle formation at this time. Based on the findings discussed above, we propose the possibility of a summative effect between bta-miR-222 and LRBP, which promotes LHCGR downregulation in the developing bovine fetal ovary.

The production of steroids in the fetal ovary is supported by CYP19A1 (Garverick et al., 2010), CYP11A1, HSD3B1, and CYP17A1 (Quirke et al., 2001) in ruminant fetal ovaries. Nevertheless, in the present study, the mRNA abundance of HSD3B1, CYP17A1, and CYP19A1 was not affected by gestational age, but we could not exclude the post-transcriptional pathways as an explanation of steroid production by the fetal ovary, as described by Yang and Fortune (2008).

Although the mRNA encoding steroidogenic enzymes was not regulated, the abundance of STAR was higher on day 150 of gestation than day 60, which coincided with an increase in the number of secondary follicles. In cows, STAR mRNA was previously detected in oocytes, and theca and granulosa cells from early antral follicles (Braw-Tal and Roth, 2005). The formation of the thecal compartment and intense granulosa cell proliferation occurs during the transition from the primary to the early antral stage (Braw-Tal and Yossefi, 1997) at approximately days 150–210 of gestation. Therefore, the consecutive increases in mRNA abundance of STAR could be related to the differentiation and proliferation of granulosa and theca cells.

Although fetal ovarian development occurs independently of gonadotrophins, the findings regarding the differential expression of LHR/LRBP suggest that this system could be one more factor that must be regulated to allow the establishment of germ cells and fetal ovarian development. In addition, it could enable better strategies, using hormonal protocols, to take advantage of the reproductive potential of cattle.

5. Conclusion

These results suggested the involvement of LHCGR/LRBP regulation with mechanisms related to the development of preantral follicles, especially during the establishment of secondary follicles. Furthermore, the present data reinforced that the reduced expression of LHR mRNA in bovine fetal ovaries on day 150 was related to the higher expression of LRBP and bta-miR-222.

6. Conflicts of interest

The authors declare no conflicts of interest and financial, personal, or other relationships with other people or organizations.

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