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Original

Corporal condition and restart of the ovarian postpartum on Holstein cows in Ecuador

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ABSTRACT

Objective. To determine the influence of corporal composition at delivery (CCD) on the ovarian postpartum restart (OPR) of the Holstein cow in the Andes Region in Ecuador. **Materials and methods.** 30 cows were produced. All of the following moments were determined: the appearing of the dominant follicle (DF), the ovulation and luteal activity; the duration of the estrous cycle, the volume of the luteum body (LC) and the progesterone concentrations (P_4) on blood serum. Descriptive statisticians of the OPR variables and its indicators were compared according to the BC, by means of a t-Student test for independent samples. The relationship between the BC and the postpartum OPR through a case-control case was assessed. **Results.** The duration of the oestrous cycle was 23.10 days, 46.67% of the cows had regular cycles and 53.33% were abnormal cycles. The DF, the ovulation and the luteal activity were seen at 16.63, 27.76 and 41.38 after postpartum, respectively; before ($p<0.05$) on cows with BC ≥ 3.5;in which both the ($p<0.05$) and the volume of the LC and P_4 concentrations were higher. The BC was correlated with ($p<0.05$) with the OPR parameters. The cows with <3.5 BC points, are 10.50 times more prompt to have a late OPR than those with a ≥ de 3.5 points BC. **Conclusions.** The OPR was early, BC had an influence on it, which constituted a major ($p<0.05$) risk factor on cows having a late OPR.

Keywords: Dominant follicle; ovulation; luteal body; progesterone; corporal condition; oestrus cycle (*Source: DeCS, CAB).*

RESUMEN

Objetivo. Determinar la influencia de la condición corporal al parto (CCP) en el reinicio de la actividad ovárica (RAO) posparto de la vaca Holstein en la región andina de Ecuador. **Materiales y métodos.** Se trabajaron 30 vacas. Se determinaron el momento de aparición del folículo dominante (FD), de ovulación y de actividad luteal; la duración del ciclo estral, el volumen del cuerpo lúteo (CL) y las concentraciones de progesterona (P_4) en suero sangíneo. Los estadígrafos descriptivos de las variables del RAO y sus indicadores se compararon según la CCP mediante una prueba de t-Student

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para muestras independientes. Se evaluó la asociación entre la CCP y el RAO posparto mediante un estudio caso control. **Resultados.** La duración del ciclo estral fue 23.10 días, el 46.67% de las vacas tuvo ciclos normales y el 53.33% ciclos anormales. El FD, la ovulación y la actividad luteal ocurrieron a los 16.63, 27.76 y 41.38 días posparto, respectivamente; antes ($p<0.05$) en vacas con CCP ≥ 3.5; en las que fueron mayores ($p<0.05$) el volumen del CL y las concentraciones de P_4. La CCP se correlacionó ($p<0.05$) con los parámetros del RAO. Las vacas con CCP <3.5 puntos, tienen 10.50 veces más probabilidades de tener RAO tardío que las que tienen CCP ≥ de 3.5 puntos. **Conclusiones.** El RAO fue temprano, sobre el influyó la CCP, la que constituyó un factor de riesgo ($p<0.05$) para que las vacas tengan un RAO tardío.

Palabras clave: Folículo dominante; ovulación; cuerpo lúteo; progesterona; condición corporal; ciclo estral (*Fuentes: DeCS, CAB*).

INTRODUCTION

The most notorious events on the ovarian activity restart (OPR) are jealousy episodes, ovulation and development of the luteal body with progesterone secretion (P_4) (1). In the OPR of the cow, the main limitation is the reproductive efficiency and several factors can be considered as highly relevant. Between them, nutrition and corporal condition are two major examples (CC) (2).

Nowadays, there is not enough information about postpartum OPR on cows with pastoral activities; for this reason, it is necessary to establish the factors that influence the OPR in bovine females under these production conditions, in order to provide them with a reproductive and nutritional management that allow them to reach their maximum reproductive efficiency.

