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RELATIONSHIP BETWEEN RETAINED PLACENTA AND BODY CONDITION CHANGES DURING THE TRANSITION PERIOD IN HOLSTEIN DAIRY COWS IN NORTH-EASTERN ALGERIA

Relación entre la retención de placenta y los cambios de condición corporal durante el periodo de transición en vacas lecheras Holstein del noreste de Argelia

Ahmed Hadef[1,2*], Kamel Miroud[1,3], Hocine Sebihi[4]

[1] Department of veterinary sciences, Faculty of nature and life sciences, University Chadli Bendjedid- El Tarf, , El Tarf, Algeria.
[2] Laboratory of Development and Control of Hospital Pharmaceutical Preparations, Faculty of Medicine, Badji Mokhtar University of Annaba, Algeria
[3] Laboratory « Epidémio-Surveillance, Santé, Productions et Reproduction, Expérimentation et Thérapie Cellulaire des Animaux Domestiques et Sauvages (ESSPRETCADS) », University Chadli Bendjedid- El Tarf, Algeria.
[4] DMV, veterinary practitioner, El Tarf province, Algeria.

* Corresponding author: Ahmed Hadef; e-mail: hadef_vet@yahoo.fr or hadef-ahmed@univ-eltarf.dz

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ABSTRACT

Retained placenta (RP) is a common complication of bovine parturition. It is a multifactorial reproductive disorder that predisposes to infection and hence, to infertility. The impact of body condition changes during the transition period on the risk of RP appearance was evaluated in 12 Holstein dairy cows calving from October to January in north-eastern Algeria. The animals were body condition scored (BCS) 3 weeks antepartum and two weeks postpartum to assess the peripartum body fat loss (BCS loss). The statistical analysis involving variables related to BCS and parity was performed using the principal component analysis (PCA). An RP critical occurrence of 41.66% exceeding significantly the threshold prevalence of 10% ($p<0.05$) was recorded. This could be related to the BCS loss that exceeded one unit over the transition period ($r=0.84$, $p<0.01$), expressing the high body fat mobilisation in response to stressor factors such as negative energy balance, feed availability and climatic condition. Scoring the body condition, particularly around the peripartum, may serve as a tool to monitor the risk factors related to the energetic status of dairy cows.

Keywords: *Body condition, dairy cow, retained placenta, transition period.*

RESUMEN

La retención de placenta (RP) es una complicación común del parto en bovinos. Es un trastorno reproductivo multifactorial que predispone a la infección y, por tanto, a la infertilidad. Se evaluó el impacto de los cambios de condición corporal durante el periodo de transición en el riesgo de aparición de RP en 12 vacas lecheras Holstein que parieron de octubre a enero en el noreste de Argelia. Los animales fueron sometidos a una puntuación de condición corporal (BCS) 3 semanas antes del parto y dos semanas después del mismo para evaluar la pérdida de grasa corporal periparto (pérdida de BCS). El análisis estadístico de las variables relacionadas con la condición corporal y la paridad se realizó mediante el análisis de componentes principales (ACP). Se registró una ocurrencia crítica de RP del 41.66% que superaba significativamente el umbral de prevalencia del 10% ($p<0,05$). Esto podría estar relacionado con la pérdida de BCS que superó una unidad durante el periodo de transición ($r=0,84$, $p<0,01$), expresando la alta movilización de grasa corporal en respuesta a factores de estrés como el balance energético negativo, la disponibilidad de alimento y las condiciones climáticas. La puntuación de la condición corporal, especialmente en torno al periparto, puede servir como herramienta para controlar los factores de riesgo relacionados con el estado energético de las vacas lecheras.

