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Size of Ovulating Follicle, Corpus Luteum and Blood Progesterone in Heifers Receiving Embryos from Three Grazing Breeds in Ecuador

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ABSTRACT

The aim of this paper was to determine the size of the pre-ovulation follicle in the corpus luteum, and in blood progesterone on the sixth and twelfth days after ovulation, in Holstein, Brown, Swiss and Criollo breeds grazing in Ecuadoran Highlands. The size of the pre-ovulation follicle and corpus luteum, and the blood progesterone levels were determined on days six and twelve, in nine heifers from each breed. Assessment of ovarian structures was made by ultrasound scanning. The concentration of blood progesterone was determined by electrochemiluminescence. The pre-ovulation follicle in Criollo heifers was larger (14.6 ± 0.41 mm) than in Holstein (12.7 ±0.47 mm), and in Brown Swiss (12.7 ± 0.65 mm). The progesterone concentration on days six and twelve after ovulation of Criolla heifers was higher than for Holstein and Brown Swiss(11.0 ± 1.68 ng/ml, and 18.4 ± 2.04 ng/ml, respectively). The linear association values were high and significant ($P < 0.05$) in the pre-ovulation follicle, corpus luteum and progesterone, in Holstein and Brown Swiss. It was demonstrated that for every additional millimeter above the Criollo mean on the sixth day, 1.67 ng/ml more of progesterone was generated in Criollo heifers ($P < 0.05$) than in the rest. It was concluded that the Criollo heifers had higher progesterone levels than Holstein and Brown Swiss on the sixth and twelfth days, thus producing better conditions in the uterus, with lower early embryo losses.

Keywords:*dairy cattle, ovary, follicle dynamics, estrus cycle, breeding, blood progesterone*

INTRODUCTION

One of the main causes of dairy cow infertility is embryonary mortality (30-40% of losses occur before the 17th day after fertilization) (Araujo, Bermeo, Maza and Meriño, 2005; Lonergan, Fair, Forde, and Rizos, 2016). Consequently, the cows return to estrus after completing the equivalent of a normal estrus cycle (Bridges, Wright, Buford, Ahmad, Hernández-Fonseca, McCormick, and Inskeep, 2000; Chabbert-Buffeta, Skinner, Caraty and Bouchard (2000). Thatcher (1994)determined that embryonary survival depends on optimum synchrony between the embryo and mother, in which several, autocrine, paracrine, and endocrine factors take place. Furthermore, several studies have made reference to the importance of POF to form CL at the P4 levels that would be generated, which, in turn, will determine a favorable uterine environment so the embryo develops (Gonella, 2010; Forde, Bazer,Spencer and Lonergan, 2015). This requirement becomes more evident when genetic breeding (embryo transfer) is applied, thus proving that the POF size, Cl volume, and secretion of P4 associate to establish and maintain pregnancy (Vasconcelos, Sartori, Oliveira, Guenther and Wiltbank, 2001; Brooks, Burns and Spencer, 2014). As a result, they affect the efficiency of embryo transfer (Baruselli, DesáFilho, Martins, Reis and Nasser, 2005; Kenyon, Mendonga, Lópes, Lima, Santos, and Chebel, 2013), particularly in terms of pregnancy percent. Therefore, it is important to have the receptor cows, as well as the inseminated cows, with CL values that favorproper formation of uterine environment that leads to optimum early embryo development (Walsh, Williams and Evans, 2011).

In order to better understand this areaseveral studies have been done to *Bostaurus and Bosindicus*. However, the information generated in Latin American Criollo breeds is insufficient. These animals have features that make them unique, like adaptation to diverse ecosystems for over 500 years, high yields, maternal instincts, longevity, resistance to disease, tolerance to extreme weather, nutrition on poor and scarce pastures in the area (Aguirre,Bermeo, Maza, and Merino,2011). Besides, their morpho-structural traits allow them to handle semi natural growing habitats more properly (Primo, 1992). Unfortunately, the need to increase production has demanded the introduction of other breeds (Holstein and Brown Swiss), with high yields, but also requiring more intensive management in terms of feeds and facilities which the economy cannot provide (FAO, 2015).