In the pastoral agricultural systems, the interactions among the chemical components in the soil determine the estate of the nutrients in the pasture and the animals that benefit from it. In these conditions, milky cows have an energetic deficit, as well as deficiencies, macro excesses and microelements that affect their fertility (3).

The chemical composition of the soil and in the pasture within agricultural ecosystems from the Andes region of Ecuador originate in milky cows an excess of proteins, as well as an energy deficiency, P, Cu and Zn; consequently, these show nutritional and metabolic alterations, as well as a low CC. In these herds, the main reproductive indicators indicate a postpartum anestrus prolongation (4,5). Additionally, the CC influences the uterine involution (6), which indicates that it can also affect over the postpartum OPR.

The objective of this study was to determine the influence of the corporal condition at delivery (CCD) in the postpartum OPR of the Holstein cow in the Andes Region of Ecuador.

MATERIALS AND METHODS

Research Setting. The study was carried out from November 2015 until April, 2016 at a Estate from the Tufiño Parish, Tulcan Canton, Carchi Providence; located between 1º 12′ and 43″ North Latitude (NL) and 78º 33′ 12″ West Latitude (WL), at a height of 2990 until 3450 msnm.

At the experimental area, the annual precipitations fluctuate between 1000 and 1250 mm in the rainy season (RS), from October to April, and from 700 to 850 mm in the dry season (DS), from May until September. The minimum temperatures are 2ºC and 15ºC, respectively, which range between 6º and 11ºC (7).

The area of study has an Andisol type of soil, with 70 cm of effective depth. Its terrain is ondulated with a slope percentage ranging between 10 and 20% (4).

Feeding and handle system. The animals were kept in rationed pasturing, with a global load of 2.5 UGM ha^{-1}. Naturalized pasture was predominant, *Pennisetum clandestinum L.* and *Holcus lannatus L.*, as well as the improved *Lolium perenne L.* and its cultivars "One fifty", *Dactylus glomeratta L.* and "Banquete", *Trifolium repens L.* No mineral energetic supplementation was administered.

All the pasture was watered with river water and fertilized with urea in 200 - 300 kg of N_2/ha^{-1}/$year^{-1}$. Two milkings were performed daily, manually. The first of them took place from 4 a.m. to 7 a.m. and the second one was from 3 p.m. until 6 p.m.The calves were left with their mothers until their third day of birth and from there, they were kept under artificial breeding.

Used animals. 30 Holstein cows were used, between their second and fourth lactation, with ages between 4 and 8 years, and a productive level of 15-18 L/cow^{-1}, BC between 3.0 and 4.0 in the scale of 5 points and clinically healthy. The postpartum OPR and its relationship with the BC was established.

In order to determine the general health status, all of the events linked to the birth and the puerperium were considered and carefully followed up, applying the functional invariables of the clinical method, including the review, anamnesis, inspection and palpation; as well as percussion and auscultation of organs and systems. Plus, all the deworming and vaccinations were valued, according to their area chronogram.

Blood sample taking and determination of progesterone (P_4). Blood was extracted via coccygeal venipuncture with vacutainer tubes, prior to an area cleaning and disinfection with 70% alcohol. 10 - 50 µL of blood serum was used and P_4 concentrations were determined every 7 days, from the seventh to the 120th day of postpartum (DP).

The P_4 was determined by an Electro chemiluminescence, using a Cobas e 411 analyzer (Roche, Germany), according to the manufacturer procedures. This method has an elevated precision (CV<10%), as well as a wide detection range (0-6 ng/mL).

Corporal Condition. The BC was estimated by inspection and palpation, classifying it in a 1-5 scale, as well as 0.25 divisions between them, based on the proposed methodology of Rodenburg (8).

Ovarian Activity. The postpartum OPR was established when the diameter of the dominant follicles (DF) was ≥5 mm; these were studied by means of an echography, until they were rallied or ovulated, or when the P_4 concentrations were higher than 1 ng/mL (9).

The first ovulation was established when the preovulatory follicle disappeared and, when in its place, an LC appeared (10). In addition, this first ovulation was also established when P_4 concentrations within the blood serum were higher than 1 ng/mL (9).