Palabras clave: Condición corporal, *vaca lechera, placenta retenida, periodo de transición.*

INTRODUCTION

Retained placenta is a reproductive disorder that is commonly defined as the persistence of foetal membranes longer than 12 hours (Noakes et al., 2018) or 24 hours post-calving (Fourichon et al., 2000). This multifactorial disease has been considered as one of the major risks affecting the profitability of dairy cattle farming through its impact on milk production (Rajala and Gröhn, 1998), development of postpartum diseases, reproduction performances (Han and Kim, 2005), and veterinary costs and culling rates (Guard, 1999; Tucho and Ahmed, 2017). Its incidence can overcome 10% (Kelton et al., 1998; Whitaker et al., 2000) that is why it's important to identify what causes it.

The transition period that lies from three weeks antepartum to three weeks postpartum, is a very critical period due to the physiological, metabolic and neuro-endocrinological implications of the adaptation process to the peripartum requirements (Drackley, 1999). Lipomobilisation is one of the physiological events expressed by a loss of body condition as recorded via the BCS technique. As a consequence, an inevitable negative energy balance (NEB) occurs during early lactation (Bell et al., 2018; Roche et al., 2013). When aggravated by inadequate and irrational feeding, this poor energetic status predisposes the dairy cows to a high risk of placental retention (Oetzel, 2004; Ospina et al., 2010). This has also been found in water buffaloes as reviewed by Morgado-Osorio et al. (2020).

The present work aimed to investigate the eventual relationship between changes in body condition during the transition period and the occurrence of foetal membrane retention in Holstein cows.

MATERIEL AND METHODS

Location and period of study

The study was carried out in an experimental dairy herd located in north-eastern Algeria, El Tarf province (36°46'10.9"N 7°51'14.8"E) during autumn and winter. This latter period has been characterised (Figure 1) by the lowest monthly temperature and the highest monthly rainfall over the last 20 years (Weather station-Annaba/2000-2020, retrieved from https://www.infoclimat.fr).

Animals and herd management

Out of fifty five cows present in the farm, twelve Holstein dairy cows aged from 2 to 9 years comprising four primiparous and eight multiparous that calved from October to January were followed up. Their parity has been studied as a variable. Their gestation length varied from 271 to 289 days (min-max) with an average of 277.22 ± 5.6 days.

Cows were reared in a semi-intensive way. When not at range, they were kept in barns with different boxes and exercise areas according to their physiological stages.

Food was distributed as a total mixed ration. About 8 to 12 kg of dry matter consisting mainly of silage (green barley or maize), oat-vetch mixture hay and concentrates (maize, barley, and soya) were given daily. The concentrate was supplemented with minerals and vitamins. Feeding depended on food availability and grazing which was irregularly practiced due to the climatic conditions. Ad-Lib watering was provided through automatic collective drinking troughs.

In the studied farm, all cows were periodically screened for infectious diseases such as brucellosis, tuberculosis, and leucosis. The data related to herd health did not reveal any clinical metabolic diseases affecting the periparturient cows followed up.

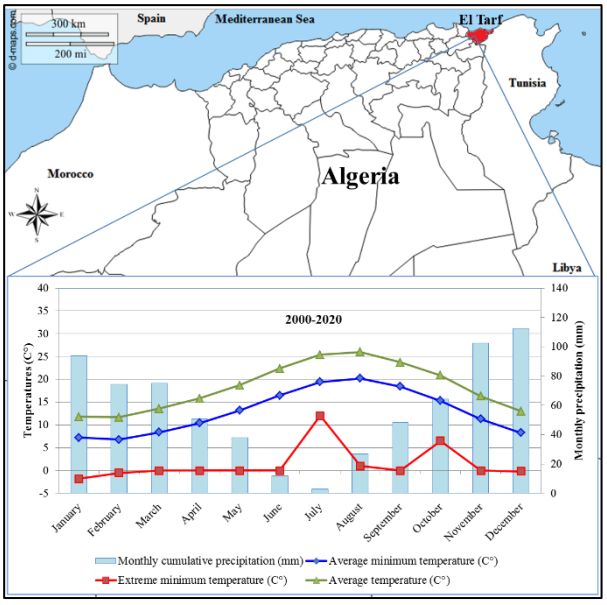

Figure 1. Area of study and its climatological data as regard to temperature and rainfall (2000-2020).