The aim of this paper was to determine the size of the pre-ovulating follicle in the corpus luteum (CL), and the levels of blood progesterone (P4) on the sixth and twelfth days post-ovulation, in Holstein, Brown, Swiss and Criollo breeds grazing in the Ecuadoran Highlands.

MATERIALS AND METHODS

This research took place on the experimental farm named "Irkis", Faculty of Agricultural Sciences, University of Cuenca, Ecuador, located on the 3044181 S and 79040813 O coordinates, 2 715 meters above sea level, 14º C mean temperature, 80% relative humidity, and 800-2 000 mm of precipitations. The study took into account the following sanitary code standard for land animals, chapter 7.8: Use of animals for research and education, by the World Organization of Animal Health (OIE, 2016).

Animals in the study

A total number of 27 heifers (Holstein (HO; n=9), Brown Swiss(BS; n=9) and Criollo (Cr; n=9)) were included. When estrus was detected, the heifers were kept in the same herd, from September 2013 to February 2015, receiving the same management and health maintenance conditions. They were fed with pasture mixture of *Pennisatum clandestinum*, *Lolium multiforum*, *Trifolium pratense*, and *Trifolium repens*, supplemented with hay and mineral salts. The initial average weight was 405.3±57.1 kg (HO); 329.8±69.1 kg(BS) and 243.3±45.0 kg (Cr), with a body condition (BC) of 2.6±0.04(HO); 2.7±0.11(BS) and 2.6±0.11(Cr), determined in a 1-5 scale (Edmonson, Lean, Weaver, Farver and Webster, 1989), with no statistical differences ($P > 0.05$). The average age of every breed group was 19.7±2.9(HO); 20.4±4.1(BS) and 19.9±4.8 (Cr) months.

Synchronizing protocol (before the beginning of the study).

Determinations were made after an estrussynchronizing protocol with prostaglandin F2α at 25mg PGF 2α doses (Lutalise®, Zoestis), intramuscularly (IM) on days 0 and 11 during the protocol. After the second application adhesive patches to detect estrus were placed on the sacrococcygeal joint and the animals were observed between the first and the seventh days after every PGF 2α injection, for 30 min, at 12 h intervals. Estrus was corroborated in the animals when they agreed to mating without moving reflexes (Roelofs, López-Gatius, Hunter, Van Ecerdenburg and Hanzen, 2010).

Size of the Preovulating follicle and the Corpus luteum (days 6 and 12)

The size of the preovulating follicle (POF) was evaluated 12 h after the beginning of estrus detection, confirming ovulation 24 h later, by echography. Ovulation was acknowledged when the follicular structure was gone from the same ovary it was observed in. The size of the corpus luteum (CL) was measured on the 6th and 12th days, according to the criteria stated by Herzog *et al*. (2010)that the structure has three stages: growth until day 4; statics (6 days), and regression (17 days and on).

Ultrasound evaluation

The study of ovarian structures was made by echography (multifrequency AlokaProsound 2, with a linear transducer of 7.5 MHz). The two ovaries from all the animals were evaluated using the scanning technique, located lateral-medial, dorsoventral and cranio-caudal, the right side first, then the left one, according to Perea *et al*. (1998). This process was performed on the estrus day to measure the POF; then on the 6th and 12th days after ovulation, to determine CL size. Each image selected was recorded, the relative position of every structure was sketched on a field record to carry out ovarian mappings, as described by Pierson and Ginther (1984). The mean of two measurements (mm) of height by width, was used to determine the size of the structures.

Progesterone measurements (P4)

On the 6th and 12th post-ovulating days, blood samples were taken from each heifer, starting time 08:00, always keeping the same order. The samples (10 ml) were collected by piercing the jugular vein, using heparinVacutainer™ tubes and 16-g needles made for these tubes. Immediately after, the sample was centrifuged for 15 minutes, and the supernatant was collected (2ml). It was placed in a vial and remained frozen (-20º C) until analysis. The concentration of P4 was determined through *in vitro* immunological test, using luminescence with heparinizedplasma. Progesterone II (Cobas®) was used as reagent.