The beginning of the luteal activity was established when the P_4 concentration in blood serum was higher than 1 ng/mL during two consecutive samples (11).

The oastrial cycles after the first ovulation were considered to be normal when its duration was between 18 and 24 days, or abnormal (disciclia) when they were ≤ to 17 or ≥ 25 days (12).

The cows were classified into two categories in accordance with the time of the postpartum OPR, in line with the recommendations made by Guáqueta et al *(*13), who proposes that the $\bar{x}$ ± from the days of the first ovulation. This event occurred within the females under study at 27.76 ± 7.73 days, so they were considered as cows with early OPR.

The DF diameter and the LC volume were calculated with echography twice a week; a CTS-800 (SIUI, China) ultrasound was used, with a lineal transductor of 5 MHz, that delivers a good correlation between the quality of the picture and the depth, up to 11 Cm (14). The ultrasound being used offers the measurements of these ovarian structures automatically.

Statistical Procedure. The average ($\bar{x}$), standard deviation (SD) and variation coefficient (VC) of each variable from the OPR were calculated. A simple correlation between the CCD and the main indicators of the postpartum OPR and its indicators were compared according to the CCD with the t-Student test for independent samples. In all these procedures, the statistical software called Statgraphics Centurion Ver. XV.II was used.

A control case study was performed in order to determine the relationship between the cows CCD and the postpartum OPR. The frequency measures were obtained, as well as the impact and statistical significance with the use of EPIDAT 3.1 statistical package.

RESULTS

The first DF appeared at 16.63 days, with a 5.67±0.30 mm diameter, which reached its biggest size at 11.26 days, and the ovulation took place 11 days after the appearance of the

DF, at 27.76 days. The estrous cycle successive to the first ovulation had a duration of 23.10±4.99 days (Table 1).

Table 1. Behaviour of the main indicators of the Postpartum OPR of Holstein milky cows.

Parameters	$\bar{x}$	ED	VC
DF appearance (Days)	16.63	3.83	23.06
DF diameter in the emergency (mm)	5.67	0.30	5.36
Biggest diameter of the DF (mm)	11.26	0.50	4.51
Ovulation occurrence (days)	27.76	7.73	28.58
Luteal Activity (days)	34.73	9.24	26.61
Luteal body Volume (cm^3)	7.02	2.07	29.51
Serum Progesterone (ng/mL)	3.74	1.42	38.16
Oastric cycle duration (days)	23.10	4.99	21.61

The 46.67% of the cows had an estrous cycle that lasted between 18 and 24 days, while the other 53.33% presented cycles with a duration inferior or superior to this range; among them, a 6.67% ≤ than 17 days, a 40% between 25 and 30 days and a 6.67% with a time span longer than 30 days (Table 2).

Table 2. Oastric cycle duration frequencies distribution (days).

Intervals	RF	AF	Classification	Goal (%)
≤ 17	0.0667	0.0667	Short	< 10
18 - 24	0.4667	0.5333	Normal	65-70
25 - 30	0.4000	0.9333	Long	< 10
> 30	0.0667	1.0000	Long	< 10

RF: Relative frequency. AF: Accumulated frequency. The classification and goal according to the established parameters of Adams et al *(*12).

In cows with a CCD ≥3.5, the FD appeared at 15.35 days, while the first ovulation and luteal activity took place at 23.94 and 29.64 days, in that order; all these events occurred earlier ($p<0.05$) than in those with a CCD < 3.5 (Table 3).

In cows with a CCD ≥ 3.5, the highest DF diameter was 11.42 mm, with a superior ($p<0.05$) CCD than those with a CC <3.5 (Table 3). All the female cows with a CCD ≥ 3.5 had a class III DF, while those with a < 3.5 CCD, the 84.6% of them were classified as a class III, while the other 15.4% as class II.

Table 3. Postpartum ovarian activity ($\bar{x}$±EE) of Holstein milky cows according to the Corporal Condition at delivery.