Cases definition of retained placenta (RP)

Cases of retained placenta recorded during the study period are those reported in cows that did not expel their foetal membranes 24 hours post calving as recorded in the farm's health data log.

All reported cases of retained placenta were medically monitored by the farm veterinarian until uterine involution was clinically completed at around day 30 PP.

Body condition scoring and lipomobilisation monitoring during the transition period

Body condition scoring during the peripartum (transition period) was based on the method described by Ferguson (1996) and Edmonson et al. (1989) using a scale from 1 to 5, where 1 corresponds to an emaciated body condition and 5 to a body condition that is too fat or obese while using a sub-unit of 0.25. BCS was done antepartum (BCS_AP) on average 20 days before calving and postpartum (BCS_PP) on average 15 days post-calving. The BCS final score retained used was the average between two scores given by two observers.

Lipomobilisation during peripartum was assessed by calculating the loss of body condition (Loss) expressed by the difference between the antepartum and postpartum BCS. This loss was considered as acceptable if not higher than 0.7 between the

drying off period and the puerperium (Kim and Suh, 2003; Šamanc et al., 2015); thus, a 0.75 unit was set as a comparison criterion between cows with and without RP.

Statistical Analysis

The Principal Component Analysis (PCA) using IBM SPSS Statistics 26 software was used to interpret the data matrix. The latter consisted of 12 observations (cows) and 5 variables (Transition Body condition Loss (Loss), Retained Placenta (RP), Antepartum Body Condition Score BCS (AP), Postpartum Body Condition Score BCS (PP), and Parity). The animals were treated as observations using identifiers (C1, C2, C3...and C12) according to the chronological order of their calving. The birth weight and sex of calves were not considered as variables since they were not intrinsically related to the energy status of the cow during the transition period.

The Student t-test was used to compare the BCS values obtained with references values as recommended by Ferguson et al. (1994) and, between groups of cows with retained placenta RP and non-retained NRP. Furthermore, the "z" test was used to compare the observed percentage of RP (cases prevalence) with the threshold RP prevalence's of 10% as cited previously. The significance level was set to 0.05 (5%).

RESULTS AND DISCUSSION

The Occurrence of retained placenta

The prevalence of RP recorded was 41.66%; among the latter 25% were primiparous and 16.66% multiparous; this was considerably higher when compared to the limit value of 10% as a tolerable standard in dairy cattle farming (Kelton et al., 1998; Rajala and Gröhn, 1998; Whitaker et al., 2000). As seen in figure 2, the observed reduced deviation (z=4.27) was greater than the theoretically reduced deviation (zα=1.96) for risk of error p=0.05. This difference means that the recorded prevalence of placental retention significantly exceeded the accepted norm. The occurrence of RP in the monitored herd was greater than the prevalence of 13.7% and 22% reported by Boudelal and Niar (2020) in the Algerian highlands (Medea, Tiaret and Tissemsilt regions) during the cold season and warm season respectively. A more or less similar percentage was recorded in Turkey (40.72%) but during the spring season (Yaşar et al., 2000).

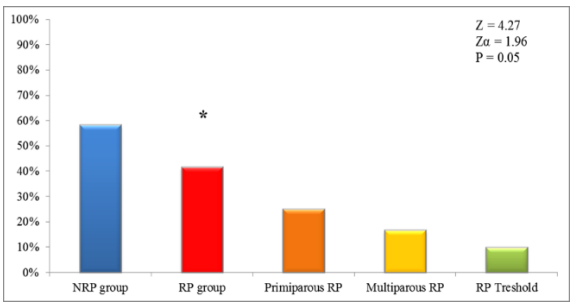

Figure 2. Prevalence of retained placenta during the study period. * Significant difference between the recorded prevalence of RP and their tolerated value using "z" test for p=0.05.

