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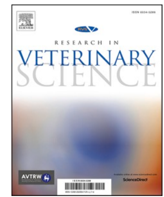

NR3C1 and glucocorticoid-regulatory genes mRNA and protein expression in the endometrium and ampulla during the bovine estrous cycle

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A B S T R A C T

The bovine reproductive tract exhibits changes during the estrous cycle modulated by the interplay of steroid hormones. Glucocorticoids can be detrimental when stress-induced but are relevant at baseline levels for appropriate reproductive function. Here, an analysis of quantitative real-time PCR was performed to study the bovine glucocorticoid-related baseline gene transcription in endometrial and ampullar tissue samples derived from three time points of the estrous cycle, stage I (Days 1–4), stage III (Days 11–17) and stage IV (Days 18–20). Our results revealed expression differences during stages, as expression observed in the ampulla was higher during the post-ovulatory phase (stage I), including the glucocorticoid receptor *NR3C1*, and some of its regulators, involved in glucocorticoid availability (*HSD11B1* and *HSD11B2*) and transcriptional actions (*FKBP4* and *FKBP5*). In contrast, in the endometrium, higher expression of the steroid receptors was observed during the late luteal phase (stage III), including *ESR1*, *ESR2*, *PGRMC1* and *PGRMC2,* and *HSD11B1* expression decreased, while *HSD11B2* increased. Moreover, at protein level, FKBP4 was higher expressed during the late luteal phase, and NR3C1 during the pre-ovulatory phase (stage IV). These results suggest that tight regulation of the glucocorticoid activity is promoted in the ampulla, when reproductive events are taking place, including oocyte maturation. Moreover, most expression changes in the endometrium were observed during the late luteal phase, and may be related to the embryonic maternal recognition. In conclusion, the glucocorticoid regulation changes across the estrous cycle and may be playing a role on the reproductive events occurring in the bovine ampulla and endometrium.

1. Introduction

Many of the physiological vertebrate functions, including metabolism, behavior, immunity, development, stress response, and reproduction, are regulated by steroid hormones (Wang and Harris, 2015). Some of these functions are exerted through the nuclear receptor's family, a diverse group of transcription factors that have steroids as ligands (Carson-Jurica et al., 1990). These receptors may have contributed to the evolution of multicellular animals, assumed to be helped by their diversification into different steroid receptors (Baker, 2019). Evolutive changes in the primordial receptor may have happen in order to provide a specialized hormonal response to the more complex and diverse physiological functions present in vertebrates (Baker et al., 2015; Bertrand et al., 2004). In reproduction, adrenal and gonadal steroids are known to regulate the physiological changes occurring in the female reproductive tract during the sexual cycle (Barton et al., 2020; Whirledge and Cidlowski, 2017). As in other animal species, in bovine, great dynamic modifications of the reproductive tract environment are taking place during the different stages of the estrous cycle (Ireland et al., 1980), both in the oviductal and uterine portions (Binelli et al., 2018; Forde and Lonergan, 2012). Although there are extensive studies focusing on gametes transport through the female tract and the interactions occurring between the maternal environment and spermatozoa and oocytes (Hunter, 2012; Talevi and Gualtieri, 2010), information

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about oviductal and endometrial regulation by steroids is especially scarce in the case of glucocorticoids (GCs) (Binelli et al., 2018).

The complex mechanisms underlying the required balance in the GC action have recently started to be deemed of much importance for reproduction (Ruiz-Conca et al., 2020a; Whirledge and Cidlowski, 2017; Whirledge et al., 2015). While sustained stress-induced levels of GCs are often detrimental for fertility (Pontes et al., 2019), baseline GC levels are necessary for an adequate physiological and reproductive function (Whirledge and Cidlowski, 2017). In comparative studies, data suggest that evolutionary changes in response to hormones occur more often by producing changes in the receptor, enzymes and co-regulators, rather than altering the hormone signal (Adkins-Regan, 2013). Many factors influence the extent of the GC action, including spatiotemporal patterns of GC exposure, which seems to variate greatly, even among closely related species (Lattin et al., 2015). Moreover, the fine-tuning of the GC regulation appears to be determined in a tissue-specific manner (Lattin et al., 2015; Martins and de Castro, 2021), displaying variations between anatomical regions of the reproductive tract (Ruiz-Conca et al., 2020a; Ruiz-Conca et al., 2020b; Whirledge and DeFranco, 2018). In humans, GC synthetic analogues are often prescribed as fertility treatments for reducing inflammatory responses when impaired endometrial receptivity is present (Robertson et al., 2016). In contrast, exposure to sustained stress-induced GC levels seem to inhibit endometrial receptivity (Park et al., 2021). Thus, these specific responses suggest the need for a necessary balance in the GC levels to differentiate between pathogenicity and reproductive physiology.

Indeed, GCs have been proposed to play a key role in the reproduction of mammals, mainly through the glucocorticoid receptor (NR3C1). The role of NR3C1 has been highlighted in both female and male reproduction on different species, where, respectively, uterine presence of the receptor is crucial for implantation in mice (Whirledge et al., 2015), and relevant for testicular function in humans (Nordkap et al., 2017). The GC availability to NR3C1 seems to be regulated by two 11β-hydroxysteroid dehydrogenases (HSD11B1 and HSD11B2), responsible for the conversion of cortisone into active cortisol, and the inactivation of cortisol to cortisone, respectively (Michael et al., 2003). Moreover, the receptor's biological mechanism of action is complex, involving a great number of co-factors and repressors, including the FK506-binding immunophilins FKBP4 and FKBP5 (Petta et al., 2016; Ratajczak et al., 2015; Wochnik et al., 2005; Zannas et al., 2019). In the absence of ligand, NR3C1 remains inactive in the cytoplasm and bound to the immunophilin FKBP5, causing no genomic effect. Nevertheless, when GCs are available, the immunophilin FKBP5 is interchanged by the FKBP4, promoting the translocation of the multimeric receptor's complex to the nucleus, promoting active transcription changes either by stimulating or repressing gene expression (Wang and Harris, 2015). On the other hand, the oocyte and embryo sensitivity to GC seems to differ substantially between species. In bovine, a regulated local GC environment seems to be necessary for the reproductive physiology of this species (Acosta et al., 2005; da Costa et al., 2016; Tetsuka and Tanakadate, 2019), although there is a lack of studies that delve into the biological mechanisms behind these findings. In addition, other factors may be involved in the GC actions, including the STAT proteins. The STAT3 and STAT5A have demonstrated roles in transcriptional actions in interaction with NR3C1 (Langlais et al., 2012; Petta et al., 2016), including the regulation of TLR2, involved in the immune actions of the reproductive tract tissues (Ezz et al., 2019). Also, the NR3C1 is closely related to other steroid receptors, such as the mineralocorticoid receptor (NR3C2), the androgen receptor (AR), the progesterone receptors components (PGRMC1 and PGRMC2) and the estrogen receptors (ESR1 and ESR2), all of them having roles in the sex steroid hormone levels during the reproductive cycle (Gibson et al., 2020; Lozovyy et al., 2021; Mukangwa et al., 2020).

Our objective is to describe the GC-related expression changes present in the bovine ampulla and uterus across the stages of the estrous cycle. We hypothesize that the expression of steroid receptors, including *NR3C1* and related genes, is modulated across the estrous cycle, conditioning the physiological dynamic modifications at tissue level occurring in the bovine female reproductive tract. Therefore, GC signaling may influence basal-related changes of the reproductive environment, which might be also relevant for later reproductive events taking place in the oviduct and uterus. For this purpose, we analyzed the mRNA and protein expression changes observed in the ampulla and the endometrium during the pre-ovulatory, post-ovulatory and late luteal phases (stage IV, I and III, respectively) of the bovine estrous cycle. We assessed the mRNA and protein expression of the glucocorticoid receptor gene (*NR3C1)* and the FK506-binding immunophilins (*FKBP4*, *FKBP5*), directly involved in receptor translocation, activation and repression; the mRNA expression of 11β-hydroxysteroid dehydrogenases, involved in GC ligand availability (*HSD11B1*, *HSD11B2*); signal transducers and activators of transcription 3 and 5A (*STAT3*, *STAT5A*), involved in the glucocorticoid receptor signaling and function; the toll-like receptor 2 (*TLR2*); and steroid receptors, including the mineralocorticoid receptor (*NR3C2*), membrane-associated progesterone receptor component 1 and 2 (*PGRMC1*, *PGRMC2*), androgen receptor (*AR*), and estrogen receptor 1 and 2 (*ESR1*, *ESR2*).

2. Materials and methods

2.1. Tissue samples collection

The tissue samples were obtained from crossbreed beef post-pubertal heifers (*Bos taurus*) ($n = 20$) sacrificed in a local slaughterhouse (Escorxador de Sabadell, Barcelona, Spain) for commercial purposes. Animals were classified according to the morphological classification established by Ireland et al. (Ireland et al., 1980) for the different stages of the bovine estrous cycle: stage I (post-ovulatory phase), from Day 1 to 4 ($n = 6$); stage II (early luteal phase), from Day 5 to 10 (not included due to low number of animals); stage III (late luteal phase), from Day 11 to 17 ($n = 8$); stage IV (pre-ovulatory phase), from Day 18 to 20 ($n = 6$). Five tissue fragments (5 mm × 5 mm, each) from the endometrium and ampulla, were obtained by dissection of the reproductive tract. The tissue samples were obtained ipsilateral to the antral follicle or corpus luteum from whole sections of the ampulla and endometrium tissue fragments obtained from intercaruncular areas at the uterine horn base. Follicular fluid was obtained by aspiration of antral follicles. All samples were processed in <1 h and stored at −80 °C in RNAlater ™ (Fisher Scientific, Gothenburg, Sweden), until RNA and protein isolation.

