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RESEARCH ARTICLE

Changes in Immune Cell Composition During the Periparturient Period in Female Dromedary Camels

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ABSTRACT

In the dromedary she-camel, the impact of parturition on composition and phenotype of blood immune cells has not been evaluated so far. Therefore, the current study aimed to investigate the composition of blood leukocytes and their cell adhesion molecules expression in she-camels during the periparturient period. Using flow cytometry and membrane immunofluorescence, leukocyte composition and adhesion molecules expression were analyzed in blood samples collected from she-camels at days -28, -14, 0, +14 and +28 relative to calving. At parturition, she-camels showed a significant rise in the number of total leukocytes with increased numbers of neutrophilic granulocytes. Two weeks after calving, the number of total lymphocytes was significantly elevated. Among lymphocytes, the numbers of CD4+ T cells and B cells were expanded. Although the cell number of total monocytes did not change significantly during the periparturient period, significant differences in the fractions of monocyte subsets were observed. The number of the $CD14^{high}$ $MHCII^{low}$ monocyte subset was significantly decreased at calving and remained at a low abundance during the postparturient time, while the numbers of the $CD14^{high}$ $MHCII^{high}$ and the $CD14^{low}$ $MHCII^{high}$ monocyte subsets was increased after calving. For neutrophils (at calving and at day +14 after calving) and monocytes (at calving), a significant increase in the expression of the cell adhesion molecule CD11a was observed. Collectively, the present study identified several alterations in the leukogram and distribution of blood monocyte and lymphocyte subpopulations during the periparturient period in dromedary she-camels.

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INTRODUCTION

The periparturient period is characterized by major changes in different body systems including the immune system (Jongh *et al.*, 1996; Lessard *et al.*, 2004; Caroprese *et al.*, 2015; Hernandez-Castellano *et al.*, 2019). For different veterinary species, including the cow (Jonsson *et al.*, 2013; Eger *et al.*, 2015), the sheep and goat (Hernandez-Castellano *et al.*, 2019), the sow (Schalk *et al.*, 2019), and the mare (Bazzano *et al.*, 2014), considerable research has been conducted into the time-dependent changes in systemic immunity around parturition. The leukogram of the dairy cow at calving is characterized by increased numbers of leukocytes, which is mainly due to higher numbers of neutrophils and monocytes (Meglia *et al.*, 2001). Also in mares, leukocyte count is significantly increased around foaling. While the neutrophil fraction continuously increased starting from the 4th week prepartal and peaked at the time of foaling, the lymphocyte fraction gradually decreased around parturition (Bazzano *et al.*, 2014). In addition, parturition is associated with significant changes in the composition of blood lymphocytes and monocytes in cows (Ohtsuka *et al.*, 2004; Eger *et al.*, 2015) and sows (Ohtsuka *et al.*, 2004).

For the dromedary camel, different subpopulations of blood leukocytes have been recently characterized (Hussen *et al.*, 2018; Hussen *et al.*, 2020). The camel monocyte population consists of three different subsets with

phenotypic and functional differences (Hussen *et al.*, 2020). The main fraction of camel monocytes expresses high levels of CD14 and CD163, but low levels of MHCII (Mo-I, $CD14^{high}$ $CD163^{high}$ $MHCII^{low}$). Camel inflammatory monocytes display a high expression density for all three markers (Mo-II, $CD14^{high}$ $CD163^{high}MHCII^{high}$). The third monocyte fraction shows low expression of CD14 and CD163 but high MHCII expression (Mo-III, $CD14^{low}CD163^{low}MHCII^{high}$) (Hussen *et al.*, 2020).

Adhesion molecules are cell surface molecules with essential roles in leukocytes adhesion and migration (Ley *et al.*, 2007; Soehnlein and Lindbom, 2010; Amulic *et al.*, 2012; Muller, 2013; Mitroulis *et al.*, 2015; Eger *et al.*, 2016; Hussen *et al.*, 2016; Kourtzelis *et al.*, 2017). CD11a dimerizes with CD18 to form the adhesion molecule lymphocyte function antigen-1 (LFA-1) expressed on all leukocytes (Roos and Law, 2001; Ley *et al.*, 2007; van de Vijver *et al.*, 2012; Muller, 2013).

