Acta Scientiae Veterinariae, 2020. 48: 1750.

RESEARCH ARTICLE
Pub. 1750

ISSN 1679-9216

Physiological and Pathological Alterations in the Mammary Gland of Holstein Cows during Transition Period

Jean Silva Ramos[1], Karina Medici Madureira[2], Vinicius Alvim Passos Baldacim[1], Cynthia Pereira da Costa e Silva[1], Mailson Rennan Borges Dias[1], Cláudia Regina Stricagnolo[1] & Viviani Gomes[1]

ABSTRACT

***Background*:** Physiological and immunological adaptations occurred in peripartum period may be responsible for susceptibility to intramammary infections during colostrogenesis. Increase in blood flow and hormones cause physiological edema by accumulation of lymphatic fluid in interstitial space. Specific composition of mammary secretion increases the susceptibility to mastitis. So, the study of physical, cellular and microbiological changes in the mammary gland in this period are fundamental for understanding, diagnosing and adopting prophylactic measures for bovine mastitis. This study aimed to characterize physiological and pathological changes that occur in the mammary gland during transition period.
***Materials, Methods & Results*:** Thirteen cows, in second to fourth lactation were followed from 3 weeks pre-calving to 3 weeks post-calving. Cows were submitted weekly to physical examination of mammary gland, strip cup test, California Mastitis Test (CMT), Microscopic Somatic Cell Count (MSCC), and bacteriological examination of mammary secretion. Mammary quarters were divided into two groups: BAC^-: samples that did not present bacteriological growth throughout transition period; BAC^+: samples that presented bacteriological growth in at least two isolates of the same bacterial genus. Regarding physical examination of mammary gland, the parameters localized or generalized alteration in volume, asymmetry, reddish color, reduced skin elasticity, increased temperature, and swollen consistency presented higher frequencies in the BAC^- group. On the other hand, infection in mammary quarters of cows from BAC^+ group promoted a decrease in frequencies of increased volume, decreased elasticity, and elevated temperature in the mammary gland, in addition to a higher positivity to CMT and higher cellularity. Overall rate of intramammary infection was 25.82%, with non-aureus Staphylococci (NAS) being the predominant agent (69.2%). Maximum peak of bacterial isolates occurred immediately after calving (33.3%). Differences were observed between groups ($P = 0.021$) for CMT, showing a higher frequency of positive tests in BAC^+ group (36.5%) compared to BAC^- group (18%). MSCC varied between weeks ($P = 0.001$), groups ($P = 0.001$), and between the interaction of the two variables ($P = 0.019$). Highest median value of MSCC was observed at calving (1.5×10^6 cells/mL), being different ($P = 0.001$) from the +1, +2, and +3 weeks and the lowest median value of MSCC (0.08×10^6 cells/mL) was observed in +3 compared to previous weeks ($P < 0.05$).
***Discussion*:** NAS was predominantly isolated in mammary secretions of cows during peripartum, it is commonly found in the skin and canal of the teat and are associated with subclinical mastitis. Peak of mammary infection was observed on calving, probably by the cortisol peak resulting in a stress leucogram and decrease in the functional activity of immune cells. Changes observed in the physical examination of mammary gland of cows from BAC^- group are compatible with physiological edema caused by circulatory changes that occurs with the proximity of calving, increasing blood flow necessary for production of colostrum. Alterations in the mammary quarters of cows from BAC^+ group may be associated with functional loss of mammary parenchyma or persistent infectious process, characterizing clinical findings of pathological changes during transition period. Mammary quarters of the BAC^+ group presented higher percentages of positive reactions to CMT in weeks +1 and +2, and higher MSCC values and are associated with infection of the mammary gland.

Keywords: edema, intramammary infection, peripartum, physical examination.

DOI: 10.22456/1679-9216.102863

Received: 27 April 2020 Accepted: 8 July 2020 Published: 31 July 2020

[1]Faculdade de Medicina Veterinária e Zootecnia (FMVZ), Universidade de São Paulo (USP), São Paulo, SP, Brazil. [2]Escola de Medicina Veterinária e Zootecnia (EMEVZ), Universidade Federal da Bahia (UFBA), Salvador, BA, Brazil. CORRESPONDENCE: K.M. Madureira [karinamedici@yahoo.com.br]. EMEVZ - UFBA. Av. Adhemar de Barros n. 500. CEP 40170-110, Salvador, BA, Brazil.