Statistical analysis

Statistical analysis (SPSS 22 for Windows) was performed twice, at different times. The first analysis included data analysis by simple ANOVA, and the Tukey´s range test (5% significance), considering the three breed groups by separate. In the second analysis, parametric correlations (Pearson) were made among the groups and within them. Besides, the corresponding slope coefficients were determined for the simple linear regression analysis among specific variables. Accordingly, the heifers were pooled in three different ways: all the animals(three breeds grouped), HO and BR heifers, and finally, the Criollo breed.

RESULTS AND DISCUSSION

The average size of the POF of the 27 heifers was 13.3±0.34 mm. Analysis of the values from each breed revealed that Cr showed a significantly larger POF ($P < 0.05$) than the other two. On the 6th day the CL was 17.5±0.62 mm, reaching 23.1±0.55 mm on the 12th day, without significant differences among the breeds in either moment. Furthermore, the mean blood concentration of P4, on the 6th day (11.0±1.68ng/ml), and the 12th (18.4±2.04ng/ml) was significantly higher ($P < 0.05$) in the Cr heifers (Table 1).

A significant positive correlation ($P <0.05$) was observed for POF, CL, and blood concentration of P4 on the 6th and 12th days, in all the 27 heifers (Table 2). Moreover, the correlation values among POF, CL and blood P4 on the 6th and 12th days, for BS and HO heifers, had a very similar behavior in general terms (Table 3). However, the correlation values in Cr heifers had a different behavior (Table 4).

Table 5 shows the linear model for the dependent variable CL size on the 6th day, in relation to POF size for BS and HO, and the group in general, with significant values ($P < 0.01$). The values of blood P4 on the 6th day accounted for 34 and 41% of the size theCL would reach in BS, HO and the whole group. The Cr heifers showed 62% ($P<0.05$).

For the P4 concentrations on days 6 and 12, the relation values were medium and high ($P < 0.05$). Finally, these values forCL size between days 6 and 12 were low and significant ($P<0.05$).

The POF diameter differed among BS, HO and Cr; however, they generated corpus luteum with the same sizes on the 6th and 12th days, though they were higher for Cr.

Pre-ovulation follicle (POF)

Perry *et al.* (2005) and Chacón,Vargas, Otero and Villami(2005) determined that the kind of management, breed traits, and probably other factors, like the environmental conditions, have effects on the size of the POF. The general mean achieved in this study coincided with the one reported by Ireland, Mihm, Austin, Diskin and Roche (2000). In their concept review, they noted that the size of POF in heifers is 13 mm.

The POF size in HO (12.7±0.47 mm), BS (12.7±0.65 mm), had similar behavior patterns to the general mean in the study ($P > 0.05$), and coincided with the values achieved bySirois and Fortune (1988), and Ginther, Beg, Bergfelt, Donadeu and Kot (2001), who determined a mean of 12.8±0.3 mm in Holstein heifers. However, it differed from Sartori, Haughian, Shaver, Rosa and Wiltbank (2004), and Echternkamp, Cushman and Allan (2009), who reported 15.0±0.2 mm and 14.0±0.3 mm, respectively, in HO heifers. It also coincided with the 11.8±2.5 mm described by Perea *et al.* (1998) in crossbreed heifers (Holstein x Brown Swiss x Brahman).

The Cr heifers had a POF of 14.6±0.41 mm, ($P < 0.05$) higher than HO (12.7±0.47) and BS (12.7±0.65) (Table 1),as well as from the results found by Chasombat, Nagai, Parnpai and Vongpralub (2014), in native Thai heifers (8.14±0.25 mm), Bosindicus beef type, different from the Cr animals used in this dairy study.