In the dromedary she-camel, the impact of parturition on the composition of blood immune cells has not been evaluated so far. Therefore, the current study aimed at the evaluation of the leukogram and the composition of blood lymphocytes and monocytes during the periparturient period in she-camels.

MATERIALS AND METHODS

Animals and blood sampling: Seven multiparous she-camels (*Camelus dromedarius*) housed at the farm of the Camel Research Center, King Faisal University, Saudi Arabia were involved in the present study. Blood was collected from each animal at days -28 and -14 relative to predicted calving time, at calving day, and postpartum at days +14 and +28. Blood was obtained by jugular vein puncture into EDTA-containing vacutainer tubes (Becton Dickinson, Heidelberg, Germany). All experimental procedures and management conditions used in this study were approved by the Ethics Committee at King Faisal University, Saudi Arabia (Permission number: KFU-REC/2019-10-01).

Cell separation: Separation of whole camel leukocytes was performed after removal of blood erythrocytes using hypotonic lysis (Hussen *et al*., 2017). Blood samples (4 ml) were diluted in phosphate buffered saline (PBS) (1:2) and were then centrifuged at 4°C for 10 min at 1000 xg without break. After centrifugation, the supernatant was descanted and the cell pellet, including white and red blood cells, was suspended in 10 ml distilled water for 20 sec (hypotonic shock to lyse red blood cells) followed by the addition of double concentrated PBS to restore tonicity. This was repeated (usually twice with centrifugation at 500 xg and 250 xg for each 10 min with break) until complete erythrolysis. Separated leukocytes were finally washed with 10 ml PBS (centrifugation at 100 xg for 10 min with break) and the cell pellet was suspended in membrane immunofluorescence (MIF) buffer (PBS containing bovine serum albumin (5 g/l) and NaN_3 (0.1 g/l)) at 5 x 10^6 cells/ml. The mean viability of separated cells was evaluated by flow cytometry using the dye exclusion method of propidium iodide (2 µg/ml, Calbiochem, Germany) and was consistently above 95 %.

Cell counting of leukocytes: After diluting camel blood in PBS (1: 4) and mixing with Türk's solution (final dilution 1:20; Merck Millipore), 10 µl of the mixture was poured onto the hemocytometer (Neubauer cell counter). Leukocytes were identified by microscopic analysis (cells in blue color), and cells were counted in four big squares of the cell counter. The total leukocyte count was calculated as 1000 cells per µl blood (Camacho-Fernandez *et al*., 2018).

Membrane immunofluorescence and flow cytometry: In the current study nine commercially available antibodies with cross-reactivity against camel leukocyte antigens (Hussen *et al*., 2017; Hussen *et al*., 2018; Hussen *et al*., 2020) were used for cell labeling (Table 1). Separated leukocytes (5 x 10^6 / ml) in MIF buffer were labeled with two combinations of monoclonal antibodies: (anti-CD4 & anti-WC1) and (anti-CD172a & anti-MHCII) in 96 well round-bottom microtiter plates (1 x 10^6 / well; 20 min; 4°C) (Eger *et al*., 2015; Hussen *et al*., 2018). After incubation with primary unlabeled antibodies, cells were washed twice and incubated with secondary antibodies specific for murine IgG1, IgM, or IgG2a (BD) labeled with different fluorochromes. After washing the cells, directly labeled monoclonal antibodies to CD11a and CD14 were added. Finally, cells were washed and analyzed by flow cytometry (FACSCalibur, Becton Dickinson Biosciences). For each measurement, 100 000 events were acquired and data were analyzed with the flow cytometric software FCS Express software Version 3 (De Novo Software, Thornton, Ontario). After microscopic estimation of total leukocyte counts (using Türk Solution and Neubauer counting chamber), absolute cell counts of leukocyte subsets were calculated based on their relative fractions determined by flow cytometry according to an established gating strategy (Hussen *et al*., 2018; Hussen *et al*., 2020). Briefly, camel granulocytes and mononuclear cells were gated based on their scatter characteristics in a side scatter height (SSC-H) against forward scatter (FSC)-H dot plot. After setting a gate on granulocytes, camel neutrophils and eosinophils were identified within the granulocyte population based on their different autofluorescence in FL-1. Within the mononuclear cells population, monocytes and lymphocytes were identified as CD14-positive and CD14-negative mononuclear cells, respectively.