Profile of BCS changes

The 12 cows, irrespective of whether or not they had retention of foetal membranes, calved with a BCS significantly lower (p<0.01) than the optimal score recommended by Ferguson et al. (1994). All of them, showed also a deteriorated postpartum BSC significantly lower when compared to the threshold normal value (p<0.01). However, cows of the RP group were thinner in early lactation than those without (1.77 vs. 2.18 units condition score) although this difference was statistically not significant (p>0.05).

Along the peripartum transition period, the cows mobilized their fat reserve considerably; they lost, on average, up to 0.72 units body condition score (Table 1) which was not significantly different (p>0.05) from the reference limit value established by Šamanc et al. (2015). However, when RP cows were score conditioned separately, this average body BCS_Loss became highly significant (p<0.01) when compared to that of the NRP group (0.47 units) using t-test (p=0.001).

This situation could simply reflect the impact of food shortage (Schütz et al., 2013) since cows were fed according to food availability at the farm. Moreover, this critical nutritional status was probably aggravated by the practice of grazing in the harsh conditions of the cold season which required more fat mobilization to generate enough energy to cope with low temperature and long walking on difficult terrains. Beaver and Olson (1997) reported that cows grazing in absence of shelters and suffering from food shortages loose more backfat and weight. Under cold weather conditions, changes affect the pituitary-adrenal axis; basal plasma cortisol concentrations increase, and Adrenocorticotropic hormone (ACTH) decreases due to durable deprivation and feeding restriction (Fisher et al., 2002). Some metabolic changes appear because of restriction in lay downtime under cold weather: mainly higher faecal glucocorticoid metabolites, T4 and non-esterified fatty acid (NEFA) concentrations (Tucker et al. 2007). The latter authors reported also that thinner cows spent less time eating, had lower minimum body temperature and higher NEFA levels than high-conditioned cows when at the range under cold weather.

The results strengthen the importance of BCS as an on-farm indicator to monitor NEB in dairy cows as stated by different researchers (Clark et al., 2005; Loker et al., 2012; Morgado-Osorio et al., 2020).

Table 1. Changes in prepartum and postpartum BCS and BCS loss during the transition period in RP and NRP cows.

Variables	Mean ± SD	Target value	Groups	Mean ± SD	t-test (between groups)	P value
BCS_AP (n=12)	2.73 ± 0.43**	3.5[1]	NRP (n=7)	2.64 ± 0.56**	-0.95	0.38
			RP (n=5)	2.85 ± 0.14**		
BCS_PP (n=12)	2.01 ± 0.41**	3.0[1]	NRP (n=7)	2.18 ± 0.44**	2.12	0.06
			RP (n=5)	1.77 ± 0.21**		
BCS_Loss (n=12)	0,72 ± 0,38	0.75[2]	NRP (n=7)	0.47 ± 0.26*	-4.79	0.001
			RP (n=5)	1.08 ± 0.14**		

* Significant difference with target value $p<0.05$ using t-test
** Significant difference with target value $p<0.01$ using t-test
[1] Ferguson et al. (1994)
[2] Šamanc et al. (2015)

Relationship between retained placenta and other variables

Results of the correlation test (Table 2) showed a positive correlation between antepartum BCS and that of postpartum ($r=0.60$, $p<0.05$) indicating that fat mobilization in early lactation depended on pre-calving fat body reserve. This is in line with what Clark et al. (2005) reported in Holstein–Friesian cows. They recorded a rapid increase loss of BCS postpartum due probably to a loss of appetite. A significant decrease in fat mobilisation along lactations ($r=-0.61$, $p<0.05$) appeared to be related to BCS_AP reduced-fat amount in the dried-up cows ($r=-0.62$, $p<0.05$).

The appearance of RP seemed only and highly related to the BCS_Loss recorded during the transition period ($r=0.835$ and $r2=0.697$; $p<0.01$) as indicated by the high percentage (69.7%) of association between these two variables with respect to the other variables (Table 2). These results were confirmed by PCA (Table 3, Figure 3 and Figure 4).