Corpus luteum on days 6 and 12 post-ovulation

The increase in the size of CL on days 6 and 12 was similar (Table 1) to the one reported by Herzog *et al.* (2010), who established CL of 3.7±0.2 cm^2 and 5.4 cm^2in HO heifers for days 6 and 12, respectively,. Echternkamp *et al.* (2009) determined CL diameter on the 7th day (19.6±0.7 mm) and on the 12th day (20.8±0.4 mm) in heifers. In a study of crossbred heifers (Holstein and Brown Swiss with Brahman),they set a maximum CL diameter on the 4th day (11±0.2 mm), and the 10th day (19±0.3 mm). Savio (1990) in Holstein heifers observed CL of 20–25 mmdiameter. Chasombat *et al.* (2014) in native Thai heifers established a 15.14±0.14 mm CL diameter

Progesterone blood concentrations on days 6 and 12post-ovulation

The P4 levels in Cr heifers on days 6 and 12 were higher than the determinations for HO and BS (Table 1). The studies made by Herzog *et al.*

(2010) on heifers, set values for day 6 (3.2±0.6ng/ml), and for day 12 (5.2±0.6ng/ml).Echternkamp*et al.* (2009) set values for P4 (6.5±0.6 ng/ml)on the 7th and 12th days (9.0±0.3ng/ml)in heifers.Chasombat*et al.* (2014) determined 2.59±0.03ng/ml over the estrus stage, and 4.10±0.06ng/ml for diestrous in Thai heifers. Sartori *et al*.(2004), set maximum P4 concentrations at 7.3±0.4ng/ml in Holstein heifers.Perea *et al.* (1998) (HO and BS with Brahman) set top levels at 5.1±1.3ng/ml.The findings in HO and BS coincided with the results from the above mentioned authors. However, the P4 levels in Cr were higher, which could improve the conditions in the uterus and raise the conception percent.

Correlation analysis

The correlation analysis made to the total females (in the group) determined a significant linear association (P 0.01) between the size of POF and the CL size on the 6th day (r=0.61) (Table 2). This relation was similar to the HO and BS group, named "foreign" (Table 3). This result coincided with reports by Vasconcelos *et al*. (2001), who claimed that a large CL derived from large follicle ovulation, since there is a positive correlation between the POF size and CL volume. Perry *et al.* (2005) described that the POF ≥12.8 mm generatedCL that produced bigger amounts of P4 than the POF <12.8 mm. However, the Cr heifers showed a different behavior from the two other groups, because the correlation value was low and insignificant (P > 0.05).

The ratio pattern set between the size of CL and the P4 levels in foreign heifers (HO and BS), coincided with reports by Mann (2009), who said it was often assumed that a large CL can produce high levels of P4. It was confirmed by Echternkamp *et al*. (2009) in HO heifers. Nevertheless, the Cr heifers would produce 2.5-fold more P4 than the foreign. The high levels of P4 in Cr heifers on the 6th and 12th days, might favor uterine conditions and embryo development, which might also cut down the percent of early embryo losses, a factor that could explain high fertility in this breed.

Diskin, Kenny, Dunne and Sreenan (2002) reported a positive association between the concentration of P4 and the day luteolysis occurred, as well as furtherembryo survival rate. Furthermore, P4 concentration is known to affect the volumes of uterine secretion, and it could impact on embryo development (McNeill, Diskin, Sreencin and Morris, 2006).

CONCLUSIONS

The Cr heifers had the highest blood levels of P4 on the 6th and 12th days post-ovulation, in comparison to HO and BS, which might favor uterine conditions, with ensuing reduction in early embryo losses.

REFERENCES

AGUIRRE, L.; BERMEO, A.; MAZA, D. y MERINO, L. (2011). Estudio fenotípico y zoométrico del bovino criollo de la sierra media y alta de la región sur del Ecuador (RSE).*AICA, 1*, 392-396.

ARAÚJO, M. C.; VALE, V. R.; FERREIRA, A. M.; Sá, W. F.; BARRETOFILHO, J. B.;CAMARGO, L. S. ySILVA, M. V. (2005). Secreção de interferon-tau em embriões bovinos produzidos in vitro frescos e congelados. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 57* (6), 751-756.

BARUSELLI, P. S.; DESÁ Filho, M. F.; MARTINS, C. M.; REIS, E. L.yNASSER, L. F. (2005). *Nuevos avances en los tratamientos de SOV en donadoras de embriones bovinos*. Congreso internacional de reproducción bovinaINTERVET, Bogotá, Colombia.

BRIDGES, P. J.;WRIGHT, D. J.; BUFORD, W. I.; AHMAD, N.; HERNANDEZ-FONSECA, H.;MCCORMICK, M. L. *et al*.(2000). Ability of Induced Corpora Lutea to Maintain Pregnancy in Beef Cows. *Journal of Animal Science*, *78*(11), 2942-2949.