Statistical analyses: Statistical analysis was performed using the software Graph Pad Prism 5.01 (Graph Pad Software, San Diego, CA, USA). Data were checked for Gaussian distribution using the Shapiro-Wilk normality test and analyzed with repeated measurements one-way ANOVA. The Bonferroni post-test was used to analyze time-dependent differences within groups. Data are presented as means ± SEM.

RESULTS

Cell count of blood leukocytes and their main populations in she-camels around parturition: Total and differential cell counting of blood leukocytes revealed significant alterations during the periparturient period in dromedary camels. The number of total leukocytes

increased continuously from day -28 to day +14, peaked at day +14 (P<0.05, compared to day +14) and remained slightly elevated until day +28 (Fig. 1A). The number of neutrophils raised at parturition (day 0) to a significantly (P<0.05) higher value in comparison to neutrophil count at day -28 and -14 and remained significantly higher at day +14 and +28 (P<0.05; Fig. 1B). For lymphocytes count, a significant rise was observed at day +14 (P<0.05, compared to day 0, Fig. 1D). No significant time-dependent changes were found in the number of eosinophils (Fig. 1C) or total monocytes (Fig. 1E) during the periparturient period.

Changes in the numbers of lymphocyte subpopulations during the periparturient period: Although the number of total lymphocytes did not show significant changes during the studied periparturient period, lymphocyte composition was significantly affected by parturition. The number of $CD4^+$ T cells increased significantly at day -14 (P<0.05, compared to day -28), followed by a slight decrease at day 0. After parturition, the number of $CD4^+$ T cells peaked at day +14 ((P<0.05, compared to day -28) and decreased slightly at day +28 (Fig. 2A). Although a slight decrease in their count was observed at parturition, the number of $WC1^+$ T cells (γδ T cells) did not change significantly during the studied period (Fig. 2B). For the number of B cells, a significant increase was observed at day +14 (P<0.05, compared to day -28, Fig. 2C).

Changes in monocyte subsets during the periparturient period: The periparturient period was associated with significant changes in the composition of blood monocytes. Before parturition, the monocyte population composed mainly (approximately 80% of the total monocytes count) of $CD14^{high}$ $MHCII^{low}$ monocytes (camel Mo-I) with minor fractions (4-6% of total monocytes count) of $CD14^{high}$ $MHCII^{high}$ (camel Mo-II) and $CD14^{low}$ $MHCII^{high}$ monocyte subset (camel Mo-III). The number of the $CD14^{high}$ $MHCII^{low}$ monocyte subset decreased significantly at parturition and remained low postpartum at day +14 and +28 (P<0.05, compared to day -14, Fig. 3A). The number of the $CD14^{high}$ $MHCII^{high}$ showed a significant increase at day -14 (P<0.05, compared to day -28) followed by a slight decrease at day 0. After parturition, Mo-II count started to rise at day +14 and peaked at day +28 (P<0.05, compared to day -28, Fig. 3B). For the $CD14^{low}$ $MHCII^{high}$ monocyte subset, a significant increase was found at day +14 (P<0.05, compared to day -28, Fig. 3C).

Adhesion molecules expression on the main leukocyte populations during the periparturient period: CD11a expression on neutrophils started to increase at day -14, peaked at parturition (P<0.05, compared to day -28) and remained high at day +14 (P<0.05, compared to day -28) with a slight decrease at day +28 (Fig. 4A). Although CD11a expression on lymphocytes showed a continues decrease until day +14 followed by a slight increase at day +28, the changes were not statistically significant (Fig. 4B). For CD11a expression on monocytes, the MFI values increased slightly at day -14 and remained high until day +28 (changes were only significant at day 0 compared to day -28, Fig. 4C).

