Serum concentration of sex-steroids, endometrial expression of their receptors, and endometrial morphology during the estrous cycle in Bos taurus Criollo and crossbred cows

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ABSTRACT
Romosinuano (Romo) and Costeño con cuernos (CCC) are Bos taurus criollo breeds (adapted to the conditions of the tropics) that presented higher plasmatic Progesterone (P4) concentration during the luteal phase compared to non-adapted genotypes. The central hypothesis was that these P4 concentrations could modulate the uterine receptivity. Blood and endometrial biopsy samples were obtained on days 0, 5, 10, 15, and 20 of the estrous cycle (day 0 = estrus) of animals of different genotypes (Romo (n = 14), CCC (n = 14), and Crossbreed Brahman × Holstein (Cross, n = 13)). Tissue-samples were used for morphometry and immunohistochemistry analyses. Data analyses were performed with Proc Mixed of SAS. The criollo breeds have higher P4 concentrations on days 5 and 15, higher values of superficial glandular area (all days) and density (days 0, 5, 10, and 15) than Cross cows (P < 0.05). ESR1 and PGR immunostaining were higher on days 0, 5, and 15 and on days 0 and 15, respectively for CCC and Romo when compared to Cross (P < 0.05). In conclusion, tropical adapted bovine breeds possess more receptive to embryo uterine environment than non-adapted breeds. This is mediated by a higher serum progesterone concentration, a strong P4 signaling, and greater developed uterine-gland morphology.

Introduction
Romosinuano and Costeño con cuernos are Bos taurus criollo (adapted to the conditions of the tropics) breeds that are most likely descendants of cattle of Spanish origin that were introduced to the South American continent during colonial times and have adapted near 500 years to the tropical lowlands in northern Colombia (Rouse 1977; Derr et al. 1995; Martínez-Corral 1995). These adapted breeds are known for their higher reproductive performance and their resistance to stressful environmental conditions compared to non-adapted breeds. In previous studies, Romosinuano cows were found to have higher plasmatic Progesterone (P4) concentration during the luteal phase compared to Brahman, crossbred Simental × Brahman, and crossbred Holstein × Brahman cows. Romosinuano cows also show excellent reproductive efficiency: 94% conception rate, an average interval of 46.13 days from parturition to first ovulation, and an average of 62.5 days to the first regular estrous cycle (Grajales et al. 2006; Grajales and Hernández 2008). Costeño con cuernos cattle also have a high reproductive efficiency, but information is lacking with regard to their P4 serum concentrations during the estrous cycle (Saraz et al. 2008). Despite their higher reproductive performance, both adapted breeds presented less meat and milk production when compared to other breeds and for this reason they are not commonly used in production systems in Colombia. However, studies have been carried out to introduce adapted breeds in crossbreeding programs with the Brahman breed in Colombia and in the United States, where heat and humidity depress performance of non-adapted cattle (Riley et al. 2014a, 2014b; Riley et al. 2015).

The above-mentioned highest P4 concentrations during the luteal phase in Romosinuano cows, lead to thinking about the effect of this hormone on the reproductive tract and how could be related to the higher reproductive performance of these adapted breeds. Several studies have been carried out to understand how sex-steroids modulate the receptivity in the female reproductive tract. During the estrous cycle, the endometrium undergoes morphological and physiological changes in response to ovarian steroids (Niswender et al. 1994; Barnes 2000; Gray et al. 2001a). At the beginning of the luteal phase, P4 stimulates histotroph production (Spencer, Johnson and Burghardt et al. 2004; Igwebuike 2009) by the endometrial glands that become larger during this phase (Diaz et al. 1986). Repeat breeder cows have diminished glandular sizes (Ohtani and Okuda 1995) and animals with lower serum P4 concentrations have reduced glandular development and decreased glandular secretions (Mann and Lamming 2001; McNeill et al. 2006; Morris and Diskin 2008). Also, animals which...
ovulate smaller follicles and forms smaller CL with less plasmatic P4 concentrations during the estrous cycle, posses a reduced receptivity modulated by a no-receptivity environment in both, endometrium (Mesquita et al. 2014; Mesquita et al. 2015) and oviduct (Gonella-Diaza et al. 2015; Gonella-Diaza et al. 2017).