2.2. RNA isolation and measurement

The total RNA extraction was performed using a TRIzol-based protocol previously described (Ruiz-Conca et al., 2020a). In summary, 1 mL TRIzol was added to five tissue fragments (1 mm × 1 mm, each; 80 mg) of each individual sample from the endometrium and the ampulla of every animal included in the study ($n = 20$) before being disrupted mechanically and homogenized (TissueLyser II with 7 mm stainless steel beads; Qiagen, Sollentuna, Sweden). The homogenized tissues were centrifuged at 12000 X *g* for 10 min at 4 °C. Phase separation was conducted by adding 1-Bromo-3-chloropropane and shaking thoroughly before centrifugation (12,000 X *g*, 15 min, 4 °C). Then, 2-propanol and RNA precipitation solution (1.2 M NaCl and 0.8 M $Na_2C_6H_6O_7$) was added and centrifuged (12,000 X *g*, 10 min, 4 °C). Also, 1 mL of 75% ethanol was added to each sample prior to centrifugation (7500 X *g*, 5 min, 4 °C). The RNA pellet was obtained after supernatant discard and 30 min dry in the fume hood. Finally, RNA was diluted in 30 μL of RNase free water.

Subsequently to 30 min on ice after isolation, RNA concentration was assessed spectrophotometrically by measuring the absorbance at 260 nm (NanoDrop™ 2000, Fisher Scientific, Gothenburg, Sweden). The RNA quality was determined using Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). Only samples with RNA integrity values

(RIN) higher than 8.0 were used (Schroeder et al., 2006). The RNA 260/230 and 260/280 absorbance ratios, and the RIN values for each sample are shown in Supplementary Table 1. The synthesis of the cDNA first strand was completed by using the High-Capacity RNA-to-cDNA™ Kit (Fisher Scientific, Gothenburg, Sweden) following the manufacturer's indications. A total of 5 μg RNA was mixed in a final volume of 50 μL for the reverse transcription reaction. All cDNA samples were kept at −20 °C until further analyses.

2.3. Quantitative real-time PCR (qPCR)

Quantitative real-time PCR (qPCR) gene expression was performed using a Real-Time PCR Detection System (CFX96™; Bio-Rad Laboratories, Inc.; Kabelsketal, Germany) following the steps described elsewhere (Ruiz-Conca et al., 2020a). The mRNA relative expression of each sample was calculated using the $2^{-\Delta\Delta CT}$ method for relative quantification described elsewhere (Livak and Schmittgen, 2001). The reactions prepared consisted of 5 μL of PowerUp™ SYBR™ Green Master Mix (Applied Biosystems™, CA, USA), 2 μL cDNA, 0.5 μL of each primer, and in a final volume of 10 μL. The PCR protocol setup performed consisted of uracil-DNA glycosylase activation at 50 °C for 2 min (1 cycle); denaturation at 95 °C for 2 min (1 cycle); denaturation at 95 °C for 5 s and annealing/extension at 60.2 °C for 30 s (40 cycles), and a melting curve at 60–95 °C (0.5 °C increments) for 5 s during each step. Fifteen commercial gene-specific qPCR primers for bovine were used (*G3PDH, NR3C1, FKBP4, FKBP5, HSD11B1, HSD11B2, NR3C2, AR, ESR1, ESR2, PGRMC1, PGRMC2, TLR2, STAT3, STAT5A*; PrimePCR™ SYBR® Green Assay, Bovine; Bio-Rad Laboratories, Inc.; Kabelsketal, Germany). Preliminary, four different housekeeping genes were used for cDNA data normalization (*G3PDH, β-ACTIN, HPRT1, TBP*). The glyceraldehyde-3-phosphate dehydrogenase (*G3PDH*) gene was chosen as the housekeeping gene used for normalization due to the most constant expression showed in the tissue samples included in the study. Two technical replicates were performed for each sample and each primer used. Specific primer sequences belong to the company (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Chromosomal location of the primers, amplicon lengths and design details are shown in Table 1. The amplicons of qPCR were loaded into an agarose gel after mixing with GelRed® Nucleic Acid Gel Stain (Biotium, Fremont, CA, USA) to confirm the product sizes. The gel was imaged by an imaging system (ChemiDoc XRS+ System, BioRad Laboratories, Inc.; Kabelsketal, Germany). All the qPCR plates included No Template Controls (NTC) as a quality control for detecting contamination, non-specific amplification or primer dimer formation.

2.4. Protein extraction and Western blot for FKBP4 and FKBP5

Three tissue fragments (1 mm × 1 mm) from each ampulla and endometrium individual sample of every animal included in the study ($n = 20$) were plunged into a mix of RIPA lysis buffer (Fisher Scientific, Gothenburg, Sweden) containing protease inhibitor and EDTA (Ethylenediaminetetraacetic acid; Thermo Scientific™ Halt™ Proteinase Inhibitor Cocktail (100×); Fisher Scientific, Gothenburg, Sweden). Samples were then homogenized (10 s vortex every 15 min) for 1 h while maintained in ice, prior to centrifugation at 13000 X *g*, 10 min, 4 °C for protein separation. The concentration of proteins was assessed by using a commercial colorimetric protein assay (DC Protein Assay; Bio-Rad Laboratories, Inc.; Kabelsketal, Germany). For Western blot preparation of each sample, 25 μg of protein were mixed with sample buffer (4× NuPAGE LDS; Fisher Scientific, Gothenburg, Sweden) and dithiothreitol (500 mM), and heated at 70 °C for 10 min. Denatured proteins and molecular marker (Odyssey® One-Colour Protein Molecular Weight Marker; LI-COR Biosciences, Inc.; Hamburg, The Netherlands) were then loaded into an 12–15% SDS-polyacrylamide gel and run for electrophoresis (1 h 15 min, 150 V). Separated proteins in gel were transferred (1 h, 100 V) into an activated 0.2 μm polyvinylidene difluoride membrane (PVDF membrane; Fisher Scientific, Gothenburg, Sweden). The membranes were then blocked at room temperature for 1 h (Intercept™ Tris-buffered saline blocking buffer, LI-COR Biosciences, Inc.; Hamburg, The Netherlands), and incubated with rabbit polyclonal antibody anti-FKBP52 (FKBP4) at 1:500 dilution (ab97306; Abcam, Cambridge, UK) or rabbit monoclonal antibody anti-FKBP51 (FKBP5) at 1:1000 dilution (ab126715; Abcam, Cambridge, UK) at room temperature for 2 h. Additionally to the primary antibodies against target proteins, rabbit monoclonal antibody anti-vinculin at 1:5000 dilution (926–42215; LI-COR Biosciences, Inc.; Hamburg, The Netherlands) for 1 h at room temperature, was used as loading control for Western blot normalization. After membranes were incubated for 1 h at room temperature with goat anti-rabbit secondary antibody at 1:10000 dilution (WesternSure® Goat anti-Rabbit HRP Secondary Antibody, 926–80011; LI-COR Biosciences, Inc.; Hamburg, The Netherlands). Finally, the membranes were incubated with chemiluminescent substrate mix for 5 min (WesternSure® PREMIUM Chemiluminescent Substrate, 926–95000, LI-COR Biosciences, Inc.; Hamburg, The Netherlands) and scanned with C-DiGit™ Blot Scanner (LI-COR Biosciences, Inc.; Hamburg, The Netherlands). Western blot bands normalization and quantification was performed by using Image Studio Lite software version 5.2.5 (LI-COR, Biosciences, Inc.; Hamburg, The Netherlands).

Table 1
Primers used for gene expression qPCR analysis.

Gene	Chromosome location	Product size (bp)	Splice variants targeted	Primer design
G3PDH	5:104239749–104241657	92	ENSBTAT00000037753	Intron-spanning
NR3C1	7:56236814–56247471	85	ENSBTAT00000025941	Intron-spanning
FKBP4	5:107449938–107450858	119	ENSBTAT00000009998	Intron-spanning
FKBP5	23:9522465–9522614	120	ENSBTAT00000064387	Exonic
HSD11B2	18:35163620–35163719	70	ENSBTAT00000007470	Exonic
HSD11B1	16:75465890–75506713	120	ENSBTAT00000020078	Intron-spanning
NR3C2	17:9743910–9953168	109	ENSBTAT00000003291	Intron-spanning
ESR1	9:90220286–90250637	112	ENSBTAT00000009422	Intron-spanning
ESR2	10:76757251–76757370	90	ENSBTAT00000005899	Exonic
PGRMC1	X:3476798–3476910	83	ENSBTAT00000026053	Exonic
PGRMC2	17:29888146–29889663	81	ENSBTAT00000014390	Intron-spanning
AR	X:88418395–88425354	116	ENSBTAT00000030067	Intron-spanning
TLR2	17:3950948–3951081	104	ENSBTAT00000010530	Exonic
STAT3	19:43063874–43065290	114	ENSBTAT00000028687	Intron-spanning
STAT5A	19:43047935–43048136	65	ENSBTAT00000034831	Exonic

bp: base pair; *G3PDH*: glyceraldehyde-3-phosphate dehydrogenase; *NR3C1*: nuclear receptor subfamily 3 group C member 1; *FKBP4*: FK506-binding prolyl isomerase 4; *FKBP5*: FK506-binding prolyl isomerase 5; *HSD11B1*: hydroxysteroid 11-beta dehydrogenase 1; *HSD11B2*: hydroxysteroid 11-beta dehydrogenase 2; *NR3C2*: nuclear receptor subfamily 3 group C member 2; *ESR1*: estrogen receptor 1; *ESR2*: estrogen receptor 2; *PGRMC1*: progesterone receptor membrane component 1; *PGRMC2*: progesterone receptor membrane component 2; *AR*: androgen receptor; *STAT3*: signal transducer and activator of transcription 3; *STAT5A*: signal transducer and activator of transcription 5A.