DISCUSSION

For the dromedary she-camel, limited information is available on the impact of parturition on immune cell composition and phenotype in peripheral blood. The aim of the current study was to follow up the time-dependent changes in the leukogram pattern and the composition of lymphocytes and monocytes subsets in blood of she-camels during the periparturient period.

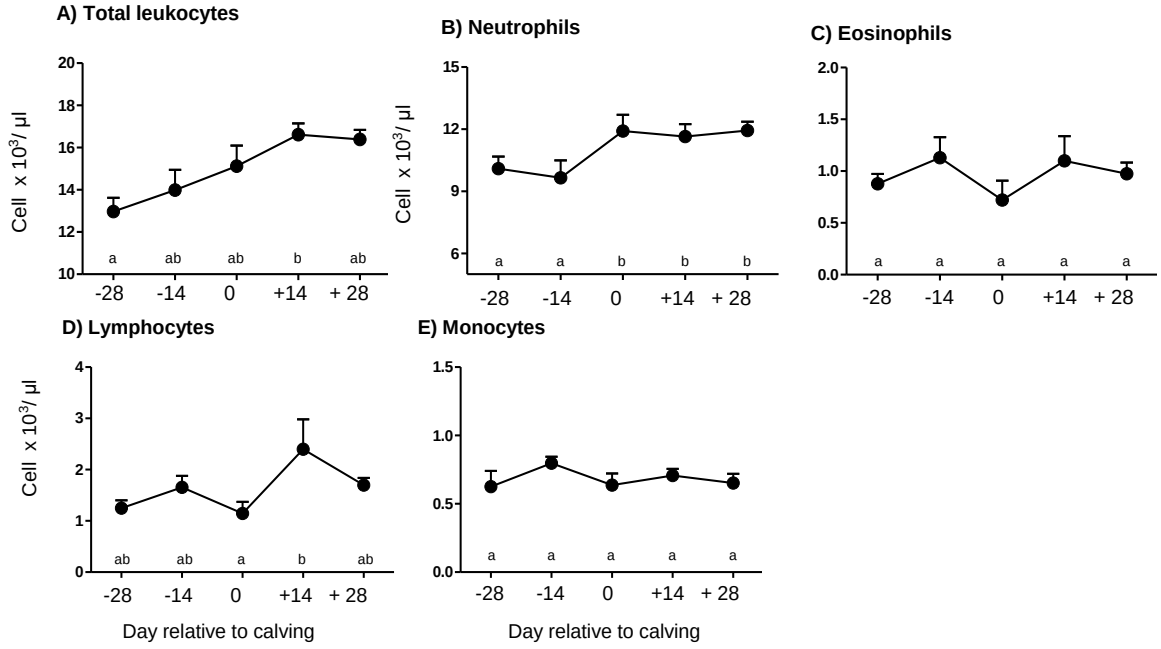

Fig. 1: Absolute cell count of the main populations of camel blood leukocytes. Blood samples were collected from dromedary she-camels at days -28 and -14 relative to predicted calving, at calving day and postpartum at days +14 and +28. Total blood leukocytes of she-camels were counted under microscope and were presented graphically (A). Absolute counts of blood neutrophils (B), eosinophils (C), lymphocytes (D) and monocytes (E) were calculated after flow cytometric estimation of their percentages and calculating their absolute numbers relative to total leukocyte count. Cell counts were presented graphically. Differences between groups were considered significant (*) if P<0.05.