BROOKS, K.; BURNS, G.ySPENCER, T. E. (2014). Conceptus Elongation in Ruminants: Roles of Progesterone, Prostaglandin, Interferon Tau and Cortisol. *Journal of Animal Science and Biotechnology*, *5*(1), 1-12.

CHABBERT-BUFFETA, N.; SKINNER, D. C.; CARATY, A.yBOUCHARD, P. (2000). Neuroendocrine Effects of Progesterone. *Steroids*, *65*(10), 613-620.

CHACÓNJARAMILLO, L.;VARGASRONCANCIO, M.; OTERO, R.yVILLAMIL, A. (2005). Seguimiento de la dinámica del ovario por ultrasonografía en novillas de la raza Gir. *U.D.C.A Actualidad y Divulgación Científica, 8*(2), 103-110.

CHASOMBAT, J.; NAGAI, T.; PARNPAI, R.yVONGPRALUB, T.(2014).Ovarian Follicular Dynamics and Hormones Throughout the Estrous Cycle in Thai Native (*Bos indicus)*Heifers. *Animal Science Journal*, *85*(1), 15-24.

DISKIN, M. G.; KENNY, D. A.; DUNNE, L. Y SREENAN, J. M. (2002). *Systemic Progesterone Pre and Post AI And Early Embryo Survival in Cattle*. Proceeding of the Agricultural Research Forum. Tullamore, Ireland.

ECHTERNKAMP, S. E.; CUSHMAN, R. A.yALLAN, M. F. (2009). Size of Ovulatory Follicles in Cattle Expressing Multiple Ovulations Naturally and its Influence on Corpus Luteum Development and Fertility. *Journal of animal science*, *87*(11), 56-68.

EDMONSON, A. J.; LEAN, I. J.; WEAVER, L. D.;FARVER, T.yWEBSTER, G. (1989). A Body Condition Scoring Chart for Holstein Dairy Cows. *Journal of dairy science*, *72*(1), 68-78.

FAO(2015)**.** *Segundo informe sobre la situación de los recursos zoogenéticos mundiales para la alimentación y la agricultura*. Roma, Italia: FAO.

FORDE, N.; BAZER, F. W.;SPENCER, T. E. yLONERGAN, P. (2015).Conceptualizing the Endometrium: Identification of Conceptus-Derived Proteins During Early Pregnancy in Cattle 1. *Biology of Reproduction*, *92*(6), 152-156.

FORDE, N.; BAZER, F. W.; SPENCER, T. E.yLONERGAN, P. (2015). Conceptualizing the Endometrium: Identification of Conceptus-Derived Proteins During Early Pregnancy in Cattle. *Biology of reproduction*, *92* (6), 156-160.

GINTHER, O. J.; BEG, M. A.; BERGFELT, D. R.; DONADEU, F. X.yKOT, K.(2001).Follicle Selection in Monovular Species. *Biology of Reproduction*, *65*(3), 638-647.

GONELLA, A. G. (2010). Ambiente receptivo uterino: control materno, control embrionario, muerte embrionaria. *MVZ Córdoba, 15*(1), 1976-1984.

HERZOG, K.; BROCKHAN-LÜDEMANN, M.; KASKE, M.; BEINDORFF, N.; PAUL, V.; NIEMANN, H.yBOLLWEIN, H. (2010). Luteal Blood Flow is a More Appropriate Indicator for Luteal Function During the Bovine Estrous Cycle than Luteal Size. *Theriogenology*, *73*(5), 691-697.

IRELAND, J. J.; MIHM, M.; AUSTIN, E.; DISKIN, M. G.yROCHE, J. F. (2000). Historical Perspective of Turnover of Dominant Follicles during the Bovine Estrous Cycle: Key Concepts, Studies, Advancements and Terms. *Journal Dairy Science, 83*(7), 1648-1658.

KENYON, A. G.; MENDONÇA, L. G. D.; LOPES, G.; LIMA, J. R.; SANTOS, J. E. P.yCHEBEL, R. C.(2013)**.** Minimal Progesterone Concentration Required for Embryo Survival after Embryo Transfer in Lactating Holstein Cows. *Animal reproduction science*, *136*(4), 223-230.