Parameter	CCD		p-Value
	<3.5 (n=13)	≥3.5 (n=17)	
Appearance of DF (days)	18.30 ± 0.99[a]	15.35 ±0.87[b]	0.0340
DF diameter in the emergency (mm)	5.57 ±0.08[a]	5.74 ±0.07[a]	0.1456
Highest DF diameter (mm)	11.05 ±0.13[b]	11.42 ±0.11[a]	0.0466
Occurrence of the ovulation (days)	32.76 ±1.85[a]	23.94 ±1.62[b]	0.0012
Luteal activity (days)	41.38 ±2.00[a]	29.64 ±1.75[b]	0.0001
Lutean body volume (cm^3)	5.79 ±0.49[a]	7.97 ±0.43[b]	0.0025
Serum Progesterone (ng/mL)	3.16 ±0.37[a]	4.18 ±0.32[b]	0.0521

CCD: Corporal Condition at Delivery; ab different letters in the super indexes within each row, they indicate significant differences (t-Student for independent samples).

The CCD had a medium correlation with the appearance of the DF (r= -0.3881, R^2=15.06%, p=0.0341), the highest diameter of DF (r=0.366147; R^2 = 13.40%, p=0.0466), the occurrence of the first ovulation (x = -0.5605; R^2= 31.41%, p=0.0013) and high with the luteal activity (r = -0.6399, R^2 = 40.95%, p=0.0001), which indicates that the higher the CCD is, the time in which these ovarian activity indicators took place, expressed in days, was inferior. Plus, the more superior the CCD is, the higher FD diameter and, therefore, the bigger secretion of E_2 appears.

Likewise, the CCD had an average correlation (p=0.0026) with the VCL (=0.5299, R^2=28.08%), this last with P_4 concentrations of (r=0.4331, R^2=18.76%, p=0.0168); which indicates that, those animals with a better CCD have a higher P_4 secretion and, consequently, a superior postpartum fertility as well.

The CCD inferior to 3.5 points, constituted a risk factor for cows to have a late OPR (Table 4).

The Odds ratio was highly significant (p=0.0043), it indicates that animals with a CCD < 3.5 points have 10.50 more chances of having a late OPR from those who have a CCD ≥ of 3.5 points.

Table 4. Association of the postpartum OPR in the studied Holstein cows.

Parameter	Estimated value	IC 95%
Odds ratio (relative probability)	10.50	1.88-58.35
Attributable fraction in exposed ones	0.90	0.47-0.98
Population attributable fraction	0.67	-0.11-0.82

The attributable fraction over exposed ones (AFE), denotes the percentage of the cows in which the late OPR was provoked by having a CCD<3.5, while the population attributable fraction (PAF) shows the percentage of them within the total population that has been caused by a CCD< than 3.5 points, and, additionally, the magnitude in which the problem could be reduced provided that the cows had a CCD with ≥3.5 points.

DISCUSSION

The DF in this research appeared later than what it has previously been reported about Holstein cows in Colombia, in which it was detected at 9.58 ± 0.6 days when they had had an early OPR and at 10.8±1.50 days in which the OPR had appeared even later (13). The difference could be linked to the fact that in those consulted works, the animals used to receive supplementation with concentrates and mineral salts.

In this study, every DF that reaches the biggest size are qualified as class II, which are the ones with the biggest diameter ≥10 mm (15,16); considering the DF diameters in the emergency, the follicle growth rate was of one millimeter per day, similar to the one reported in Angus and Creole cows from Argentina by Robson et al (17).

The first postpartum ovulation (Table 1) with respect to the Holstein cows in Colombia occurred later than in those who had an earlier OPR, but sooner than those in which the period started later, 21.55 and 48.70 days, respectively (13).

Even if the postpartum OPR of the cow occurs earlier than expected and the first DF can be detected between 10 and 20 days after delivery; the percentage of the ovulation is low, occurring nearly 30 days after the delivery, this might actually be conditioned by a poor HL concentration and pulsatile discharge within this period (18).

At the early postpartum, the follicles are developed so they express receptors for the gonadotropic hormones (19); nevertheless, the first DF after the delivery does not generally ovulates due to a lack of HL. However, when it does, it happens after a silent zeal more than 70% of the time, with a low fertility at Artificial Insemination (15).