Therefore, the central hypothesis of this study is that the higher P4 levels during the luteal phase funded in the Bos taurus Criollo breeds could modulate the uterine environment. The aim of the present study was to evaluate blood P4 and Estradiol (E2) concentrations, endometrial transcript abundance of ESR1 and PGR, endometrial protein immunolocalization of ESR1 and PGR, and endometrial glands morphology in three breeds of cattle that are commonly raised in lowland tropical conditions in Colombia (Romosinuano, Costeño con Cuernos, and crossbreed Holstein × Brahman).

Methodology

All procedures involving animals were approved by the local institutional Bioethics committee (Comité de Bioética, Facultad de Medicina Veterinaria y de Zootecnia. Universidad Nacional de Colombia, Bogotá, Colombia, South America).

Animals and samples

The present work was conducted at the Turipaná Research Center located in the Department of Córdoba (Colombia) at 20 meters above sea level, with an annual average temperature of 27.5°C, 1200 mm of annual precipitation and an average of 83% relative humidity. All healthy cows received the same adequate diet, management, and environmental conditions. After clinical examination, cows with any abnormal symptoms, history of previous delivery problems or a body condition score of 3 or lower were discarded. Animals of three different genotypes were studied in the present experiment, including Romosinuano (n = 14), Costeño con Cuernos (n = 14), and Crossbreed Brahman × Holstein (n = 13). All selected cows had experienced 3–6 parturitions prior to the research study. All births in this population occurred from January to March, and the calves remained with their mothers during all experimental phases. After parturition, each animal was monitored to detect the restoration of ovarian cyclicity by transrectal palpation and ultrasound examinations every three days. Furthermore, visual estrus detection was monitored on a daily basis for 1 h in the morning and 1 h in the afternoon. Following the establishment of cyclicity through observations of 2 consecutive estrous cycles (regular estrous cycle = 19–21 days), samples were obtained during the third regular estrous cycle. Blood and endometrial biopsy samples were obtained on days 0, 5, 10, 15, and 20 of the same estrous cycle (day 0 = estrus).

Blood samples were taken from the coccygeal vein into a 10 mL tube without anticoagulant (Vacutainer; Becton Dickson, NJ, USA) and immediately placed at 4°C to allow clotting. The samples were centrifuged at 2500 × g for 10 min to separate the serum from the formed elements, and they were then stored at −20°C until analysis of the hormonal serum concentration by RIA. After sacrococcygeal epidural anesthesia (lidocaine 2%), an endometrial biopsy was performed with special forceps. Samples were always taken from the medial region of the ipsilateral horn to the corpus luteum or the dominant follicle. This region was chosen because it was shown in previous studies that embryo implantation occurred there in the majority of cases (Díaz et al. 1986; Escobar and Hernández 1996). To avoid taking samples from the same site, which could potentially cause an adverse inflammatory reaction, tissues were taken from four zones of the same endometrial region: the dorsal, lateral, medial, and ventral zones as employed previously by Boos et al. (1996) and Mann and Lamming (1994). Initially, tissue-samples were washed with cold (4°C) RNase-free PBS (No. AM9624, 10× PBS pH 7.4; Ambion, Japan) and dissected in order to obtain two tissue sections. One of the tissue sections was immersed in the RNAlater storage solution (No.7021, Ambion) at 4°C for 24 h and then was stored at −20°C until it was processed for the molecular analyses. The other tissue section was fixed in buffered formalin solution (pH 7.4) for 24 h and embedded in paraffin.