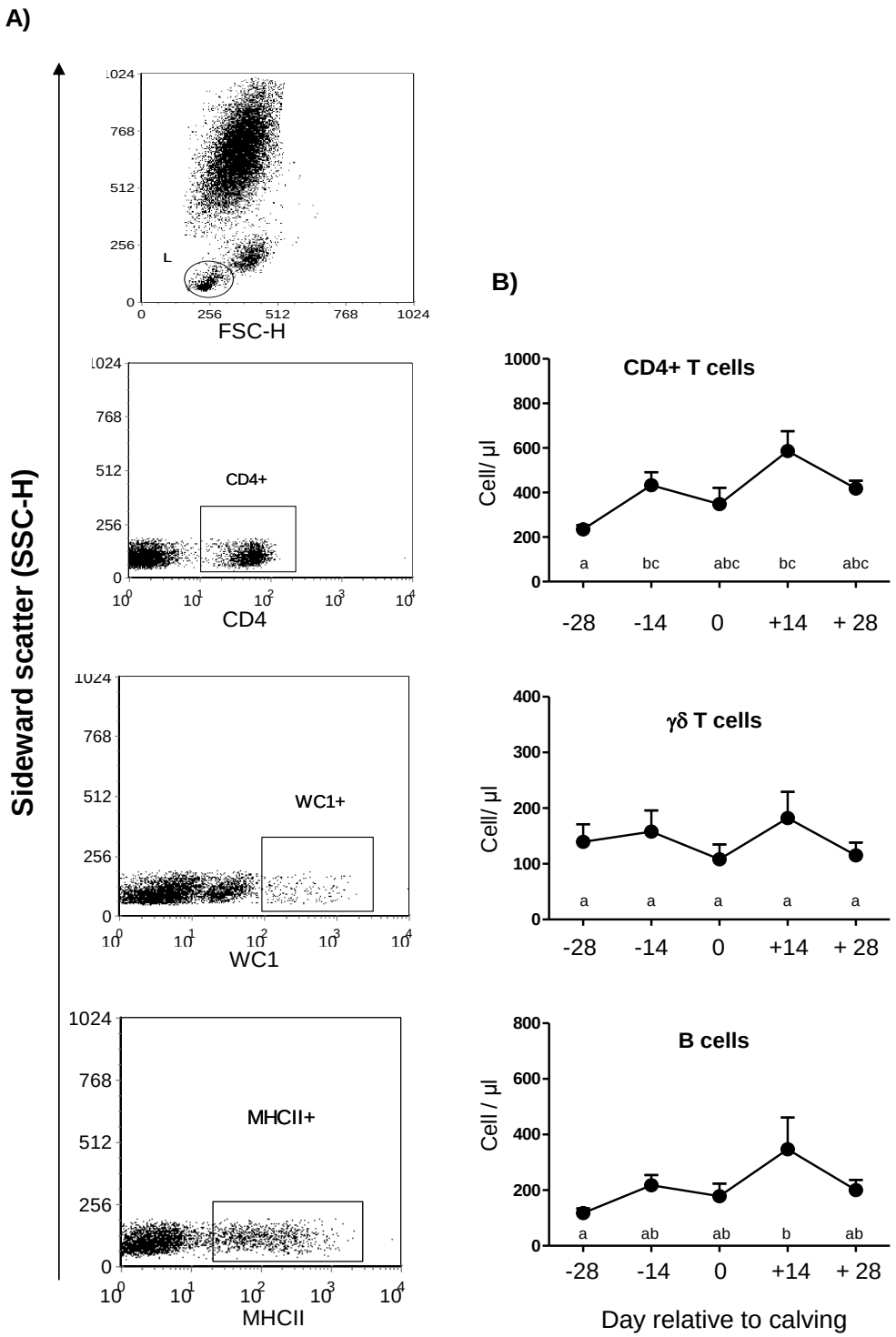

Fig. 2: Cell count of camel lymphocyte subpopulations around parturition. A) After gating on lymphocytes based on their FSC/SSC properties, camel CD4+ T helper cells, γδ T cells (WC1+), and B cells (MHCII+) were identified based on their specific labeling with monoclonal antibodies. B) The absolute numbers of CD4-positive T helper cells (A), γδ T cells (B), and B cells (C) were calculated and presented for the days -28 and -14 relative to predicted calving, for the calving day and postpartum for the days +14 and +28. Differences were considered significant (*) if P<0.05.