LONERGAN, P.; FAIR, T.; FORDE, N. yRIZOS, D. (2016). Embryo Development in Dairy Cattle.*Theriogenology*,*86* (1), 270-277.

MANN, G. E.(2009).Corpus Luteum Size and Plasma Progesterone Concentration in Cows. *Animal reproduction science*, *115*(1), 296-299.

MCNEILL, R. E.; DISKIN, M. G.; SREENAN, J. M.yMORRIS, D. G.(2006).Associations Between Milk Progesterone Concentration on Different Days and with Embryo Survival During the Early Luteal Phase in Dairy Cows. *Theriogenology*, *65*(7), 1435-1441.

OIE(2016).*Código sanitario para los animales terrestres*.Retrieved on January 21, 2016, from http://www.oie.int/es/normas-internacionales/codigo-terrestre.

PEREA, F.; GONZÁLEZ, R.; CRUZ, R.; SOTO, E.; RINCÓN, E.;GONZÁLEZ, C.yVILLAMEDIANA, P. (1998). Evaluación ultrasonográfica de la dinámica folicular en vacas y en novillas mestizas.*Revista Científica FCV-LUZ, 8*(1), 14-24.

PERRY, G. A.; SMITH, M. F.; LUCY, M. C.; GREEN, J. A.; PARKS, T. E.; MACNEIL, M. D.yGEARY, T. W.(2005).*Relationship Between Follicle Size at Insemination and Pregnancy Success*. Proceedings of the National Academy of Sciences of the United States of America, *102*(14), 5268-5273.

PIERSON, R. A.yGINTHER, O. J.(1984).Ultrasonography of the Bovine Ovary. *Theriogenology*, *21*(3), 495-504.

PRIMO, A. T.(1992).El ganado bovino ibérico en las Américas: 500 años después. *Archivos de zootecnia*, *41*(154), 421-432.

QUEZADA-CASASOLA, A., AVENDAÑO-REYES, L., RAMÍREZ-GODÍNEZ, J. A., MACÍAS-CRUZ, U.yCORREA-CALDERÓN, A.(2014)**.** Behavioural, Follicular and Hormonal Characteristics of the Oestrous Cycle of Mexican Criollo Cattle. *Animal Production Science*, *54*(3), 277-284.

ROELOFS, J.; LÓPEZ-GATIUS, F.; HUNTER, R. H. F.; VANEERDENBURG, C. M.yHANZEN, C.(2010). When is a Cow in Estrus? Clinical and Practical Aspects. *Theriogenology*, *74*(3), 327-344.

SARTORI, R.; HAUGHIAN, J. M.; SHAVER, R. D.; ROSA, G. J. M.yWILTBANK, M. C.(2004). Comparison of Ovarian Function and Circulating Steroids in Estrous Cycles of Holstein Heifers and Lactating Cows. *Journal of dairy science*, *87*(4), 905-920.

SAVIO, J. D.; BOLAND, M. P.yROCHE, J. F. (1990)**.** Development of Dominant Follicles and Length of Ovarian Cycles in Post-partum Dairy Cows. *Journal of Reproduction and Fertility*, *88*(2), 581-591.

THATCHER, W. S. D. (1994). Embryo Health and Mortality in Sheep and Cattle. *Journal Animal Science, 72*(3), 16-30.

SIROIS, J.yFORTUNE, J. E.(1988).Ovarian Follicular Dynamics During the Estrous Cycle in Heifers Monitored by Real-time Ultrasonography. *Biology of reproduction*, *39*(2), 308-317.

VASCONCELOS, J. L. M.; SARTORI, R.; OLIVEIRA, H. N.; GUENTHER, J. G.yWILTBANK, M. C.(2001). Reduction in Size of the Ovulatory Follicle Reduces Subsequent Luteal Size and Pregnancy Rate. *Theriogenology*, *56*(2), 307-314.

WALSH, S. W.; WILLIAMS, E. J.yEVANS, A. C O. (2011). A Review of the Causes of Poor Fertility in High Milk Producing Dairy Cows. *Animal reproductionscience*, *123*(3), 127-138.