INTRODUCTION

Mammary gland of cows can undergo successive renovations during cycles that comprise lactation: lactogenesis I (growth, differentiation and colostrogenesis), lactogenesis II (full lactation) and involution (non-lactating state). In lactogenesis I gestation induces changes in endocrine system responsible for proliferation of the mammary parenchyma [14] and IgG1 are transported by mammary epithelial cells through transcytosis four weeks before calving until calving [2].

Increase in blood flow and action of hormones involved in lactogenesis I cause accumulation of lymphatic fluid in interstitial space, promoting physiological edema in udder, making it more susceptible to physical damage [12].

Composition of colostrum and transitional milk are also unfavorable to defense system of mammary gland during peripartum, increasing the susceptibility to mastitis. Despite large number of somatic cells present in these secretions, there is a low proportion and reduced viability of neutrophils [4]. Predominant IgG1 in colostrum does not contribute to phagocytosis of bacteria by polymorphonuclear cells due to its low opsonizing activity [5].

Colostrogenesis may be a risk phase for mastitis in immediate postpartum and it is extremely difficult to differentiate physiological from pathological edema of udder, due to the similarity of clinical signs and inviability of tests that estimate the cellularity of mammary secretion in this period [17]. The aim of this study was to characterize physiological and pathological alterations in mammary gland of Holstein cows during transition period.

MATERIALS AND METHODS

Animals and conditions of study

The study was conducted between September and November on an experimental farm belonging to the Paulista Agency of Agribusiness Technology (APTA) located in the city of Nova Odessa - São Paulo, Brazil (latitude 22°75′S and longitude 47°27′W). Thirteen Holstein cows were selected, between second and fourth lactation, according to the date of insemination and calving prediction. Cows were monitored weekly at seven different times during the transition period, including calving (week -3 to week +3), and were submitted to physical examination of the mammary gland (n= 52 mammary quarters, 364 evaluations, weeks -3 to +3), examination of mammary secretion that included strip cup test (n= 156 samples, weeks +1 to +3), California Mastitis Test - CMT (n= 156 samples, weeks +1 to +3), microscopic somatic cell counts - MSCC (n= 208 samples, from calving to +3), and bacteriological examination (n= 364 samples, weeks -3 to +3).

Management procedures adopted during study were according to farm routine, including dry cow therapy using a product (Mastijet fort)[1] containing the antimicrobials tetracycline (200 mg), neominine (250 mg), and bacitracin (2000 IU) with 10 mg of prednisolone.

Cows were transferred to the maternity pen composed of *Cynodon* grass, 30 days before the expected calving. Regarding the welfare conditions, the absence of coverage was verified; except for the existence of some trees, there were no specific areas for parturition and the deliveries were not attended at night. After calving, animals were transferred to paddocks for newly calved cows and separated in batches according to dairy production.

Composition of the diet offered to cows during pre and postpartum was the same; however, the amounts of corn silage (prepartum: 20 kg, postpartum: 35 kg) and concentrate (prepartum: total equivalent to 3 kg, postpartum: 1 kg for each liter of milk produced) were different. The anionic balance of diet was not considered due to low rates of occurrence of hypocalcemia and retained placenta. Drench postpartum was also not provided.

Physical examination of the mammary gland was performed in a containment trunk, according to Grunert [8]. All mammary quarters were examined by inspection and palpation. Parameters used in the physical examination are presented in Table 1.

Samples and parameters

After physical examination, the cows were transferred to milking parlor in order to ensure the hygiene of the samples for bacteriological examination. Teats were predipped with 2% sodium hypochlorite solution and dried with individual paper towels; then the first mammary secretion jets were discarded for the strip cup test.

For bacteriological examination, 3 mL of mammary secretion was obtained in sterile plastic bottles with screw caps, after disinfecting teats with 70% alcohol [9] and it was performed according NMC [14].

Samples were considered to have an intramammary infection (IMI) if they had three or more colonies of the same type. Samples with three or more species on a plate were considered contaminated. If no bacterial growth was observed after 72 h on the initial culture the incubated sample was replated.

California Mastitis Test (CMT) and Microscopic Somatic Cell Count (MSCC) were performed from parturition. For CMT, samples with negative or traces results were considered negative because of the high cellularity expected for this phase of lactation, whereas 1+ to 3+ reactions were considered positive [21]. For MSCC, 50 mL of mammary secretion was obtained in conical plastic tubes [6].