2.5. Competitive ELISA for protein quantitative determination of NR3C1

Competitive ELISA (Bovine Glucocorticoid Receptor (NR3C1) ELISA Kit; MBS7263720; MyBiosource; San Diego, CA, USA) was used for quantitative determination of NR3C1 protein abundance. Briefly, three tissue fragments (1 mm × 1 mm, each; 30–50 mg) from each sample were weighted and homogenized (10 s vortex every 15 min) in 250 μL of a mix of RIPA lysis buffer (Fisher Scientific, Gothenburg, Sweden) containing protease inhibitor and EDTA (Ethylenediaminetetraacetic acid; Thermo Scientific™ Halt™ Proteinase Inhibitor Cocktail (100×); Fisher Scientific, Gothenburg, Sweden) and centrifugated at 13000 X *g* for 15 min, 4 °C. Then, 100 μL of protein extracts from each sample were pipetted into the wells of a pre-coated plate, and 100 μL of balance solution and 50 μL of enzyme conjugate (except the blank) were added. The plate was incubated in the dark for 1 h at 37 °C. After incubation, the wells were decanted and washed prior to be incubated with a substrate for the HRP enzyme. Finally, after 15 min at 37 °C, an acid solution was added to stop the enzyme-substrate reaction. The absorbance values were spectrophotometrically measured at 450 nm using a microplate reader (Sunrise-147 Basic Tecan; Tecan Austria GmbH, Grödig, Austria). The NR3C1 concentration of each sample was interpolated from a standard curve. This standard curve was plotted using the absorbance measured in different dilutions of standard samples of known concentrations within the detection range of the kit. Moreover, a weight-normalization was performed to express the concentration of NR3C1 protein per total concentration of protein extracted. All samples and standards were assayed in duplicate. The precision within the test was assessed by calculating coefficients of variation (CV, where CV = SD/mean × 100) from duplicate samples. The CV for NR3C1 was 3.23% and a sensitivity of 0.1 ng/mL was obtained. No significant cross-reactivity or interference between NR3C1 and analogues has been described by the kit manufacturer.

2.6. Progesterone and estradiol measurement in follicular fluid

Follicular fluid was obtained by aspiration of healthy antral follicles visible in the ovarian cortex using an 18 G needle with a 10 mL syringe. After collection, follicular fluid samples were centrifuged at 5000 X *g* for 10 min and stored at −20 °C until being analyzed. Progesterone (P4) and estradiol (E2) levels on follicular fluid were measured by means of enzyme immunoassay (EIA) following protocols previously described by our group (Maya-Soriano et al., 2013). Commercial kits were used for hormonal determination (Progesterone ELISA KIT and Estradiol ELISA KIT; Neogen Corporation, Ayr, UK; estimated sensitivity of 0.23 ng P4/mL and 0.012 ng E2/mL). Manufacturer's indications estimated that cross-reactivity (>0.02%) of the commercial P4 antibody with other steroid hormones was 2.5% for deoxycorticosterone, 2.0% for corticosterone, 2.0% for pregnenolone, 1.0% for 4-androstenedione, 0.4% for 17-hydroxyprogesterone, 0.29% for testosterone, 0.2% for cortisol, 0.2% for cortisone, 0.2% for dehydroepiandrosterone, 0.2% for E2, and 0.2% for estrone. Manufacturer's indications estimated that cross-reactivity (>0.02%) of the commercial E2 antibody with other steroid hormones was 1.0% for testosterone, 0.41% for estriol, and 0.1% for estrone.

2.7. Statistical analysis

The software CFX Maestro™ 1.1 version 4.1.2433.1219 (Bio-Rad Laboratories, Inc.; Kabelsketal, Germany) was used for the complete genetic data analysis and Image Studio software version 5.2.5 (LI-COR, Biosciences, Inc.; Hamburg, The Netherlands) was used for the Western blot analyses. The statistical analysis was performed in R software version 3.6.1 (R Core Team, 2019). Normal distribution and homoscedasticity were checked using the Shapiro–Wilk Normality test and Levene's test, while log(x + 1) was used for data transformation into normal distribution. The packages *nlme* (Pinheiro and Bates, 2011) and *multcomp* (Hothorn et al., 2008) were used to perform linear mixed effects models (LME), and to conduct pairwise comparisons adjusted by Tukey's test, respectively. The estrous cycle stages (stage I, III and IV) were considered as the fixed factor of the LME, and the samples were included in the random part of the LME. Stage I (post-ovulatory phase) was set as an arbitrary reference for relative gene expression. Comparisons between specific pairs of genes (*HSD11B1/HSD11B2* and *FKBP4/FKBP5*) was conducted using *t*-test when data showed normal distribution, and Mann-Whitney *U* test for non-normal data. HSD11B1 and FKBP4, respectively, were set as arbitrary references for relative gene expression of each specific pair of genes. Data are presented as mean ± standard error of the mean (SEM). The significance threshold was established at $p < 0.05$. Correlation of the qPCR expression data was analyzed using multiple Spearman's rank correlation coefficient to find the connection between the relative fold change of each studied gene, and also with the follicular fluid concentrations of E2 and P4. Relative protein expression analyses of NR3C1, FKBP4 and FKBP5 were conducted by performing one-way ANOVA test, followed by Tukey's multiple comparisons test. Data are presented as mean ± SEM. The significance threshold was established at $p < 0.05$. Additionally, the correlations between relative mRNA levels and relative protein expression of each sample were calculated using multiple Spearman's rank correlation coefficient.

3. Results

3.1. Ampullary and endometrial mRNA expression in the pre-ovulatory phase vs. post-ovulatory phase (stage IV vs stage I)

The observed ampullary mRNA expression levels of *NR3C1, ESR1,* and *AR* were significantly higher ($p < 0.05$) in stage I, compared to stage IV (Fig. 1). Regarding endometrium, we found that the mRNA expression of *HSD11B2* was significantly lower ($p < 0.05$) in stage I than in stage IV (Fig. 2).

3.2. Ampullary and endometrial mRNA expression in the post-ovulatory phase vs. late luteal phase (stage I vs stage III)

The observed ampullary mRNA expression level of *NR3C2* in stage III was significantly higher ($p < 0.05$) in comparison to stage I. Also, mRNA expression level of *NR3C1*, *FKBP4*, *FKBP5*, *HSD11B1*, *HSD11B2, ESR1*, *AR,* and *PGRMC2* ($p < 0.05$) was higher in stage I, than in stage III in the ampulla (Fig. 1). In the endometrium, the mRNA expression level of *FKBP4, NR3C2, HSD11B2, ESR1, ESR2, PGRMC1, PGRMC2, TLR2,* and *STAT3* in stage III, was significantly higher ($p < 0.05$) in comparison to stage I. Also, mRNA *HSD11B1* expression observed in stage III was significantly lower ($p < 0.05$) than in stage I in the endometrium (Fig. 2).

3.3. Ampullary and endometrial mRNA expression in the late luteal phase vs. pre-ovulatory phase (stage III - stage IV)

In the ampulla, during stage IV, we observed higher mRNA expression level of *NR3C1*, *FKBP4*, *FKBP5*, *ESR1*, *AR,* and *PGRMC2* ($p < 0.05$) compared to stage III. In the same tissue, we found in stage IV lower ($p < 0.05$) mRNA expression of *NR3C2*, *TLR2,* and *STAT5A,* when compared to late diestrus (stage III) (Fig. 1). In the endometrium, higher mRNA expression level of *HSD11B1* in stage IV was described, when compared to stage III ($p < 0.05$). Also, our results show lower ($p < 0.05$) mRNA expression of *NR3C2*, *HSD11B2*, *ESR1*, *ESR2*, *PGRMC1,* and *STAT3* in stage IV compared to stage III in the endometrium (Fig. 2).

3.4. Ampullary and endometrial mRNA expression of HSD11B1 vs. HSD11B2 during each estrous cycle stage

In the ampulla (Fig. 3; A-C), higher mRNA expression ($p < 0.05$) of *HSD11B2* was found during stage I, stage III and stage IV compared to