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Table 1. Mean (±SE) of POF (mm), CL (mm) and blood levels of P4 (ng/ml) in Holstein, Brown Swiss and Criollo heifers

	Breeds			
	Criollo	Brown Swiss	Holstein	P
Pre-ovulating follicle (POF)	14.6±0.41[b]	12.7±0.65[a]	12.7±0.47[a]	0.02
Corpus luteum on day 6 (CL)	18.3±0.79	17.2±1.41	16.9±0.98	0.67
Progesterone on day 6 (P4)	11.0±1.68[b]	3.2±1.44[a]	3.1±0.93[a]	0.00
Corpus luteum on day 12 (CL)	22.8±0.74	22.1±0.91	24.2±1.15	0.30
Progesterone on day 12 (P4)	18.4±2.04[b]	9.2±1.71[a]	7.2±0.85[a]	0.00

Different a;b letters mean a significant difference according to the Tukey´s test (5%); POF (mm); CL (mm); P4 (ng/ml); *Results corresponding to variance analysis (ANOVA)

Table 2. Correlation values between the pre-ovulating follicle, corpus luteum and blood progesterone on the 6th and 12th days post-ovulation, in all heifers (pooled).

	CL dia-meteron day 6 (mm)	P4 levels on day 6 (ng/ml)	CL diameter on day 12 (mm)	P4 level on day 12 (ng/ml)
POF size (mm)	0.61**	0.65**	0.38[ns]	0.67**
CL diameter on day 6 (mm)	--------	0.58**	0.53**	0.50**
P4 levels on day 6 (ng/ml)	--------	--------	0.38[ns]	0.89**
CL diameteron day 12 (mm)	--------	--------	-------	0.17[ns]

*The correlation is significant at 0.05; **the correlation is significant at 0.01; Pearson´s correlation test

Table 3. Correlation values between the pre-ovulating follicle, the corpus luteum and blood progesterone on days 6 and 12 post-ovulation in Holstein and Brown Swiss (foreign).

	CL diameter on day 6 (mm)	P4 levels on day 6 (ng/ml)	CL diameter on day 12 (mm)	P4 level on day 12 (ng/ml)

POF size (mm)	0.66**	0.60**	0.48*	0.59*
CL diameter on day 6 (mm)	---------	0.64**	0.51*	0.58*
P4 levels on day 6 (ng/ml)	---------	---------	0.53*	0.87**
CL diameteron day 12 (mm)	---------	--------	-------	0.29^{ns}

*The correlation is significant at 0.05; **the correlation is significant at 0.01; Pearson´s correlation test

Table 4. Correlation values between the size of the pre-ovulating follicle, corpus luteum and blood progesterone on the 6th and 12th days post ovulation in Criollo

	CL diameteron day 6 (mm)	P4 levels on day 6 (ng/ml)	CL diameter on day 12 (mm)	P4 level on day 12 (ng/ml)
POF size (mm)	0.35^{ns}	0.25^{ns}	0.45^{ns}	0.42^{ns}
CL diameter on day 6 (mm)	-------	0.79*	0.72*	0.62^{ns}
P4 levels on day 6 (ng/ml)	--------	--------	0.78*	0.71*
CL diameteron day 12 (mm)	-------	--------	-------	0.39^{ns}

*The correlation is significant at 0.05

Table 5. Simple linear regression analysis of pre-ovulating follicle size, corpus luteum and progesterone levels on days 6 and 12 in Criollo, foreign and pooled animals.

	Breeds					
	Pooled		**Foreign**		**Criollo**	
	R^2	**b >b**	**R^2**	**b >b**	**R^2**	**b >b**
Pre-ovulating follicle and corpus luteum on day 6	0.37	1.11**	0.46	1.46**	0.12	0.66^{ns}
Corpus luteum and progesterone on day 6	0.34	1.00**	0.41	0.63**	0.62	1.67*
Progesterone on days 6 and 12	0.8	1.11**	0.75	1.00**	0.51	0.87*
Corpus luteum on days 6 and 12	0.28	0.47**	0.26	0.46*	0.52	0.67*

**Significance at 0.01; *Significance at 0.05