Table 2. Matrix of correlation between the variables investigated.

Variables		RP	BCS_AP	BCS_PP	BCS_Loss	Parity
RP	r	1.00	0.25	-0.51	0.84**	-0.57
	P		0.44	0.09	0.001	0.05
BCS_AP	r	0.25	1.00	0.60*	0.50	-0.62*
	P	0.44		0.04	0.10	0.03
BCS_PP	r	-0.51	0.60*	1.00	-0.40	-0.09
	P	0.09	0.04		0.20	0.77
BCS_Loss	r	0.84**	0.50	-0.40	1.00	-0.61*
	P	0.00	0.10	0.20		0.03
Parity	r	-0.57	-0.62*	-0.09	-0.61*	1.00
	P	0.054	0.03	0.77	0.03	

**Correlation is significant at $p<0.01$.
*Correlation is significant at $p<0.05$

The factor analysis (Table 3) showed that factor axes 1 and 2 gave 90.09% of the total variance (54.70% and 35.39% respectively).

Axis 1 was well correlated with RP, BCS_Loss and parity. The variables RP and BCS_Loss were opposed to parity, which could indicate that axis 1 characterized the evolution of the body condition loss and the frequency of RP depending on parity (Figure 3).

Axis 2 was associated with BCS_AP and BCS_PP and indicating that the evolution of the postpartum body condition is related to the pre-partum BCS (Figure 3).

Table 3. Factors pattern of variables following factor analysis using PCA.

Variables	Factor 1	Factor 2
RP	**0,87**	-0,38
BCS_AP	0,62	**0,75**
BCS_PP	-0,21	**0,97**
BCS_Loss	**0,94**	-0,18
Parity	**-0,82**	-0,30
Eigenvalues	2,73	1,77
Proportion (%)	**54,70**	**35,39**

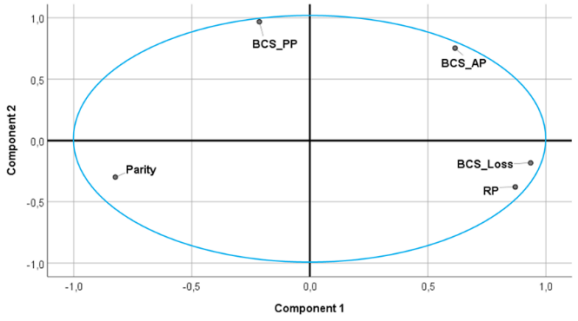

Figure 3. Component plot of studied variables using PCA.

The distribution of observations on factorial plan 1 and 2 revealed that RP group cows with the highest body condition loss that exceeds one unit of body condition (C1, C8, C9, C10) were contrary to cows without RP (C2, C4, C7 and C12) and with less fat loss (Figure 4).

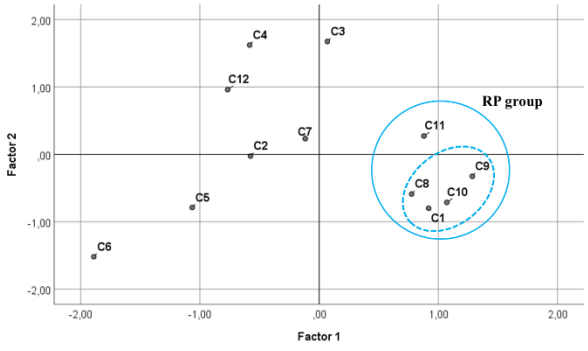

Figure 4. Observations plot using PCA. Continuous line circle includes RP cows (C1, C8, C9, C10, and C11) and discontinued line circle includes RP cows (C1, C8, C9, and C10) whose BCS loss ≥ 1 unit.