Radioimmunoassay

The solid-phase radioimmunoassay (RIA) technique was used to determine the serum concentrations of E2 and P4. The kit Coat-a-Count Progesterone (Siemens, Los Angeles, CA, USA), which has a sensitivity of 0.02 ng/mL, was employed for the measurement of P4 (as previously reported by Bridges et al. (2010) and Mariano et al. (2010)). The CT 17β-estradiol kit (MP Biomedicals, Irvine, CA, USA), with a sensitivity of 0.5 pg/mL, was used for serum E2 measurements (as previously reported by Papeszambito et al. (2008), Tremblay et al. (2009), and Stevenson (2011)). Serum E2 and P4 were each measured with one assay. The intra-assay coefficients of variation for serum E2and P4 were 6.8% and 4.2%, respectively.

Morphometry

Four-micrometer-thick endometrial sections were stained with a standard hematoxylin and eosin method for histological and morphometric analyses. A computerized image analysis system (Leco 2001, MI, USA) that was connected to a light microscope (Labophot-2, Nikon, Tokyo, Japan) and a video camera (DXC-151A, Sony, Tokyo, Japan) was employed to capture the images. Area (Avoiding the luminal area; µm2) and glandular density (number of glandular sections per optical field) were measured in 20 randomly chosen fields: 10 from a superficial endometrial region, and 10 from a deep region. Measurements were performed using the 10× microscope objective.

Immunohistochemistry

Standard immunohistochemistry procedures were carried out in order to immune-localize ESR1 and PGR proteins in the endometrial biopsy samples. A monoclonal antibody to the amino terminal of the ESR1 (ER Ab1, clone AER314, human sequence) was used (MS-168-B, Thermo Scientific, Surrey, UK, diluted to 20 µg/mL in 1× PBS) to identify the ESR1 protein. And, to visualize PGR protein, a monoclonal antibody produced in mouse against the N-terminal PGR sequence (PgR, clone hPRA-2, a human protein) was used (MS-192-P, Thermo Scientific; diluted to 10 µg/mL in 1× PBS). Both antibodies are suitable for bovine
tissues according to the manufacturers and have previously been demonstrated by others (PGR: Saruhan et al., 2009; Akbalik et al., 2011; Sagsoz et al., 2011; and ESR1: Abbondanza et al., 1993; Kimmins et al., 2003). Briefly, histological sections (4 μm) were deparaffinized and dehydrated using decreasing concentrations of ethanol. Subsequently, antigen retrieval (Epitope Retrieval Solution, pH 9.0, Ref. 7119, Novocastra, New Castle, UK) and blocking endogenous peroxidase [6% H2O2 solution (Merck, USA) for 30 min] were conducted. To avoid non-specific staining, a blocking step was carried out using 2% BSA in PBS for 30 min. Then, the slides were incubated in a humidified chamber overnight at 4°C with the primary antibody. Mouse immunoglobulin diluted 1:200 was employed as a negative control for each sample and human breast cancer tissue was used as a positive control. Incubation was performed at room temperature for 2 h with a 1:200 dilution of the concentrated secondary antibody in the antibody diluent. To visualize all of the reactions, a polymer (SuperSensitive Polymer-HRP, No. QD430-XAKE, Biogenex, Fremont, USA) and a chromogen (3,3′ diaminobenzidine, Novocastra) were used. Afterwards, Harris hematoxylin was used in all samples as a counterstain.

Next, a digital camera (DCMD510, Advanced Optical) adapted to a microscope (Eclipse E400, Nikon) was used to capture images from 10 optical fields at 400× magnification in each endometrial sample. Each image was captured under the same reproducible conditions (640 × 480 pixels, 24-bit colour mode, 16.7 million colours) and saved in uncompressed tagged image format file (TIFF). Semi-quantification of the immunostaining was performed on the digitalized images of the randomly selected fields. Using the computerized image analysis software Image-Pro Plus 7.0 (Media Cybernetics. Silver Springs, USA), the number of positive and blank nuclei per optical field was obtained and the percentage of positive nuclei was calculated. The nuclei were considered positive if their immunostaining were larger than 50% of the nuclear area. Weak brown stains were excluded from the counting.