Table 1: List of antibodies

Antigen	Antibody clone	Labelling	Species reactivity	Source	Isotype
CD172a	DH59b	-	bovine	Kingfisher	mIgG1
CD14	Tuk4	PerCP	bovine	Biorad	mIgG2a
MHCII	TH81A5	-	bovine	Kingfisher	mIgG2a
CD11a	G43-25B	PE	human	BD	mIgG2a
WC1	BAQ128A	-	bovine	Kingfisher	mIgG1
CD4	GC50A	-	bovine	Kingfisher	mIgM
mIgG2a	polyclonal	PE	mouse	Invitrogen	gIgG
mIgG1	polyclonal	FITC	mouse	Invitrogen	gIgG
mIgM	polyclonal	APC	mouse	Invitrogen	gIgG

Ig: Immunoglobulin; m: mouse; MHC-II: Major Histocompatibility Complex class II, g: goat.

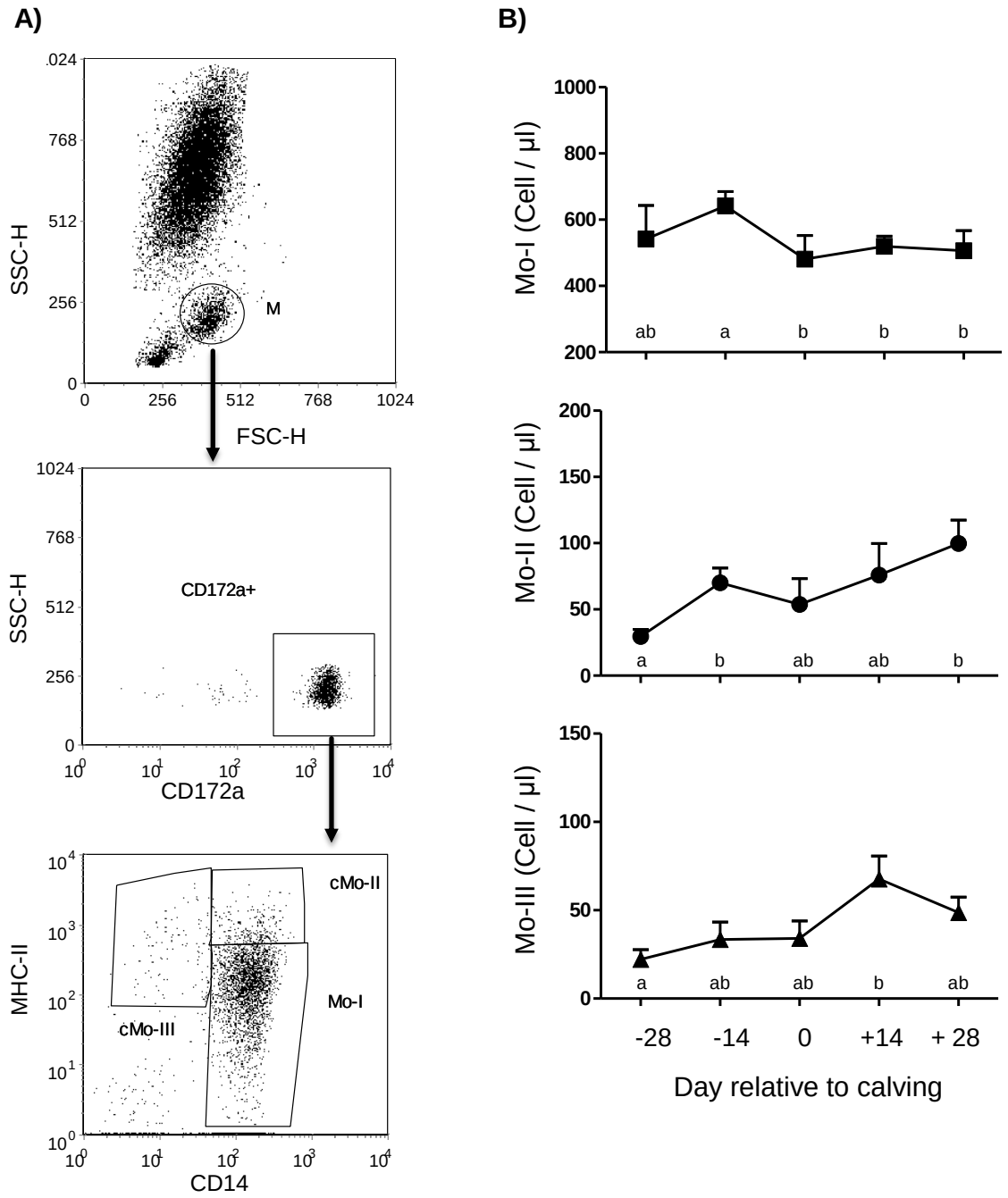

Fig. 3: Cell number of camel monocyte subsets in blood of she-camels around calving. A) In a FSC/SSC dot plot, a gate was formed on camel monocytes. In a separate dot plot, monocytes were identified as CD172a+ cells. In a CD14/MHCII dot plot, camel Mo-I ($CD14^{high}MHCII^{low}$), Mo-II ($CD14^{high}MHCII^{high}$), and Mo-III ($CD14^{low}MHCII^{high}$) were identified. B) Total numbers of camel monocyte subsets in blood of she-camels were calculated and presented for the periparturient period. Differences between groups were calculated using the one-way ANOVA test. Different lowercase superscript letters indicate statistical significance ($P<0.05$).

In the current study, the increased number of total leukocytes and neutrophils and the decreased numbers of lymphocytes at parturition are in line with the observation in dairy cows, where parturition was associated with leukocytosis, neutrophilia and a slight decrease in the lymphocyte fraction (Meglia *et al.*, 2001). However, the unchanged number of monocytes during the periparturient period contrasts with the reported monocytosis in dairy cows at calving (Meglia *et al.*, 2001; Ohtsuka *et al.*, 2004). Whether the higher expression of CD11a on neutrophils at calving and during the postparturient period contributes to an enhanced migration of this innate immune cell to the reproductive tract around parturition still to be investigated.

In the present study, the significant rise in lymphocyte count two weeks after calving seems to be due to increased numbers of $CD4^+$ T cells and B cells. Similar expansion of bovine $CD4^+$ T cells and IgM+ cells (B cells) was observed in dairy cows at the end of first month after calving (Ohtsuka *et al.*, 2004).

Although the cell number of total monocytes did not change significantly during the periparturient period, significant differences in the fractions of monocyte subsets were observed. In contrast to bovine monocytes, where all subsets followed the same time-dependent course around parturition (Eger *et al.*, 2015), camel monocyte subsets showed different subset-specific time-dependent charges