Mammary quarters that did not present bacterial isolation at any of the study times (-3 to +3) were included in the BAC^- group, while those with at least two isolates of the same bacterial genus were grouped in the BAC^+ group.

Statistical analysis

Statistical analysis was performed using the Statistical Social Package Science program - version 18. Variables of the study were qualitative (binary and ordinal) and quantitative. Generalized estimating equations (GEE) from a generalized linear model (GLM) was used with a logistic model for qualitative binary and ordinal variables. The quantitative MSCC variable was tested for the type of distribution and adjusted for the best model according to the Akaike information criterion (AIC). For the latter, the model of distribution Gama on the GEE was adopted. Time and group were the predictor variables tested for effect and interaction. In the definition of the model, exponential parameters were proposed to obtain odds ratio (OR) and confidence interval.

RESULTS

During the study, some samples were discarded due to insufficient volume and manifestation of disease (ketosis) in three cows. In addition, during the sample division into the experimental groups (BAC^+ and BAC^-), eight mammary quarters needed to be excluded from the study because they had only one bacterial isolation, making it impossible to perform appropriate statistical tests. Thus, 302 samples were evaluated for bacteriological examination (151 samples in the BAC^+ group and 151 samples in the BAC^- group, between weeks -3 to +3), 303 physical evaluations of the mammary quarters (152 in the BAC^+ group and 151 in the BAC^- group, between weeks -3 to +3), 128 samples for CMT (63 in the BAC^+ group and 65 in the BAC^- group, between weeks +1 to +3), and 174 samples for MSCC (86 in the BAC^+ group and 88 in the BAC^- group, between the week of calving to the week +3).

Overall infection rate during transitional period was 25.82% (78/302), considering all mammary quarters evaluated between weeks -3 and +3. Mixed infections and those with a single bacterial agent were observed in 2.64% (8/302) and 23.17% (70/302) of the samples, respectively. Non-aureus Staphylococci (NAS) was isolated from 69.2% (54/78) of the total positive samples for bacteriological examination (n = 78); 10.3% (8/78) was *Corynebacterium* spp., 6.4% (5/78) was *Streptococcus* spp., 3.8% (3/78) was *Serratia marcescens*, and 10.3% (8/78) comprised associations between different microorganisms.

Distribution of bacterial isolates in each week of the transitional period revealed a maximum peak immediately after calving (33.3%, 16/48), with NAS being the main agent isolated. On the other hand, the second week postpartum (+2) was characterized by a lower infection rate (7.7%, 4/52). For other studied moments (weeks -3, -2, -1, +1 and +3), frequencies of bacterial isolates were 27.1; 23.1; 25.5; 21.2 and 17.3%, respectively.

The effect of the group (BAC^+ and BAC^-), evaluated weeks (-3 to +3), and interaction between groups and weeks on the physical parameters of the mammary gland, besides the odds ratio are presented in Table 1. The parameters generalized volume alteration, elasticity of the skin, and increase in temperature of the mammary gland differed between the experimental groups (P = 0.001; 0.011 and 0.003, respectively), with the highest frequencies occurring in the BAC^-. Evaluation of the effect of time (weeks) on the physical parameters revealed higher frequencies in the BAC^- group for generalized volume alteration (P = 0.001), localized volume alteration (P = 0.044), asymmetry (P = 0.001), reddish coloration of the mammary gland (P = 0.019), decreased elasticity (P = 0.001), increased temperature (P = 0.001), swollen consistency of the mammary gland (P = 0.001), and altered consistency of the teats (P = 0.001). No significant differences were observed when the interaction between groups and time was evaluated. The strip cup test was positive for only one mammary quarter at weeks +1 and +2 (BAC^+ group), setting as the only positive samples for clinical mastitis.

Table 1. Results of the physical examination of mammary gland of cows during the transition period.