mRNA extraction and reverse transcription

Samples stored in RNAlater buffer were thawed and washed with cold RNase-free PBS (Ambion) before homogenizing with a tissue homogenizer (Tissue Ruptor, Qiagen, Germany). The extraction of mRNA was performed with the MicroPoly (A)Purist kit (AM1919, Ambion) and then stored at −80°C in the RNA storage solution (AM7000, Ambion) following the manufacturer’s instructions. The concentration of mRNA was quantified in a NanoVue (GE Healthcare, UK) and 200 ng mRNA were treated with DNase I (18068-015, Invitrogen, USA) and then, used to generate the cDNA. For the reverse transcription reaction, random hexamer primers (Promega, USA) and Superscript III reverse transcriptase (18080-093, Invitrogen, USA) were used according to the manufacturer’s protocol. cDNA was obtained in a final volume of 20 μl.

Analysis of real-time PCR

The real-time PCR test was conducted on a LightCycler 2.0 system (Roche, Mannheim, Germany) using the SYBR Green (LightCycler® FastStart DNA MasterPLUS SYBR Green I, No. 03515885001, Roche) methodology. Specific primers were used to amplify each gene of interest: ESR1, PGR, and the reference gene β-actin (Table 1). The Primer 3 software package (http://www.simgene.com/Primer3) was employed to design the primers based on the Bos taurus genome. The primers were designed in order to target a position over an exon-exon junction to prevent genomic DNA amplification. The substitution of cDNA template with PCR-grade water (Ambion) was used as a negative control to verify that no contaminating nucleic acids were introduced into the master mix or the samples during the process. All reactions were performed under the following conditions: denaturation programme (95°C for 10 min), 45 amplification cycles (95°C for 10 s, 67°C for 30 s and 72°C for 25 s), melting curve programme (60–95°C with a heating rate of 0.1°C/s and continuous fluorescence acquisition), and finally a cool-down programme (40°C). To determine the crossing point (CP) of each transcript the ‘Fit point method’ of the Light cycler 3.3 software (Roche) was used. In each sample, the ESR1 and PGR mRNA expression levels were normalized to the levels of gene β-actin, obtaining the n-fold value for statistical analysis. Finally, the specificity of the obtained amplification products was verified through melting curves and electrophoresis on a 1.5% agarose gel to obtain the sizes of the products.

Statistical analysis

The cow was the experimental unit in all models. Data were checked for normality and homogeneity of variance using the UNIVARIATE procedure of SAS (version 9.3; SAS Institute, Cary, NC, USA) and the Bartlett and Levene’s test, respectively. Logarithm transformations were performed when the data were not normally distributed (specifically for the variables: PGR and ESR1 transcript abundance, PGR protein abundance, and E2 serum concentration). In those cases, the transformed data were used to calculate P-values and the corresponding mean ± standard error (S.E.) of the non-transformed data are presented in the results for clarity. Data were analyzed using repeated-measures with the MIXED procedure of SAS. Fixed effects included breed, day, and their interaction. When the interaction term was not significant it was subsequently excluded from the final model. Cow within treatment was included as a random

| Target Gene | Primers | Amplicon size | Gene identification number |
|-------------|---------|---------------|---------------------------|
| ESR1        | Sense   | ACGGAGGCTCTTATTTGCTCC | 231 | NM_001001443.1 |
|             | Antisense | CGGTGATGGTCCTCCTCCT |          |                  |
| PGR         | Sense   | GAGACGTGATCAAGCCAAATTG | 227 | NM_001205356.1 |
|             | Antisense | CATCCTGCAATATATCTT |          |                  |
| Actin-beta  | Sense   | GGATGAGGCTCTGAGCAAGAGA | 77  | NM_173979.3     |
|             | Antisense | TCGTCCCAGTTGGTGACGAT |          |                  |
effect. The type of variance–covariance structure used was chosen depending on the magnitude of the Akaike information criterion (AIC) for models run under compound symmetry, unstructured, autoregressive, or Toeplitz variance–covariance structures. The model with the least AIC value was selected. Differences between treatments were determined by F-tests using Type III sums of squares. The PDIFF command incorporating the Tukey test was applied to evaluate pairwise comparisons between treatment means.