PCA showed that the alarmingly high prevalence of RP (41.66%) was strongly associated with the peripartum intensive body loss condition (loss ≥ 1 unit). As a result, an increase in ketones and blood lipid concentrations develops. This rise in NEFA level observed mainly during prepartum was reported to be associated with a high risk of placental retention occurrence (Ospina et al., 2010). The immunosuppressive effects of NEFA and ketones in dairy cattle during the periparturient period have been largely discussed by Ingvartsen and Moyes (2015). The effects of BCS and level of feeding before calving on the indicators of metabolic changes (liver function, stress) and immune function have been reported (Roche et al. 2015) and changes in neutrophil and Interleukin (IL)-8 were recorded in cows with RP (Kimura et al., 2002).

These changes seem to be related to the immune-suppressive effect of the rising cortisol level during the periparturient period leading to decreased phagocytosis of neutrophils, decreased cytotoxic ability of lymphocytes, as well as depressed activity of their cytokines, causing inefficient rejection of fetal membranes and their retention. Moreover, the activation of the hypothalamic-pituitary-adrenal cortex axis (HPAA) following the peri-calving stress causes metabolic modifications with a decrease or suppression of uterine tonicity via catecholamines (adrenalin) has also been considered in the physiopathology of RP not related to infectious disease (Mordak and Stewart, 2015).

Recently, Chebel (2021) confirmed the association between RP and functional deficiency of the immune system after noticing a diminution of the risk of this RP to happen in the absence of calving problems with an intense phagocytic activity of polymorphonuclear leukocyte and a low prepartum NEFA concentration.

Given the feeding practice applied in the herd investigated (food shortages and irregular food availability), a relationship between energy deficit and placental retention in Holstein dairy cows could be attributed to a decrease in the activity of enzymes involved in prostaglandin synthesis (PgF2α) due to liver steatosis (Chassagne and Barnouin, 1992).

In transition dairy cows (2 weeks before calving), fat mobilization due to a shortage of dietary energy intake led to an insufficient NEFA oxidation generating excessive amounts of ketones (β-hydroxybutyrate: BHB). These disorders have been associated with an increased risk of retained placenta (LeBlanc, 2006; LeBlanc et al., 2004; Schütz et al., 2013).

The results obtained were different from those reported by Chebel (2021) who observed that the BCS changes in the last 28 days of gestation ($p \geq 0.68$) were not associated with the likelihood of RP. However, the latter author noted his result in conditions where the prevalence of RP was close to the accepted limits (between 6% and 13% with the absence of cases in the Holstein cows).

Furthermore, the fact that PCA indicated that RP and parity were opposed led to a lesser risk of RP occurring which could be due to the negative correlation between parity and BCS loss in the present study ($r=-0.61$, $p<0.05$). However, it appeared from the correlation matrix that parity (lactation number) was not significantly correlated with placental retention occurrence ($r=-0.57$, $p>0.05$). This is in agreement with the results of Badinand and Sensenbrenner (1984), Han and Kim (2005) and Chebel (2021) who observed no difference in the occurrence of RP between primiparous and multiparous dairy cows, but not with the results of Gröhn et al. (1990) and Yaşar et al. (2000). A significant effect of parity on RP develops with age and its frequency increases with twin calvings, but not with single ones (Chassagne et al., 1996). The latter authors along with others (Gröhn et al., 1990) reported, as well, that the risk of placental retention increases with rising milk yield in dairy cows. In the present study, cows with RP were too young (2 or less than 4 years old) to consider the increase of the parity effect with age.

CONCLUSION

In conclusion, the higher fat mobilization during the transition period in the cold-weather season, the higher risk of RP occurrence usually claimed as a consequence of metabolic disorders and immune system dysfunction. Intensive fat mobilization (>1 unit body condition), is the major risk factor of RP appearance. In the absence of metabolic profiling and immune system assessment, body condition scoring appears to be a practical tool to monitor critical body fat loss and reduce the risk of RP occurrence.

CONFLICT OF INTEREST
The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS
AH and HS designed and carried out the study. AH analysed the data, KM and HS aided in the interpretation of the results. AH wrote the manuscript in consultation with KM and HS. All authors approved the final version of the paper.

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