Parameter		BAC+	BAC-	OR[1]	CI(95%)	Effects		
						Week (W)	Group (G)	W x G
Generalized V.	Changed	23(35/152)	45.7(69/151)	0.245	0.134-0.447	0.000*	0.000*	0.944
	Normal	77(117/152)	54.3(82/151)					
Localized V.	Changed	3.3(5/152)	4.6(7/151)	0.69	0.170-2.797	0.044*	0.604	0.959
	Normal	96.7(147/152)	95.4(144/151)					
Asymmetry	Changed	27.6(42/152)	31.8(48/151)	0.829	0.486-1.413	0.000*	0.49	0.687
	Normal	72.4(110/152)	68.2(103/151)					
Color	Reddish	2.0(3/152	8(12/150)	0.372	0.074-1.859	0.019*	0.228	0.342
	Normal	98(149/152)	92(138/150)					
Elasticity	Decreased	61.8(94/152	74.2(112/151)	0.474	0.259-0.868	0.000*	0.011*	0.092
	Normal	38.2(58/152)	25.8(39/151)					
Temperature	Increased	19.7(30/152	32.5(49/151)	0.349	0.173-0.703	0.000*	0.003*	0.509
	Normal	80.3(122/152)	67.5(102/151)					
Consistency	Swollen	46.7(71/152)	53(80/151)	0.430	0.181-1.020	0.001*	0.291	0.373
	Firm	17.1(26/152)	17.2(26/151)	0.939	0.398-2.214			
	Normal	36.2(55/152)	29.8(45/151)					
Injuries	Present	3.9(6/152)	7.3(11/151)	0.541	0.192-1.527	0.629	0.246	0.419
	Absent	96.1(146/152)	92.7(140/151)					
Lymph nodes	Increased (1)	27.6(42/152)	20.5(31/151)	0.544	0.235-1.260	0.387	0.453	0.908
	Increased (2)	13.8(21/152	30.5(46/151)	1.508	0.652-3.488			
	Normal	58.6(89/152)	49(74/151)					
Injuries - teats	Present	25(38/152)	19.9(30/151)	1.344	0.777-2.324	0.132	0.29	0.886
	Absent	75(114/152)	80.1(121/151)					
Supernumerary teat(s)	Present	0(0/152)	4.6(7/151)	-	-	-	-	-
	Absent	100(152/152)	95.4(143/151)					
Consistency - teat(s)	Thelitis	0.7(1/152)	2.6(4/151)	0.430	0.181-1.020	0.001*	0.291	0.373
	Cisternitis	3.3(5/152)	3.3(5/151)	0.939	0.398-2.214			
	Normal	96.1(146/152)	94(142/151)					

*$P < 0.05$ by test GEE; Generalized V.= Generalized volume; Localized V.= Localized volume; [1]OR= Odds ratio, 95% confidence interval (CI).

According CMT, differences were observed between groups ($P = 0.021$), with a higher frequency of positive tests in the BAC+ (36.5%, 23/63) compared to BAC- (18%, 12/65). BAC+ group had a greater chance of presenting a positive result during transition period (OR = 2,585, 95% CI = 1,153–5,797). However, time and interaction between time and groups were not significant for CMT ($P = 0.560$ and 0.640, respectively).

MSCC varied between studied weeks ($P = 0.001$), between groups ($P = 0.001$), and between interaction of the two variables ($P = 0.019$). Highest median value of MSCC was observed at the calving week (1.5×10^6 cells/mL, minimum value = 0.24×10^6 cells/mL and maximum value = 59.0×10^6 cells / mL) being different ($P = 0.001$) from the weeks +1, +2, and +3. The lowest median value (0.08×10^6 cells/mL, minimum value = 0.007×10^6 cells/mL and maximum value = $19{,}0 \times 10^6$ cells/mL) was observed in +3, compared to all previous weeks ($P < 0.05$) [Figure 1]. Comparing groups, in the postpartum period, the BAC+ had median values of 5.0×10^5 cells/mL (minimum value = 0.07×10^5 cells/mL and maximum value = 30.4×10^6 cells / mL), while the BAC- group had cell counts equivalent to 2.87×10^5 cells/mL (minimum value = 0.23×10^5 cells/mL and maximum value = 59.2×10^6 cells/mL).

DISCUSSION

This study evaluated physiological and pathological changes occurred in the mammary gland and milk examination of Holstein cows during transition period. To our knowledge, there are no published studies addressing the differentiation between physiological and pathological edema of the mammary gland, which directly aids in the diagnosis of clinical mastitis.