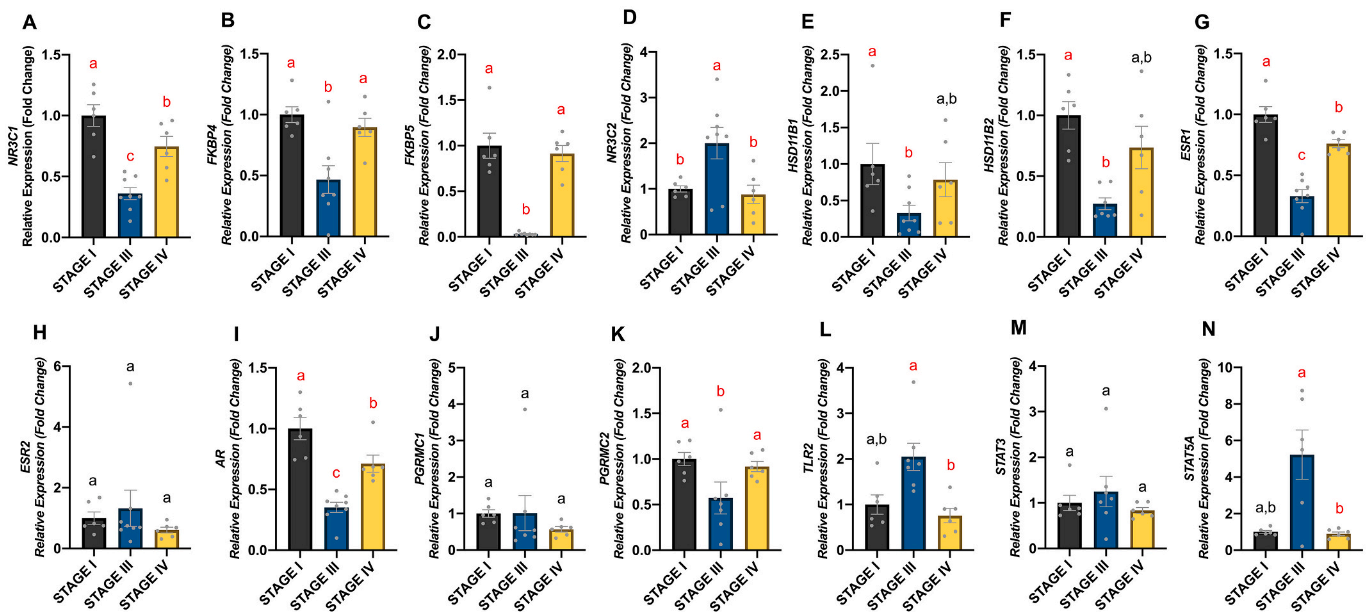

Fig. 1. Relative gene expression (fold change) of the target genes (A: *NR3C1*; B: *FKBP4*; C: *FKBP5*; D: *NR3C2*; E: *HSD11B1*; F: *HSD11B2*; G: *ESR1*; H: *ESR2*; I: *AR*; J: *PGRMC1*; K: *PGRMC2*; L: *TLR2*; M: *STAT3*; N: *STAT5A*) in the ampulla of each individual, comparing stage I (black) vs. stage III (blue) vs. stage IV (yellow); (mean ± SEM). Stage I was set as the reference group. Significant differences are represented by different letters (red; $p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

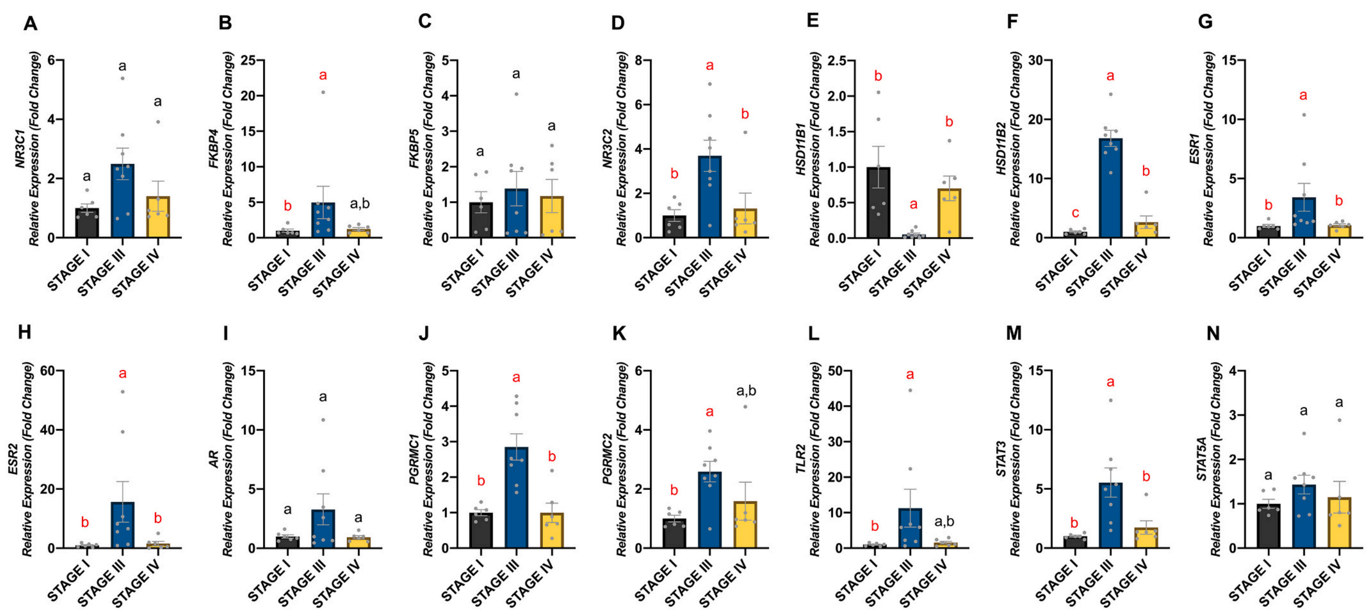

Fig. 2. Relative gene expression (fold change) of the target genes (A: *NR3C1*; B: *FKBP4*; C: *FKBP5*; D: *NR3C2*; E: *HSD11B1*; F: *HSD11B2*; G: *ESR1*; H: *ESR2*; I: *AR*; J: *PGRMC1*; K: *PGRMC2*; L: *TLR2*; M: *STAT3*; N: *STAT5A*) in the endometrium of each individual, comparing stage I (black) vs. stage III (blue) vs. stage IV (yellow); (mean ± SEM). Stage I was set as the reference group. Significant differences are represented by different letters (red; $p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

HSD11B1. In the endometrium (Fig. 3; D—F), *HSD11B2* mRNA expression was higher in stage III, but lower in stage I and IV, when compared to *HSD11B1* mRNA expression ($p < 0.05$).

3.5. Ampullary and endometrial mRNA expression of FKBP4 vs. FKBP5 during each estrous cycle stage

In the ampulla, higher mRNA expression of *FKPB5* ($p < 0.05$) compared to *FKBP4* (Fig. 4; A-C) was observed during stage I and stage IV. On the other hand, in stage III we detected lower mRNA expression levels of *FKPB5* ($p < 0.05$) compared to *FKBP4*. In the endometrium (Fig. 4; D—F), lower *FKBP5* during stage III was found when compared to *FKBP4* ($p < 0.05$), while no significative changes were detected during stage IV and stage I.

3.6. Correlations between mRNA expression and P4 and E2 concentrations

The P4 concentration measured in follicular fluid was 119.69 ± 42.81 ng/mL in stage I; 153.11 ± 95.20 ng/mL in stage III; and 194.49

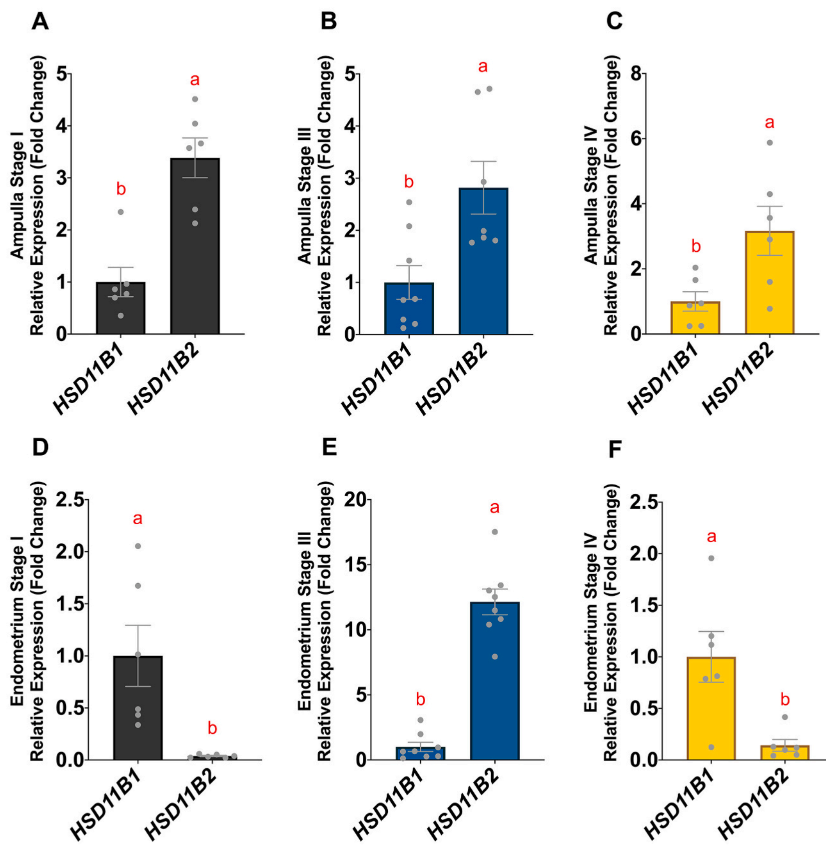

Fig. 3. Relative gene expression (fold change) of *HSD11B1* (green) and *HSD11B2* (red), in the ampulla (A, B, C) and endometrium (D, E, F) during stage I (A, D), stage III (B, E) and stage IV (C, F) of the bovine estrous cycle; (mean ± SEM). *HSD11B1* mRNA expression was used as a reference for relative *HSD11B2* expression. Significant differences ($p < 0.05$) are represented by different letters (red). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