Results

Resumption of reproductive cyclicity

No clinical problems were detected in the cows during the parturition and postpartum periods. The Costeño con Cuernos and Romosinuano cows exhibited the first estrus earlier ($P < 0.01$) than the crossbred Holstein × Brahman cows. The mean interval from calving to first estrus was 36.67 days, and there were differences ($P < 0.01$) among the 3 genotypes (Table 2). The overall interval from calving to the onset of the first estrus cycle was 78.59 days, and the Costeño con Cuernos and Romosinuano cows had shorter ($P < 0.05$) intervals than the crossbred Holstein × Brahman cows. The mean duration of the regular estrous cycles were 21.4 ± 3.26, 20.8 ± 2.75 and 22.7 ± 1.98 days for the Costeño con Cuernos, Romosinuano and crossbred Holstein × Brahman cows, respectively.

Serum progesterone and 17β-estradiol concentrations

There was a time × genotype interaction for the P4 serum concentrations ($P < 0.02$). There were significant differences ($P < 0.05$) among the breeds in the P4 concentrations on days 5 and 15 (Figure 1). These results indicated that the crossbred Holstein × Brahman animals had a lower P4 concentration on day 5 and 15 than the Costeño con Cuernos and Romosinuano cows.

There was not time × genotype interaction for the E2 serum concentrations, however this variable was affected by time effects. E2 concentrations were higher on days 0 and 20 for all genotypes. No significant differences in serum E2 concentrations were observed between the genotypes (Table 3).

Morphometry of the endometrial glands

All the biopsy samples collected during the estrous cycle contained endometrial tissue, which consists of the luminal epithelium, superficial glands, deep glands, and endometrial stroma. However, certain samples did not contain luminal epithelium, and no samples contained myometrial tissue. A secreted material was observed in the glandular epithelium on days 5 and 10 of the estrous cycle in all genotypes. By day 15, that tissue has formed cilia and was fully developed.

The two-way interaction (time × genotype) was not detected for any of the variables evaluated in the endometrial glands. The morphology of the endometrial glands changed according to the day of the cycle. For all breeds studied, the superficial glands had lower area and density values on days 0 and 20 compared to days 5, 10 and 15 ($P < 0.01$).

Also, superficial glandular area and density of superficial glands were different among genotypes. Higher values of the superficial glandular area were recorded during all the estrous cycle in Costeño con Cuernos and Romosinuano cows compared to crossbreed Holstein × Brahman (Figure 2). Also, the density of superficial glands was greater in Costeño con Cuernos and Romosinuano cows on days 0, 5, 10 and 15 than in crossbred Holstein × Brahman cows. The deep glands morphology was no different among breeds.

Localization and semi-quantification of ESR1 and PGR proteins in the immunohistochemical preparations

The ESR1 protein was localized in the nuclei of the stromal cells, glandular epithelium and luminal epithelium of all three

![Figure 1. Mean ± SE values of serum progesterone concentrations (ng/ml) in three bovine genotypes during the estrous cycle. * Indicates differences among genotypes ($P < 0.05$).](image-url)
A weak cytoplasmic reaction was observed in some preparations, mainly in the deep stromal cells. The PGR protein was also located in the nuclei of the stromal cells, luminal and glandular epithelia. A positive reaction was observed in all genotypes during all days of the estrous cycle.