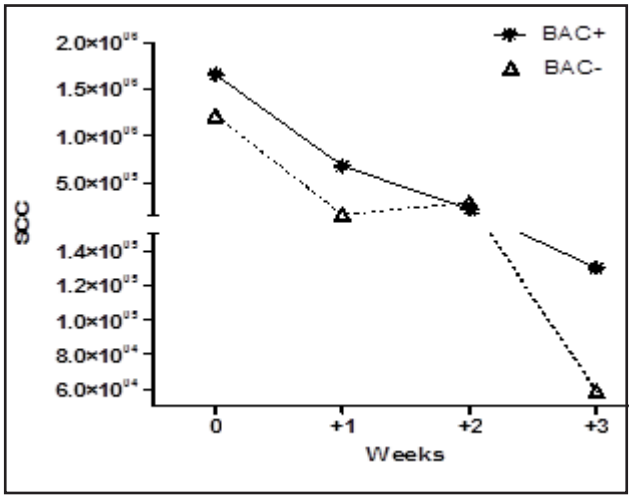

Figure 1. Median values of somatic cell count (SCC) of milk from BAC⁺ and BAC⁻ Holstein cows in postpartum period.

Overall rate of mammary infection for the transitional period was 25.82%. NAS were predominantly isolated in the mammary secretion of cows during peripartum (69.2%), similar to the frequency of 76.92% observed by Reis *et al*. [17] in the same property selected for the present research, while Odenstsen *et al*. [15] found frequencies of 31.7%, indicating that sanitary management of the property directly impacts the rates and type of bacterial agents that cause mammary infections.

NAS bacteria are commonly found in the skin and canal of the teat and are associated with subclinical mastitis [20]; this aspect was verified in the present study, since of the 78 positive samples in the bacteriological examination, only one presented a positive result in the strip cup test. A previous study showed that some factors are associated with NAS infections, such as primiparous cows and a history of high SCC in cows [16].

Cows evaluated in the present study were treated during the dry period with a commercial product containing antibiotics and corticoid[1]. However, although dry cow therapy was used to prevent new occurrence of intramammary infection between the dry period and subsequent onset of lactation [23], this was not sufficient to avoid bacterial growth occurring during the transition period. The antimicrobials present in the formulation of commercial product are broad-spectrum, but probable failures in milking management and resistance to antimicrobials may have compromised the effectiveness of dry cow therapy [19].

The peak of mammary infection was observed on the day of parturition (33.3%); the cortisol peak occurred due to the stress of calving, combined with the negative energy balance of the transition period, resulting in a stress leucogram and probable decrease in the functional activity of immune cells [7]. In addition to changes in immunity, physiological changes in the mammary gland itself such as increased volume, increased skin tension, and pendulous udders are risk factors for intramammary infection [12].

Circulatory changes occur with the proximity of calving, increasing blood flow to the mammary gland necessary for the production of colostrum. This event occurs weeks before calving and ceases abruptly on calving under hormonal influences [1]. These phenomena justify the changes observed in the physical examination of the mammary gland of cows belonging to BAC⁻ group, such as generalized or localized increase in mammary gland volume, asymmetry, reddish coloration, decrease in skin elasticity, increase in temperature, and swollen consistency, which are compatible with the postpartum mammary gland physiological edema observed in 95% of the cows [10].

The intensity of the physiological edema of the mammary gland can vary according to the severity of the changes, and can be classified in scales varying from 1 to 5 [3] or from 1 to 10 [22]. The presence of positive Godet signal is observed in edema, which mainly accompanies enlargement of the mammary gland, decrease in skin elasticity, and increase in temperature; these parameters were altered during the study period for the BAC⁻ group.

Mammary edema may also vary according to number of lactations, race, and season of the year; however, few studies have demonstrated changes in physical parameters during transition period, in terms of the differentiation between physiological and pathological edema [3, 10].

On the other hand, infection in the mammary quarters of cows belonging to the BAC⁺ group promoted a decrease in the frequencies of increased volume, decreased elasticity, and elevated temperature of the mammary gland and may be associated with functional loss of the mammary parenchyma or persistent infectious process, characterizing the clinical findings of pathological changes in the mammary gland during the transition period.

Some studies have demonstrated the impact of infection on the health of mammary gland, such as changes in physical characteristics of the milk, damage

to the mammary epithelial cells, and gene expression in these tissues [9,18]. Fundamental in the regulation of immune response, endothelial cells of mammary gland exert major changes in episodes of mammary infection, facilitating the passage of leukocytes and changing blood flow, factors that are directly associated with loss of mammary tissue [18]. Other impacts such as expression of genes in cases of infection of mammary gland have been studied, indicating that levels of expression depend on the duration of infection and type of bacteria [9].