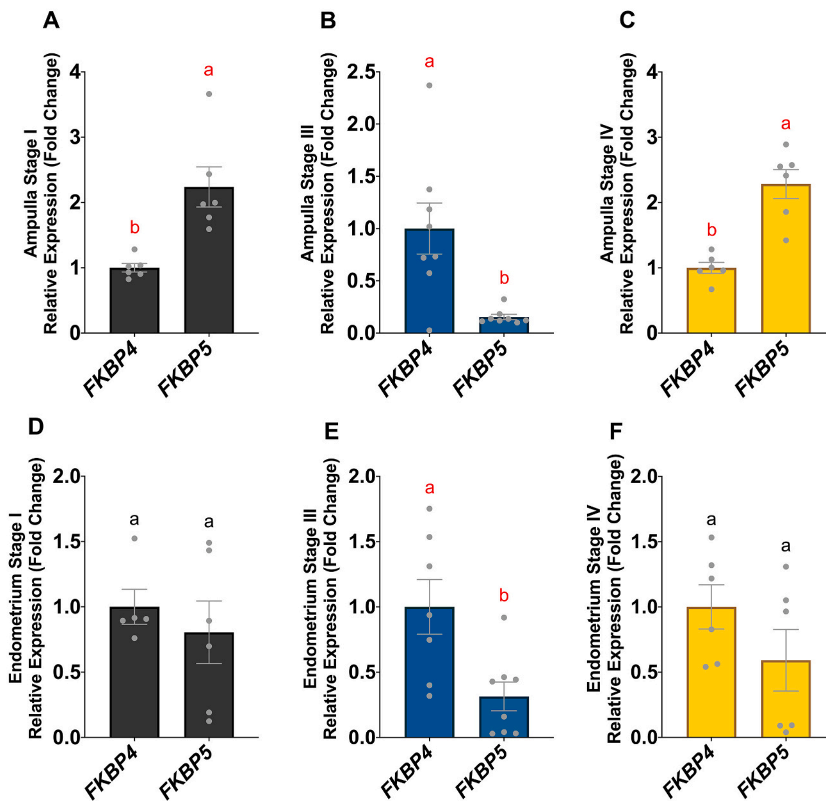

Fig. 4. Relative gene expression (fold change) of *FKBP4* (blue) and *FKBP5* (purple), in the ampulla (A, B, C) and endometrium (D, E, F) during stage I (A, D), stage III (B, E) and stage IV (C, F) of the bovine estrous cycle; (mean ± SEM). *FKBP4* mRNA expression was used as a reference for relative *FKBP5* expression. Significant differences ($p < 0.05$) are represented by different letters (red). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

± 78.50 ng/mL in stage IV (mean ± SD). Regarding E2, concentrations of 42.15 ± 32.86 ng/mL were observed for stage I; 56.59 ± 72.44 ng/mL for stage III, and 110.32 ± 90.65 ng/mL for stage IV (mean ± SD). Additionally, progesterone/estradiol (P4/E2) ratios were calculated at stage I (6.92 ± 5.94), stage III (11.46 ± 14.1), and stage IV (6.30 ± 10.1).

One-to-one correlations with each mRNA and follicular fluid hormonal concentrations were performed in the ampulla (Fig. 5, A-C) and in the endometrium (Fig. 5, D—F). The highest number of positive and negative significant correlations were found in stage I. During this stage, in the ampulla, *NR3C1* and a high number of genes, including *ESR1*, *AR*, *PGRMC1*, *PGRMC2*, *STAT3*, *STAT5A*, were positively correlated with the P4/E2 ratio, while negatively correlated with E2, *NR3C2*, *ESR2* and *HSD11B1*. In stage III, *ESR2* was correlated with *NR3C1*, in contrast to *ESR1*, that was negatively correlated to *NR3C1* mRNA. Also, *HSD11B1* and *HSD11B2* were positively correlated with *NR3C1* in both stage III and stage IV. In the endometrium, during stage I, a positive correlation was found between *NR3C1* and *HSD11B1*, *HSD11B2*, *PGRMC2*, *NR3C2*, *AR*, *PGRMC1*, *PGRMC2*, while the *NR3C1* mRNA negatively correlated with *FKBP4*, *FKBP5*, *STAT3* and *ESR2*. Interestingly, both *FKBP4* and *FKBP5* were positively correlated with *NR3C1* in the ampulla during stage I, and in the endometrium, both were also positively correlated during stage III. Moreover, a negative correlation was found between *ESR1* and *ESR2* in the ampulla, irrespectively to the estrous cycle phase. In the endometrium, a positive correlation was found between *PGRMC1* and *PGRMC2*, also in all the stages. Finally, E2 was negatively correlated to *STAT5A* in stage I and stage IV in both tissues, whereas this correlation was positive for stage III. P4/E2 ratio and *PGRMC1* were negatively correlated in both tissues during stage III, but positively correlated during stage IV.

3.7. Endometrial and ampullary protein expression of NR3C1 during each estrous cycle stage

In the ampulla, we did not detect significant differences ($p < 0.05$) in the NR3C1 protein expression between stages (Fig. 6; A). In the endometrium, we found that NR3C1 levels were significantly higher ($p < 0.05$) in stage IV, compared to stage I and III (Fig. 6; B).

3.8. Correlations between NR3C1 protein abundance and mRNA expression levels

The NR3C1 protein expression quantified in the ampulla showed significant positive correlation with the mRNA relative expression (Spearman's rank correlation coefficient = 0.51; p = 0.039). In the endometrium, correlation between the protein expression levels detected and mRNA levels was not significant (Spearman's rank correlation coefficient = −0.16; p = 0.528) (Fig. 7; A,B).

3.9. Endometrial and ampullary protein expression of FKBP4 and FKBP5 during each estrous cycle stage

The protein expression levels of FKBP4 (FKBP52) observed in the ampulla were significantly higher ($p < 0.05$) in stage I, compared to stage III (Fig. 8; A,B). Regarding endometrium, we found that the protein expression of FKBP4 was significantly lower ($p < 0.05$) in stage I than in stage III (Fig. 8; C,D). For FKBP5 (FKBP51), the ampullary protein expression levels observed in stage III were significantly lower ($p < 0.05$) in comparison to stage I (Fig. 9; A,B). In the endometrium, we did not find significant differences ($p < 0.05$) in the FKBP5 protein expression between stages (Fig. 9; C,D).

3.10. Correlations between FKBP4 and FKBP5 protein abundance and their mRNA expression levels

The relative FKBP4 protein expression detected in both the ampulla and the endometrium, showed significant positive correlation with their respective ampullary (Spearman's rank correlation coefficient = 0.52; p = 0.024) and endometrial (Spearman's rank correlation coefficient = 0.49; p = 0.046) mRNA relative expression levels (Fig. 7; C,D). Regarding the relative protein expression of FKBP5 levels, we also found a significant positive correlation with their respective mRNA relative expression levels both in the ampulla (Spearman's rank correlation coefficient = 0.57; p = 0.011) and the endometrium (Spearman's rank correlation coefficient = 0.50; p = 0.026) (Fig. 7; E,F).

4. Discussion

Glucocorticoids (GCs), and also P4 and estrogen levels, modulate the female reproductive tract during the estrous cycle (Whirledge and Cidlowski, 2017). Our results confirm the presence of changes on the GC-related expression during the post-ovulatory phase (stage I) in the ampulla, including an increase in the mRNA expression of *NR3C1*, the main mediator of the GCs actions, and other related genes involved in the GC actions (*HSD11B1*, *HSD11B2, FKBP4* and *FKBP5)*. In contrast, endometrial mRNA expression changes were predominantly observed during the late luteal phase (stage III), including lower expression of *HSD11B1* and higher expression in *HSD11B2* and *NR3C2*.

We found that, at the mRNA level, the glucocorticoid receptor *NR3C1* expression is progressively increasing in the ampulla, being higher in the post-ovulatory stage. In this sense, we detected that there is a promotion of *NR3C1* mRNA expression in the bovine ampulla after ovulation, coinciding in time with the oocyte presence in the oviduct (Siemieniuch et al., 2010). This increase on *NR3C1* during the post-ovulatory phase can be linked to the local GC environment surrounding the bovine oocyte that is promoted during the oocyte maturation and transport through the oviduct in this species (Tetsuka and Tanakadate, 2019). In this sense, the GCs may have an influence on the oocytes that can be considerably despair depending on the species (Gong et al., 2017; Scarlet et al., 2017; Yang et al., 1999), but seems to be positive for the bovine oocytes (da Costa et al., 2016) and embryos (Santana et al., 2014). Moreover, during the late luteal phase, when the oocyte is no longer in the oviduct and high levels of P4 are present in the reproductive tract (Stevenson and Lamb, 2016), we observed a decrease in the ampullary *NR3C1* mRNA expression, which can be related to the avoidance of the deleterious effects produced by a prolonged exposure to GC (Okret et al., 1986; Rosewicz et al., 1988), as cortisol can be produced from P4 by the action of steroidogenic enzymes (Amweg et al., 2017). In contrast to our results in mRNA, we did not detect differences in the NR3C1 protein expression across the different stages of the estrous cycle in the ampulla. Discrepancies in mRNA and protein results are common and can be explained by post-transcriptional, post-translational, and protein-degradational regulatory mechanisms (Vogel and Marcotte, 2012). In the ampulla, where mRNA expression differences were found, but not at the protein expression level, discrepancies may be explained by post-transcriptional regulatory mechanisms, such as the control of the mRNA half time and decay (Basu et al., 2021; Schoenberg and Maquat, 2012), post-translational regulatory mechanisms, such as variations in the initiation site of translation, and modifications (Livingstone et al., 2010), including ubiquitination, phosphorylation, glycosylation and/or SUMOylation, which can produce a vast number of NR3C1 protein isoforms (Druker et al., 2013; Duma et al., 2006; Oakley and Cidlowski, 2011; Tian et al., 2002), that may be playing particular roles in the GC function in reproduction (Čikoš et al., 2019). Another plausible explanation may rely on compensatory mechanisms, as evolutionary selection for constant protein levels has been shown to be greater than selection for constant mRNA levels (Khan et al., 2013). However, ampullary NR3C1 mRNA and protein abundances were