The immune-localization and semi-quantification results are shown in Figure 3. The two-way interaction (time * genotype) was not significant when the ESR1 and PGR immunostaining were evaluated. Nevertheless, both variables were affected by time and genotype effects. In general, the maximal immunostaining for ESR1 and PGR proteins was observed on days 0 and 5, respectively. ESR1 protein achieved its maximal level on day 0 and then gradually decreased, reaching its lowest level on day 15. ESR1 protein then had a clear intense reactivity on day 20. PGR protein reached its maximal reactivity level on day 5 before decreasing on days 10 and 15. PGR protein levels again increased on days 20 and 0 of the estrous cycle.

There were significant differences among the genotypes in the number of immunostaining nuclei positive to ESR1 and PGR proteins. On days 0, 5, and 15, the Costeño con Cuernos and Romosinuano animals showed a higher ESR1 protein immunostaining than the crossbred Holstein × Brahman animals (P < 0.05). Also, a greater proportion of PGR protein immunolabelled nuclei were detected in the Costeño con Cuernos and Romosinuano cows than in the crossbred Holstein × Brahman cows on days 0 and 15 (P < 0.01).

**ESR1 and PGR mRNA expression**

The two-way interaction (time * genotype) was not significant for the ESR1 and PGR mRNA expression. Nevertheless, both variables were affected by time effect and PGR mRNA expression affected by genotype effects. The ESR1 mRNA expression levels were significantly higher on days 0 and 20 compared to days 5, 10 and 15 in all genotypes (P < 0.01). The expression of the PGR mRNA presented higher values on days 5 (Figure 4) and 10 than those obtained on days 0 and 20 (P < 0.001) for all genotypes.

The Romosinuano and Costeño con Cuernos genotypes had higher levels of expression compared to the crossbred Holstein × Brahman animals on days 10 and 20 of the estrous cycle (P < 0.05). Although there were no significant differences, the level of ESR1 mRNA expression on day 0 was lower in the crossbred Holstein × Brahman animals compared to the Costeño con

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Table 3. Mean ± SE values of serum estradiol concentrations (pg/ml) in three bovine genotypes during the estrous cycle.

| Genotype              | N     | Day 0       | Day 5       | Day 10      | Day 15      | Day 20      |
|-----------------------|-------|-------------|-------------|-------------|-------------|-------------|
| Costeño con Cuernos  | 14    | 14.34 ± 1.47| 4.54 ± 0.94 | 3.67 ± 0.74 | 6.56 ± 1.19 | 9.66 ± 1.731|
| Crossbreed Holstein × Zebu | 13    | 15.20 ± 3.57 | 3.39 ± 1.30 | 6.09 ± 1.65 | 8.65 ± 1.18 | 7.38 ± 1.84 |
| Romosinuano           | 14    | 14.16 ± 1.80 | 4.14 ± 0.72 | 3.87 ± 0.69 | 4.41 ± 0.84 | 6.73 ± 0.83 |

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Figure 2. Mean and SE of the area (µm²) and density (number of glandular sections per optical field) of the superficial and deep endometrial glands during the estrous cycle in three bovine genotypes in the Colombian tropics. Different letters indicate P < 0.05.

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Figure 3. Mean ± SE values of serum estradiol concentrations (pg/ml) in three bovine genotypes during the estrous cycle.
Cuernos and Romosinuano animals. Significant differences ($P < 0.05$) were also observed when comparing the PGR mRNA expression levels on days 5 and 10 among the crossbred Holstein × Brahman animals and the Costeño con Cuernos and Romosinuano animals.