During the first postpartum weeks, there are no limitations related to follicle development caused by FSH deficiency. However, there are some related to HL deficiency, especially on BEN cows. The HL deficiency inhibits growth, maturation and ovulation of the DF from the first cohort (20).

The obtained results in this study show that the DF development until a pre-ovulatory size is not limited to a restart of the postpartum reproduction in Holstein cows, under these production conditions. These females have a more favorable reproductive behavior than those in which the postpartum OPR is produced after 35 DPP, as well as being those with the lowest pregnancy rates and, therefore, those with the highest delivery-pregnancy intervals (21).

According to Adams et al (12), 46.67% of the studied cows had regular cycles, between 18-24 days and the other 53.33%, the cycles were abnormal; in 6.67% of them were ≤ than 17 days, which allows them to be classified as short, while the rest are therefore classified as long, 40% of them between 25 and 30 days and 6.67% longer than 30 days.

The duration of the estrous cycle between two consecutive estrous ≤ 17 days could have been attributed to the fact that the first postpartum DF is developed, but in limited times this causes estrous manifestations. Nevertheless, ovulation occurs, LC is formed and an early luteolysis happens because of the premature liberation of endometrial $PgF_2\alpha$ (1). The 40% of those with ≥25 days are caused by port-service anestrus, just like the 6.67% of those with more than 30 days, which can also be attributed to a precocious embryonic death rate (22).

Differences within the postpartum ovarian activity parameters (table 3) can be motivated by changes in the CCD, this last for the energetic concentrations of the cows. Those with a CC <3.5 at the moment of delivery will have a more pronounced BEN and, even if the first postpartum ovulation was produced in them and consequently the LC formation, this would produce low levels of P_4 (1).

A P_4 deficit diminishes the secretion of GnRH and the hypophysis response to that hormone; this causes atypical waves and an insufficient HL preovulatory peak that impede growth, maturation and ovulation of the DF (20, 23). For these reasons, cows on BEN have lower P_4 and Estrogen (E_2) circulatory concentrations (19).

On the contrary, a cow with a CCD ≥ 3.5 points, has energetic reserves enough that guarantee the postpartum follicle development, as the positive energetic balance favorably influences the ovary and consequently, the hypothalamus is able to release GnRH, which stimulates the synthesis and liberation of FSH and HL by the hypophysis (19).

In Argentina, cross breed cows that delivered with a CC > than 2.5 points had a higher quantity of class III DF, ≥ 10 mm of diameter, bigger percentages of these with LC and held P_4 serum concentrations superior to 0.5 ng/mL from the 30 DPP. In those animals with a delivery CC of ≤ 2.5, however, the OPR was delayed (16).

In this research, it was proved that the CCD is correlated with the postpartum ovarian activity; corroborating the data obtained by Drescher et al (24), who found a correlation (p=0.0183) between the CCD and the sum of the class III DF in both ovaries.

In this study, it was shown that the CCD constitutes a significant risk factor for the late OPR, corroborating that association studies must be incorporated to the daily jobs, because they offer an exposure impact factor to a determined risk factor (25).

The postpartum CCD and BEN can affect both the OPR and the ovary-hypophysis-hypothalamus exe physiology. Consequently, they decrease the quantity of follicles of each follicle wave, the development of the DF and the pulse liberation of HL. Because of this, the postpartum ovulation gets delayed, and the LC activity is diminished, especially the P_4 production (26).

The productive levels of the flocks within this research are 15-18 L/cow^{-1} day^{-1}, which generate requirements that are not usually covered by a pasture based diet (4) and in these conditions, BEN is presented (24), which is expressed in the CCD, and has a major influence over the OPR.

It was concluded that the OPR is early, the DF, the ovulation and the luteal activity occurs in a period of time lower than in cows with a CCD ≥ 3.5, in which the LC has a major volume and the concentrations P_4 are superior. The CCD constituted a risk factor for cows having a late OPR.

Conflict of interests

The authors declare there were no conflicts of interest.

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