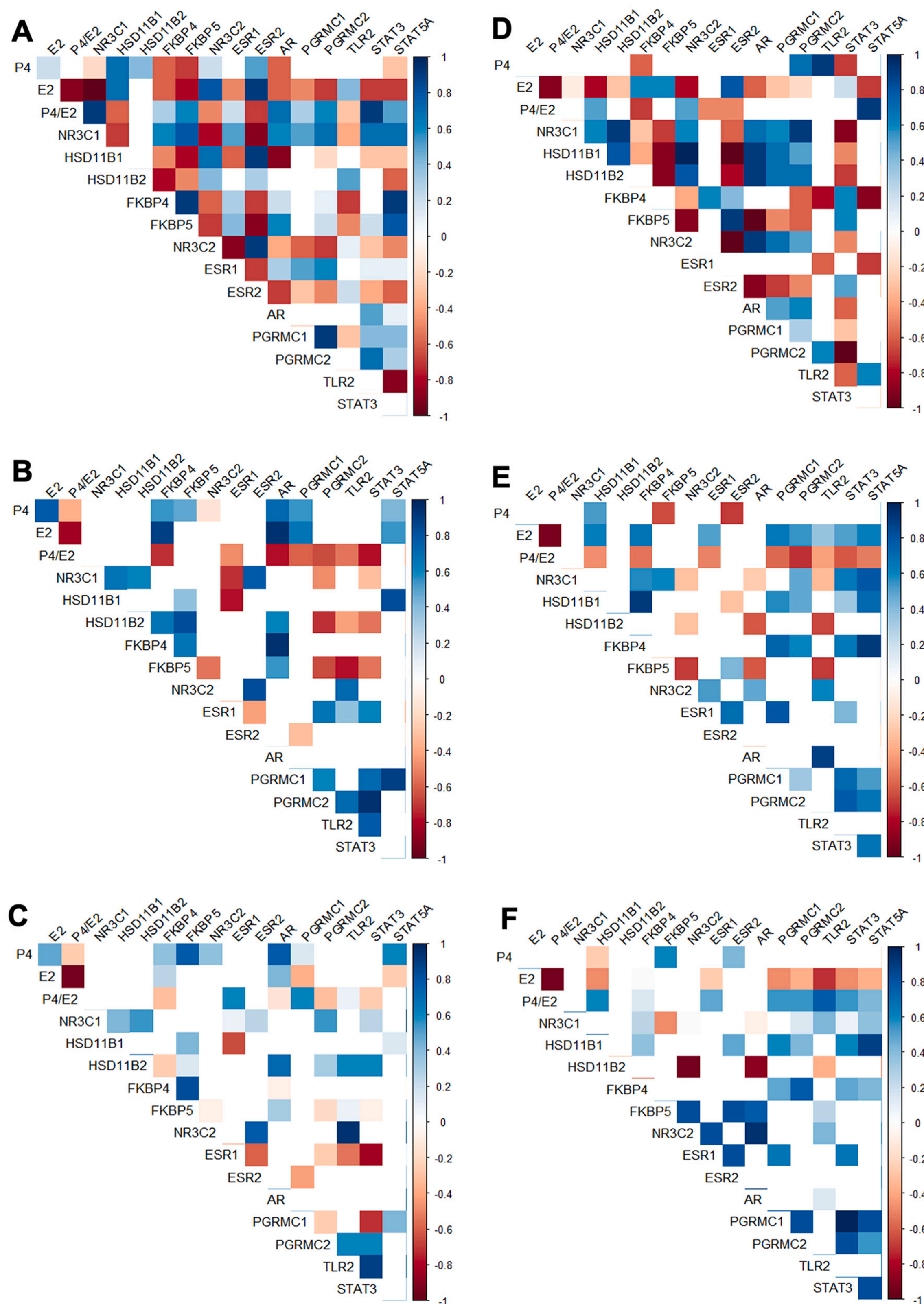

Fig. 5. mRNA relative expression one-to-one correlations (Spearman rank) with each transcript, and with follicular fluid estradiol (E2) and progesterone (P4) concentrations, in the ampulla (A-C) and the endometrium (D—F). Different stages of the estrous cycle are represented as follows: stage I (A, D), stage III (B, E), and stage IV (C, F). Significant positive correlations are shown as blue squares for each pair of genes or hormones ($p < 0.05$), and significant negative correlation coefficients are shown as red squares for each pair of genes or hormones ($p < 0.05$). Non-significant correlations are shown as empty squares. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

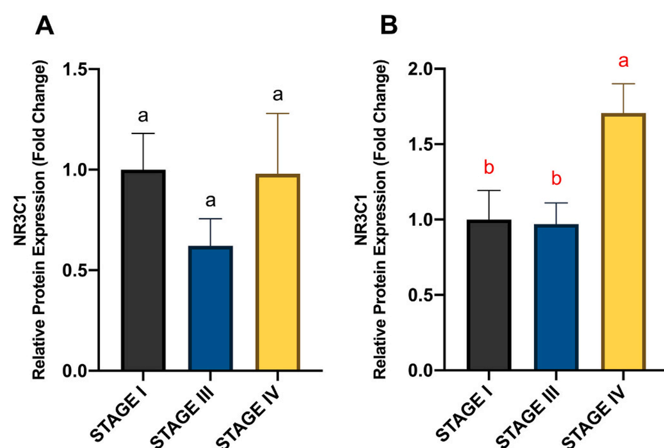

Fig. 6. Relative protein expression (fold change) of NR3C1 in the ampulla (A) and endometrium (B) comparing stage I (black) vs. stage III (blue) vs. stage IV (yellow); (mean ± SEM). Stage I was set as the reference group. Significant differences are represented by different letters (red; $p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

correlated (51% of the variation in protein abundances can be explained by knowing mRNA levels).

In the endometrium, we did not detect mRNA differences between stages, but an increased expression was found at protein level during the pre-ovulatory phase (stage IV). The presence of NR3C1 in the endometrium, and its regulation during pregnancy has been described in bovine and ovine (Kuse et al., 2013; Simmons et al., 2010), and cortisol production in this tissue has been suggested during this stage (Simmons et al., 2010). In the endometrium, where we found non-correlated constant mRNA and differences in the protein levels, divergences may be attributed to the heterogeneity of mRNA translation (Sonneveld et al., 2020), including translational rate changes under different conditions, such as the different stages of the estrous cycle. This process could also be modulated by the presence of micro RNAs and short interference RNAs, which can inhibit mRNA translation (Valencia-Sanchez et al., 2006). Moreover, the possibility of mRNA or protein degradation should not be discarded. In fact, the mRNA and protein levels of genes involved in response to an stimulus, including many fast responding genes and transcription factors, as in the case of *NR3C1*, have been described to be particularly unstable and have short half-lives (Schwanhausser et al., 2011). In this sense, future studies including sequencing the nascent transcripts (Wissink et al., 2019) and ribosome profiling (Brar and Weissman, 2015) may delve into these questions.

The availability of GCs binding to NR3C1 is controlled by the HSD11B1 and HSD11B2 enzymes (Chapman et al., 2013; Michael et al., 2003). The *HSD11B1* gene encodes for a bidirectional reductase that converts cortisone metabolites into active cortisol, and the *HSD11B2* does it for an oxidase that catalyzes the opposite reaction (Michael et al., 2003). Both enzymes, HSD11B1/2, have been described in the cattle ovary (Amweg et al., 2013; Tetsuka et al., 2010; Tetsuka et al., 2003), but evidences of their expression in the oviduct remained scarce so far (Siemieniuch et al., 2010; Tetsuka and Tanakadate, 2019). We found expression of both *HSD11B1* and *HSD11B2* in the oviduct ampulla at the mRNA level. Moreover, we detected a decrease in their expression during the luteal phase, compared to what occurs at post-ovulation, indicating tight regulation of the GC availability during the bovine oocyte transport in the oviduct. These enzymes are not equally present across mammalian species, and the presence/absence of HSD11B1 or HSD11B2 may trigger an unequal sensitivity to the deleterious effects of GC on the oocytes and embryos during the initial steps of reproduction (Gong et al., 2017; Yang et al., 1999). In humans, the fallopian tube displays only the HSD11B2-mediated cortisol conversion to cortisone, but not HSD11B1 (Muneyyirci-Delale et al., 2005). In contrast, in bovine, the HSD11B1 is expressed in the cumulus cells, while HSD11B2 seems to be restricted to the oocyte (Tetsuka et al., 2016; Tetsuka and Tanakadate, 2019). In this sense, HSD11B1-mediated promotion of GC creation in the cumulus cells have positive effects for the bovine oocyte maturation and early embryo development (da Costa et al., 2016; Santana et al., 2014; Tetsuka and Tanakadate, 2019). Interestingly, we observed ampullary 3-fold higher *HSD11B2* mRNA levels compared to the *HSD11B1* levels in all the stages, which may be related to the sensitivity of oocytes to GCs (Gong et al., 2017; Scarlet et al., 2017; Yang et al., 1999) raising questions regarding the oviduct contribution to the GC regulation. Moreover, the *HSD11B1* expression is promoted in the endometrium at the pre- and post-ovulatory phases, when compared to the late luteal phase, suggesting a role of cortisol in preventing excessive uterine prostaglandin production during the pre-ovulatory phase (Lee et al., 2007). On the other hand, during the late luteal phase, cortisol inactivation may be driven by the *HSD11B2* higher expression and *HSD11B1* lower expression that we detected in the endometrium at this stage.