**Discussion**

The processes involved in implantation and gestation are very complex, and there are currently many unknown intrinsic and extrinsic factors that affect them. Many apparently healthy and viable embryos fail to develop properly because they do not find an optimal uterine environment (Hansen 2002). Certain bovine genotypes adapted to the lowlands in the Colombian tropics, namely the Romosinuano and Costeño con Cuernos, have superior reproductive efficiencies. For instance, they typically require fewer inseminations per conception, and they have developed a resistance to stressful environmental conditions compared to non-adapted breeds (Saraz et al. 2008). In previous studies, elevated serum P4 concentrations were proposed as a possible explanation for the greater reproductive efficiency of the Romosinuano genotype compared to the Brahman breed and two of its Crossbreeds (Grajales et al. 2006; Grajales and Hernández 2008; Báez et al. 2007). In the present study, Romosinuano and Costeño con Cuernos presented higher P4 concentrations on days 5 and 15 of the estrous cycle compared to the crossbred Holstein × Brahman genotype, which supports the hypothesis that P4 could be a marker for higher reproductive efficiency in tropically adapted breeds. This observation is most likely the result of the adaptation of the Bos taurus breeds to specific climatic conditions through a natural selection acquired during more than 5 centuries. However, how these higher P4 concentrations affect the female reproductive tract was not previously studied in these genotypes.

This is the first study in which the serum sex-steroids concentrations are related to endometrial transcript and protein abundance of ESR1 and PGR and endometrial glands morphology in tropically adapted and non-adapted breeds.

The present results demonstrate that the Romosinuano and Costeño con Cuernos animals had a higher P4 concentration on days 5 and 15 and higher glandular development (area and density) than the crossbred Holstein × Brahman animals. This could mean that the tropical breeds possess a more receptive uterine environment for hosting the embryo compared to the crossbred Holstein × Brahman animals. This hypothesis is supported by previous data from Gray et al. (2001b). They established a positive correlation between endometrial gland density and the viability of the conceptus on day 14 post-mating and they also found that an endometrial gland knockout sheep was unable to maintain pregnancy. Also, Ohtani and Okuda (1995) found differences between normal and
repeat breeder cows in regard to their endometrial histology. They observed decreases in glandular mitoses on day 1 and in glandular secretions on day 8 of the estrous cycle in the repeat breeder cows group. This led to the assumption that animals with larger glandular sizes during the luteal phase contained greater amounts of nutrients and growth factors in their histotroph. The histotroph provides multiple nutrients and metabolites to the embryo for its development during preimplantation (Spencer and Burghardt et al. 2004; Spencer, Johnson and Bazer et al. 2004). Without histotroph, the embryo, which lacks mechanisms to synthesize its own nutrients, would die (Spencer, Johnson and Bazer et al. 2004). In addition, the histotroph also provides enzymes, growth factors, cytokines, lymphokines, hormones, and transport proteins to stimulate the growth, differentiation, and development of the conceptus (Morris and Diskin 2008; Spencer, Johnson and Bazer et al. 2004). During embryo development, the embryo must pass from the spherical and tubular stage and elongate to a filamentous stage, synthesizing sufficient Interferon-τ (IFN-τ) to prevent luteolysis. Blastocyst elongation begins around day 13 of pregnancy, which coincides with the late luteal phase, and is, therefore, a critical period for conceptus survival. During the late luteal phase, the availability of P4 for the endometrial glands becomes critical, given that a sufficient trophoblastic growth must occur to produce enough IFN-τ to maintain pregnancy (Mitko et al. 2008).

Moreover, animals with high serum P4 concentrations are less likely to experience EM (Mann and Lamming 2001; McNeill et al. 2006; Stronge et al. 2005; Mann and Lamming 1995). Mann and Lamming (1995) reported differences in serum P4 concentrations on days 12 and 16 between pregnant cows (5.7 and 12.3 ng/mL, respectively) and cows with EM (4.8 and 10.5 ng/mL, respectively). From the same study, it was also concluded that cows with lower concentrations of P4 during the luteal phase at slaughter had smaller embryos. Also, P4 concentrations on day 15 have been related to embryo viability. P4 concentrations on day 15 of 7.5 ng/mL versus 4.5 ng/mL were reported for animals with healthy embryos versus animals with non-competent embryos (Mann and Lamming 2001). The abovementioned studies support the idea that the Romosinuano and Costeñito con Cuernos genotypes might have advantages over the crossbred Holstein × Brahman animals because of their significantly higher P4 concentrations on days 5 and 15 of the estrous cycle. It is then possible that the uterine environment of the Romosinuano and Costeñito con Cuernos cows are better prepared to receive embryos and maintain pregnancy because their endometrial glands have more stimulation and therefore a greater secretory capacity.