Mammary quarters of the BAC^+ group presented higher percentages of positive reactions to CMT in weeks +1 and +2, in addition to higher MSCC values. These milk test results show that the increases in these parameters are associated with the infection of the mammary gland, indicating differences between groups.

The result obtained for MSCC of colostrum in the present study (1.5×10^6 cells / mL) differed from the values of 0.88×10^6 cells / mL observed by Gomes *et al*. [6]. The status of mammary healthiness and bacterial infection rate were responsible for the observed differences.

MSCC was higher in the colostrum compared to weeks +1, +2, and +3. The decrease in the MSCC in the first week postpartum has also been reported by Mehrzad *et al*. [11]. In the present study, low milk cellularity between weeks +1 to +3 is compatible with the low rate of mammary infection observed in the respective weeks. Accumulation of leukocytes during the colostrogenesis process and desquamation of the epithelial lining cells of mammary gland may be responsible for the high cellularity of colostrum. However, as milking progresses, the elimination of these cells, bacteria, and cellular debris accumulated in the mammary secretion and a subsequent decrease in milk cellularity occur. Physiological changes in cellularity in the immediate postpartum compromise the use of the MSCC for the detection of mastitis [17].

In general, the BAC^+ group presented a lower percentage in the frequencies of increased volume, decrease elasticity, elevated temperature of the mammary gland, and higher value of CMT, MSCC, and bacterial infection rate compared to the BAC^- group. These phenomena are probably related to the histological characteristics of the mammary tissue, associated with the chronic inflammatory process in the group of cows with mammary infection manifested during the transition period.

CONCLUSIONS

In this study we characterized physiological and pathological alterations in mammary gland and we observed during the physical examination higher frequencies of parameters such as generalized or localized alteration in volume, asymmetry, reddish coloration, decreased elasticity of the skin, increased temperature and swollen consistency in cows free of mammary infection and carriers of postpartum physiological edema. In the BAC^+ group, a lower frequency was observed for changes in volume, elasticity, and temperature, higher frequencies of samples positive for CMT test and higher cellularity, which are characteristics compatible with pathological alterations. This study contributes to the diagnosis of mastitis during the transition period through physical examination of the mammary gland and evaluation of its secretion.

MANUFACTURER

[1]MSD Animal Health, Boxmeer, Netherlands.

***Funding*.** This research was supported by grants from National Counsil for Cientific and Technological Development (CNPq; Project number 479381/2013).

***Ethical approval*.** The Animal Ethics Committee of the Faculty of Veterinary Medicine and Animal Science of the University of São Paulo - FMVZ/USP approved this research (protocol # 2767/2012).

***Declaration of interest*.** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of paper.

REFERENCES

1 **Barrington G.M., McFadden T.B., Huyler M.T. & Besser T.E. 2001.** Regulation of colostrogenesis in cattle. *Livestock Production Science*. 70(1): 95-104.

2 **Brandon M., Watson D. & Lascelles A. 1971.** The mechanism of transfer of immunoglobulin into mammary secretion of cows. *Australian Journal of Experimental Biology and Medical Scienc*. 49: 613-623.

3 **Dentine M.R. & McDaniel B.T. 1983.** Variation of edema scores from herd-year, age, calving month and sire. *Journal of Dairy Science*. 66(11): 2391-2399.

4 **Godden S.M., Smolenski D.J., Donahue M., Oakes J.M., Bey R., Wells S., Sreevatsan S. & Stabel J. 2012.** Heat-treated colostrum and reduced morbidity in preweaned dairy calves: results of a randomized trial and examination of mechanisms of effectiveness. *Journal of Dairy Science*. 95(7): 4029-4040.

5 **Gomes V., Madureira K.M., Soriano S., Della Libera A.M.M.P., Blagitz M.G. & Benesi F.J. 2011.** Factors affecting immunoglobulin concentration in colostrum of healthy Holstein cows immediately after delivery. *Pesquisa Veterinária Brasileira*. 31(1): 53-56.

6 **Gomes V., Madureira K.M., Della Libera A.M.M.P., Blagitz M.G., Alves M., Baptistella F. & Benesi F.J. 2011.** Dinâmica da celularidade do colostro de vacas da raça Holandesa no pós-parto imediato. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*. 63(5): 1054-1059.