Furthermore, an increased expression of the mineralocorticoid receptor (*NR3C2*) was observed in the endometrium at the late luteal phase. The NR3C2 is a promiscuous receptor that has a higher affinity for cortisol than for other hormones, such as aldosterone (Baker et al., 2013). GCs limitation by the HSD11B2 may allow mineralocorticoids to bind NR3C2 (Chapman et al., 2013). Thus, the NR3C2 signaling is directly implied in the sensitivity of NR3C1 to GCs both by ligand competition, and by modulation of other factors involved in NR3C1, such as the FKBP immunophilins (Hartmann et al., 2021). The NR3C1 regulation is driven also by the immunophilins FKBP5 and FKBP4 (Ratajczak et al., 2015). The FKBP5, together with other co-factors, is bound to the cytosolic inactive receptor multimeric complex, and it is interchanged by FKBP4 after GC ligands are bound to NR3C1 (Wochnik et al., 2005). Thereafter, FKBP4 recruits the dynein motor protein, supporting the translocation of NR3C1 to the nucleus, where gene transcription is promoted (Davies et al., 2002). NR3C1-mediated expression of *FKBP5* is then activated, reducing the affinity of the receptor for GCs, therefore controlling the response to GC (Ratajczak et al., 2015; Wochnik et al., 2005). We detected higher mRNA expression in the ampulla of both *FKBP4* and *FKBP5* during the pre- and post-ovulatory phases. Thus, FKBP4-mediated translocation of NR3C1 to the nucleus may occur, inducing the activation of *FKBP5*, which was more expressed than *FKBP4* in the pre- and post-ovulatory phases, indicating increased regulation of the NR3C1 actions when the oocyte is being transported throughout the oviduct, compared to the luteal phase. The increased levels of *FKBP5* detected in the ampulla may help to reduce the sensitivity to the deleterious effects of cortisol, similar to what occurs at systemic level in New-World monkeys compared to Old-World species (Scammell et al., 2001; Westberry et al., 2006). At the protein level, the ampullary FKBP5 and FKBP4 were also found decreased during the late luteal phase, but there were no differences regarding the pre-ovulatory phase. These slight discrepancies may be explained by the rates of protein degradation, post-transcriptional and post-translational mechanisms, or issues related to the sensitivity of the protein detection. In addition, we found a correlation between both parameters since 52% and 57% of the variation in FKBP4 and FKBP5 protein abundances, respectively, can be explained by the detected mRNA levels in the ampulla (Vogel and Marcotte, 2012). In the endometrium, *FKBP4* observed expression, but not *FKBP5*, was higher during the late luteal phase, both at mRNA and protein level. These results agree with previous findings in mice regarding the crucial role of FKBP4 expression on uterine receptivity, being knockout mutants completely infertile (Tranguch et al., 2005). In this case, mRNA and protein levels displayed similar differences and their levels were also correlated for both FKBP4 and FKBP5 (49% and 50% of the variation in protein abundances explained by mRNA, respectively) in the endometrium (Vogel and Marcotte, 2012).

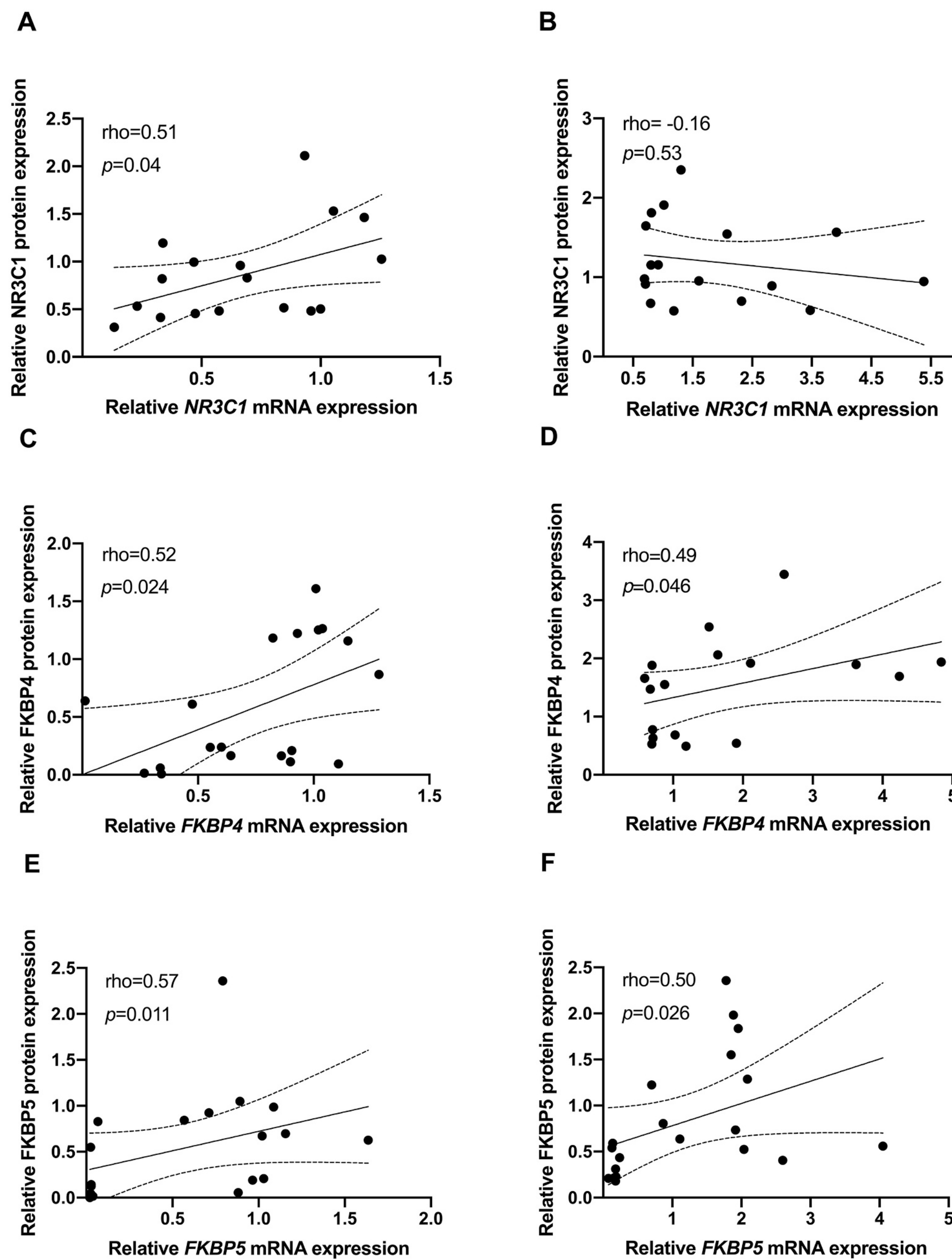

Fig. 7. Correlation (Spearman's rank) between protein relative expression and mRNA relative expression of NR3C1 (A,B), FKBP4 (C,D) and FKBP5 (E,F) in each sample of ampulla (A,C,E) and endometrium (B,D,F) in stage I, III and IV. Spearman's rank correlation coefficient (rho) shows the strength of the correlations. Scatter plot and linear regression (line) with 95% confidence interval (dashed line) is represented in each plot and shows the direction of the correlations. The *p* value represents the significance of the correlations ($p < 0.05$).

The STATs, relevant in the Janus kinase (JAK)/STAT inflammatory signaling pathways, are important in the GCs signaling since they are directly involved in the transcriptional activity of the NR3C1 complex when translocated to the nucleus (Langlais et al., 2012; Newton et al., 2017). STAT3 is critical for the embryo inner cell mass development (Meng et al., 2015) and the endometrial adherence and trophoblastic invasion (Marconato et al., 2012). It is also activated by potent anti-inflammatory cytokines (Hedl et al., 2019), having a role in the decrease of inflammatory responses (Petta et al., 2016). Our results presented an increase in the endometrium during the late luteal phase, which may correspond to avoidance of potential inflammatory damage. Regarding STAT5A, which is related to inflammatory cytokines, T-cell differentiation, reproduction and pregnancy (Bednorz et al., 2011; Maj and Chelmonska-Soyta, 2007), we observed a decreased expression during the pre-ovulatory phase, which might contribute to the promotion of tolerance towards spermatozoa in the ampullar region. Moreover, while STAT3 stimulates the transcriptional activity of NR3C1, AR and ESR, STAT5A is involved in NR3C1 recruitment and transcriptional synergism (Langlais et al., 2012; Petta et al., 2016), together with the NF-κB cells, and other factors, enhancing TLR2 expression (Hermoso et al., 2004). We observed an increase in *TLR2* mRNA during the late luteal phase that may contribute to the creation of an adequate uterine environment by polymorphonuclear neutrophil activation, which is needed for protection against pathogens and remaining sperm removal (Alderton, 2012; Ezz et al., 2019). In this context, we detected decreased *TLR2* levels during the pre-ovulatory phase vs. the late luteal phase in the ampulla, which might be associated to lower neutrophil activity prior to ovulation on this region.