Higher P4 concentrations on days 5 and 15 of the estrous cycle are related with the greater glandular development in the tropically adapted breeds. In a previous report, Wang et al. (2007) found that Holstein cows possessed large total endometrial areas during the follicular phase and a greater glandular area during the luteal phase (days 8 and 16). They did not observe significant variations in the gland duct density throughout the estrous cycle. Díaz et al. (1986) reported an increase in the area of the endometrial glands during the luteal phase in Brahman cows. Additionally, they found a greater height of the glandular epithelium during the mid-luteal phase than during the follicular phase (P < 0.05). Also, Ohtani and Okuda (1995), reported that repeat breeder cows had fewer endometrial secretions and a low rate of mitosis in the glands during the follicular phase compared to normal cows and heifers. This study validates the hypothesis that bovine genotypes adapted to a lowland tropical environment have higher P4 concentrations during the luteal phase and a concomitant glandular development during part of the critical period of rapid blastocyst elongation and preimplantation.

In the present work, the Romosinuano and Costeñito con Cuernos animals had higher expressions of PGR (both protein and mRNA) on days 5 and 10 of the cycle compared with levels expressed by crossbred Holstein × Brahman cows (P < 0.01). In fact, crossbred Holstein × Brahman animals had fewer nuclei marked for PGR protein on days 0, 10 and 15 of the cycle (P < 0.05). From the present findings, we propose that animals with higher serum P4 concentrations, PGR expression (both, transcript and protein), and higher endometrial glandular sizes result in higher histotroph secretions and a better uterine environment, which could explain their optimal reproductive performance.

In the present study, there were no differences in E2 concentrations during the estrous cycle among the genotypes. Many authors have concluded that E2 concentrations during the follicular phase modulate the expression of ESR1 and PGR (Ing et al. 1996; Ing and Torresi 1997; Robinson et al. 2001; Kombe et al. 2003; Lane et al. 2009). Robinson et al. (2001) showed that ESR1 mRNA is expressed in the luminal and glandular epithelia during estrus, followed by a quantitative decrease after ovulation. It then decreases to its lowest levels during days 12 to 15 but begins to increase again on day 16. Similar patterns of expression for both, ESR1 and PGR, were found in the present study. Xiao and Goff (1999) observed a clear effect on E2 concentration and the expression of the ESR1 and PGR genes in vitro (dose-dependent expression). It would be feasible to think that animals with higher serum E2 concentrations could express more transcripts for these genes. However, the present study fails to establish a positive correlation between E2 serum concentrations and ESR1 and PGR expression.

In conclusion, tropical adapted bovine breeds possess more receptive to embryo uterine environment. This is mediated by a higher serum P4 concentration, a strong P4 signaling, and greater developed uterine-gland morphology. All these findings could be potentially related to major histotroph production and could help to explain the higher reproductive efficiency of the Romosinuano and Costeñito con Cuernos cows in Colombian-tropical conditions.

**Acknowledgements**

The authors are grateful to Dr. Rodrigo Martinez, director of the germplasm bank in CORPOICA, for permitting access to Romosinuano and Costeño con cuernos animals and to Associate Professor Nhora Martinez (Universidad Nacional de Colombia, Bogotá-Colombia) for her assistance in the statistical analysis.

**Disclosure statement**

No potential conflict of interest was reported by the authors.
Funding

This work was supported by the División de Investigaciones de la Universidad Nacional de Colombia, Sede Bogotá [Grant number: 10588], and Programa de Posgrado en Salud y Producción Animal de la Facultad de Medicina Veterinaria y Zootecnia, Universidad nacional de Colombia, Sede Bogotá.

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