7 **Gomes V., Miranda M.S., Sousa R.S., Fagliari J.J. & Madureira K.M. 2017.** Imunidade celular em vacas Holandesas soropositivas para o Vírus da Leucose Bovina (BLV) durante o período de transição. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*. 69(6): 1367-1375.

8 **Grunert E. 1993.** Sistema genital feminino. In: Dirksen G., Gründer H.D. & Stöber M. Rosenberger (Eds). *Exame Clínico dos Bovinos*. Rio de Janeiro: Guanabara Koogan, pp.269-314.

9 **Kościuczuk E.M., Lisowski P., Jarczak J., Krzyżewski J., Zwierzchowski L. & Bagnicka E. 2014.** Expression patterns of β-defensin and cathelicidin genes in parenchyma of bovine mammary gland infected with coagulase-positive or coagulase-negative Staphylococci. *BMC Veterinary Research*. 10(1): 246.

10 **Melendez P., Hofer C.C. & Donovan G.A. 2006.** Risk factors for udder edema and its association with lactation performance on primiparous Holstein cows in a large Florida herd, U.S.A. *Preventive Veterinary Medicine*. 76(3-4): 211-221.

11 **Mehrzad J., Dosogne H., Meyer E., Heyneman R. & Burvenich C. 2001.** Respiratory burst activity of blood and milk neutrophils in dairy cows during different stages of lactation. *Journal of Dairy Research*. 68(3): 399-415.

12 **Montero A.S. 2013.** Edema de ubre en ganado bovino: I entrega. *Nutrición Animal Tropical*. 7(1): 25-39.

13 **National Mastitis Council. 1999.** *Laboratory Handbook on Bovine Mastitis*. Madison: NMC, 222 p.

14 **Neville M.C., McFadden T.B. & Forsyth I. 2002.** Hormonal regulation of mammary differentiation and milk secretion. *Journal of Mammary Gland Biology and Neoplasia*. 7(1): 49-66.

15 **Odensten M.O., Berglund B., Persson Waller K. & Holtenius K. 2007.** Metabolism and udder health at dry-off in cows of different breeds and production levels. *Journal of Dairy Science*. 90: 1417-1428.

16 **Oliveira C.S.F, Hogeveen H., Botelho A.M., Maia P.V., Coelho S.G. & Haddad J.P.A. 2015.** Cow-specific risk factors for clinical mastitis in Brazilian dairy cattle. *Preventive Veterinary Medicine*. 121(3-4): 297-305.

17 **Reis J.F., Leite R.F., Silva C.P.C., Baccili C.C., Melville P.A., Benites N.R. & Gomes V. 2016.** Somatic cell counts as an indicator of mammary infection in periparturient cows. *Brazilian Journal of Veterinary Research and Animal Science*. 53(2): 161-168.

18 **Ryman V.E., Packiriswamy N. & Sordillo L.M. 2015.** Role of endothelial cells in bovine mammary gland health and disease. *Animal Health Research Reviews*. 16(2): 135-149.

19 **Rupp R. & Boichard D. 2003.** Genetics of resistance to mastitis in dairy cattle. *Veterinary Research*. 34(5): 671-688.

20 **Santos L.L.D., Costa G.M.D., Pereira U.D.P., Silva M.A. & Silva N.D. 2011.** Mastites clínicas e subclínicas em bovinos leiteiros ocasionadas por *Staphylococcus* coagulase-negativa. *Revista do Instituto Adolfo Lutz*. 70(1): 1-7.

21 **Schalm O.W. & Noorlander D.D. 1957.** Experiments and observations leading to development of the California Mastitis Test. *Journal of American Veterinary Medical Association*. 130: 199-204.

22 **Tucker W.B., Adams G.D., Lema M., Aslam M., Shin I.S., Le Ruyet P. & Weeks D.L. 1992.** Evaluation of a system for rating edema in dairy cattle. *Journal of Dairy Science*. 75(6): 2382-2387.

23 **van Hoeij R.J., Lam T.J.G.M., Koning D.B., Steeneveld W., Kemp B. & van Knegsel A.T.M. 2016.** Cow characteristics and their association with udder health after different dry period lengths. *Journal of Dairy Science*. 99(10): 8330-8340.