Sex steroids, androgen receptor (*AR*) and estrogen receptor 1 (*ESR1*), mRNA expressions in the ampulla were observed increased in the pre-ovulatory phase. Higher levels were detected during the post-ovulatory phase, decreasing during the late luteal phase. This findings differ from previous findings that described stable expression of estrogen and progesterone receptors in the ampulla across the bovine estrous

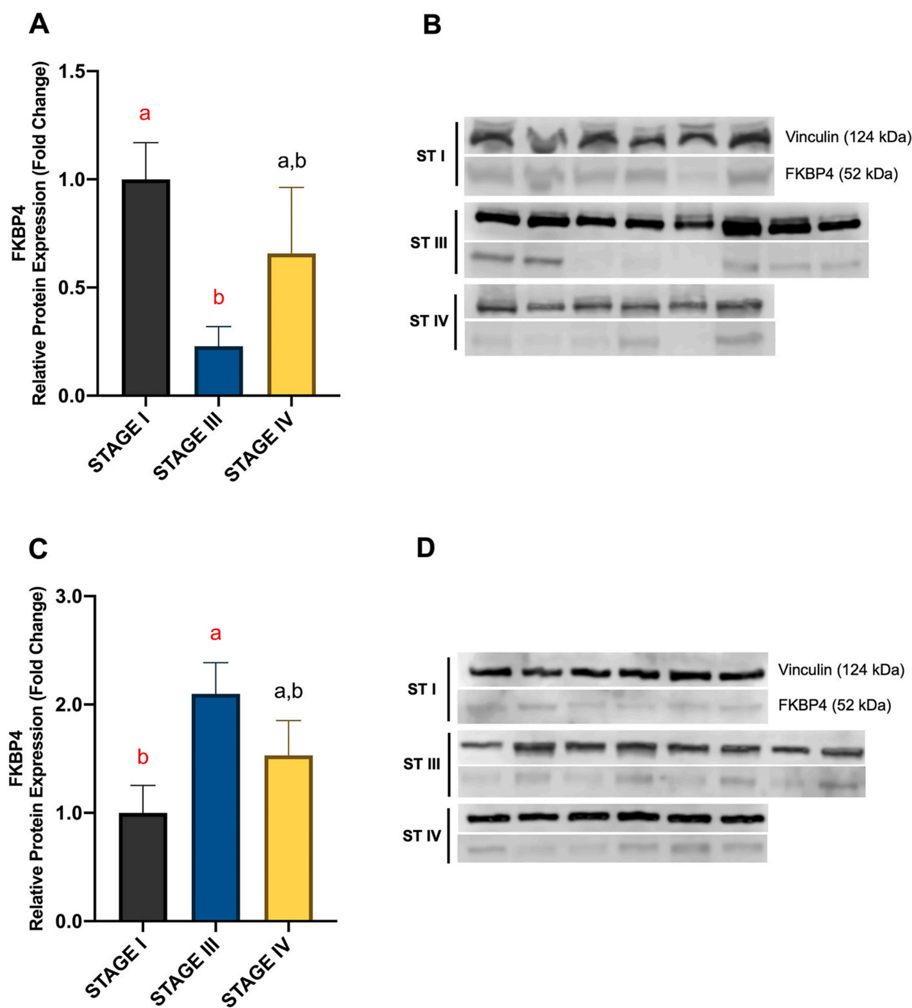

Fig. 8. Relative protein expression (fold change) of FKBP4 in the ampulla (A) and endometrium (C) comparing stage I (black) vs. stage III (blue) vs. stage IV (yellow); (mean ± SEM). Stage I was set as the reference group. Significant differences are represented by different letters (red; $p < 0.01$). Western blot analyses of stage I (ST I), stage III (ST III) and stage IV (ST IV) samples were used for FKBP4 protein (52 kDa) detection and quantification in the ampulla (B) and endometrium (D). Vinculin (124 kDa) housekeeping protein was used as loading control for Western blot normalization. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

cycle (Ulbrich et al., 2003). The results observed on *ESR1* in the post-ovulatory phase may be linked to the oviductal transport of the oocyte and embryo (Li et al., 2017), and also the sperm migration. In this context, *ESR1* knockout mice reduced by 50% the fertilization rates and produced an impaired oviduct environment for the cumulus cells (Winuthayanon et al., 2015). Regarding the *AR*, it is known to be promoted by both E2 and androgens, and it remains static across menstrual cycles in the fallopian tube in humans (Horne et al., 2009). For progesterone receptor component 2 (*PGRMC2),* we detected a decrease in the ampulla during the late luteal phase, which may be associated to oocyte development (Kowalik et al., 2016; Qiu et al., 2008), and oviductal transport (Nutu et al., 2007). Gene expression studies in the canine oviduct found higher expression in the mRNA levels of both *PGRMC1* and *PGRMC2* during the periovulatory period of the estrous cycle (Tahir et al., 2013), but we only found differences in the *PGRMC2*. In the endometrium, we observed that the mRNA expression of all four estrogen and progesterone receptors included in the present study (*ESR1, ESR2, PGRMC1, PGRMC2*) increased at the late luteal phase. Relevant activity for correct physiology may be exerted by steroid receptors during the late luteal phase, when P4 levels are elevated, and E2 levels are starting to increase (Forde and Lonergan, 2012). In contrast, *PGRMC1* does not change across the cycle, and reduced levels of *PGRMC2* have been related to endometriosis in macaques (Keator et al., 2012). In addition, P4 levels mediated by progesterone receptors have recently been linked to a beneficial induction of cortisol production in the bovine oocyte during maturation (Anbo et al., 2022), which may encourage further research regarding the interplay between the actions of steroid receptors in reproduction.

5. Conclusions

In conclusion, there is an important modulation of the GC regulatory activity in the bovine reproductive tract during the estrous cycle both in the ampulla and the endometrium. GC activity is promoted in the ampulla during the post-ovulatory phase (stage I) by increasing mRNA expression of *NR3C1*, *FKBP4* and *FKBP5*, *HSD11B1* and *HSD11B2*, claiming for a role of the oviduct in the regulation of GC actions during early reproductive events taken place on this region, including oocyte maturation. In the endometrium important changes in the GC-related mRNA expression occurred during the late luteal phase (stage III), by the time that the embryonic maternal recognition is established in the uterus. Further studies should focus on mechanistic studies to elucidate the specific contribution of each of the agents involved in the crucial steroid regulation taking place in the female reproductive tract.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rvsc.2022.09.018.

Ethics approval

Not applicable.

Funding

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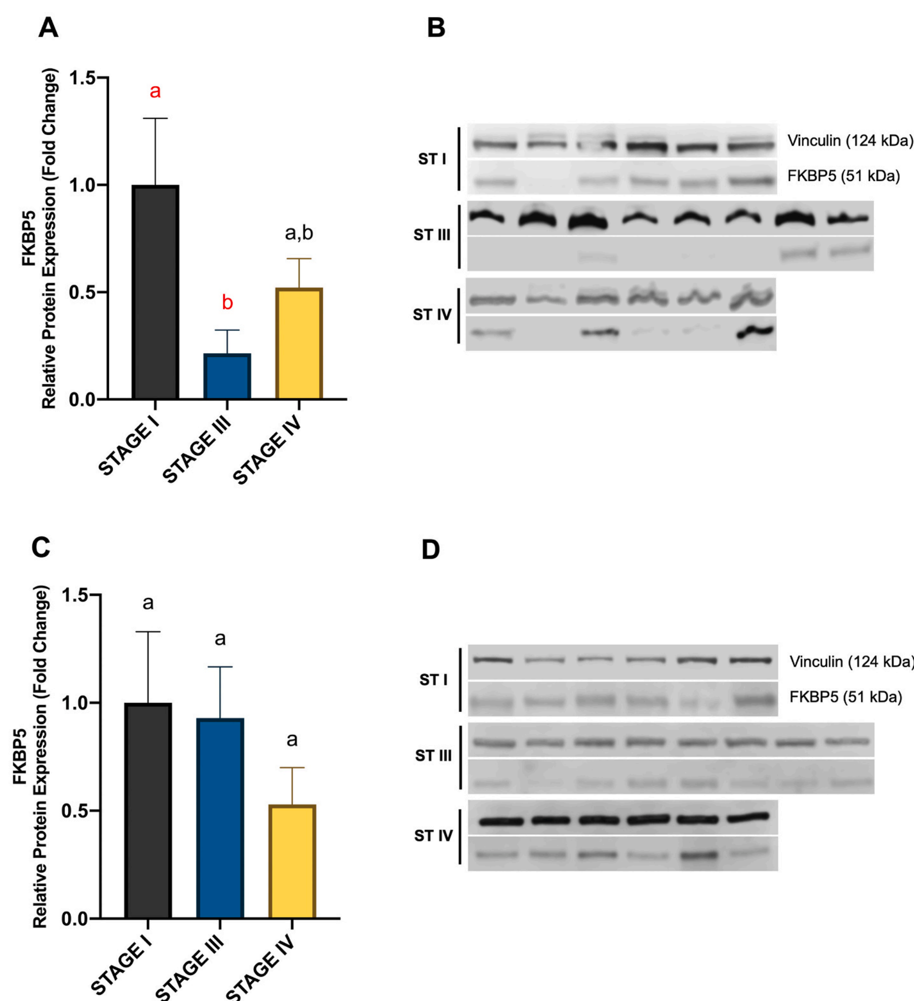

Fig. 9. Relative protein expression (fold change) of FKBP5 in the ampulla (A) and endometrium (C) comparing stage I (black) vs. stage III (blue) vs. stage IV (yellow); (mean ± SEM). Stage I was set as the reference group. Significant differences are represented by different letters (red; $p < 0.05$). Western blot analyses of stage I (ST I), stage III (ST III) and stage IV (ST IV) samples were used for FKBP5 protein (51 kDa) detection and quantification in the ampulla (B) and endometrium (D). Vinculin (124 kDa) housekeeping protein was used as loading control for Western blot normalization. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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CRediT authorship contribution statement

Mateo Ruiz-Conca: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization. **Jaume Gardela:** Methodology, Investigation, Writing – review & editing. **Sergi Olvera-Maneu:** Methodology, Investigation, Writing – review & editing. **Manel López-Béjar:** Conceptualization, Resources, Writing – review & editing, Supervision, Funding acquisition. **Manuel Álvarez-Rodríguez:** Conceptualization, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

None.

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