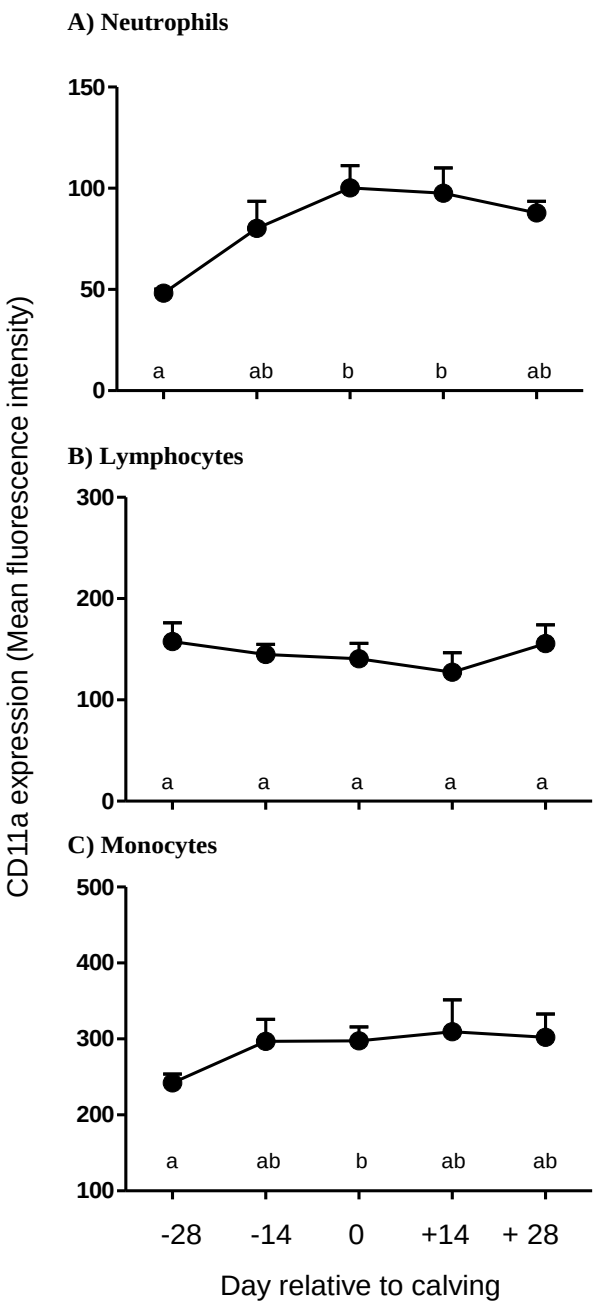

Fig. 4: Expression density of CD11a on leukocytes from periparturient she-camels. The differential expression of the adhesion molecule CD11a was estimated as the mean fluorescence intensity (MFI) of each molecule on blood neutrophils (A), lymphocytes (B), and monocytes (C). Data for different time-points during the periparturient period in she-camels were presented graphically. Differences between groups were calculated using the one-way ANOVA test. Different lowercase superscript letters indicate statistical significance ($P<0.05$).

during the periparturient period. While all bovine monocyte subsets expanded in blood of dairy cows after calving (Eger *et al.*, 2015), only camel $CD14^{high}$ $MHCII^{high}$ and $CD14^{low}$ $MHCII^{high}$ monocyte subsets increased after calving in the current study. The decrease in the main subset of camel monocytes, the $CD14^{high}$ $MHCII^{low}$ monocyte subset, at calving and during the postparturient time may be due to selective recruitment of this subset into the uterus or the mammary gland. This is supported by the significantly higher expression of CD11a on monocytes at calving. Camel monocyte subsets differ in their expression pattern of cell adhesion molecules (Hussen *et al.*, 2020). However, we did not evaluate the time-dependent change in the subset-specific expression pattern of adhesion molecules.

In several species, hormonal changes during pregnancy and parturition have shown major effects on several elements of the immune system (Arbib *et al.*, 2016; Nowak *et al.*, 2016; Gat *et al.*, 2019). In the dromedary camel, this is supported by the recently reported differences in leukogram and immunophenotype of leukocytes in pregnant and non-pregnant she-camels (Hussen *et al.*, 2019). The role of hormonal changes in the observed alterations in camel leukocyte composition and phenotype, however, needs to be investigated. Although the increased numbers of neutrophilic granulocytes, which play essential role in innate defense against bacterial infections (Ge *et al.*, 2020; Lentini *et al.*, 2020; Xie *et al.*, 2020), may indicate their contribution to enhanced antimicrobial capacities of the post-parturient dromedary she-camel, further functional studies are required to study the role of the parturition-associated changes in the susceptibility of the dromedary she-camel to post-parurient infections such as metritis and mastitis.

Conclusions: Collectively, the present study identified alterations in the composition of blood leukocyte subpopulations during the periparturient period of she-camels. Parturition was associated with a significant rise in the numbers of leukocytes and neutrophils but reduced numbers of the $CD14^{high}$ $MHCII^{low}$ monocyte subset. The postparturient period of she-camels was characterized by increased numbers of CD4+ T cells, B cells and the two monocyte subsets $CD14^{high}$ $MHCII^{high}$ and $CD14^{low}$ $MHCII^{high}$ monocytes. Alterations in the expression of cell adhesion molecules around parturition may have contributed to the changed composition of blood leukocyte subsets.

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Authors contribution: JH and TS conceived and designed the study. JH, TS and NA collected the samples. JH, TS, FA and AIA prepared the samples for flow cytometry. JH, NA, AIA and FA analyzed the labelled cells by flow cytometry. All authors read and approved the final manuscript.

REFERENCES

Amulic B, Cazalet C, Hayes GL, *et al.,* 2012. Neutrophil function: from mechanisms to disease. Annu Rev Immunol 30:459-89.

Arbib N, Aviram A, Gabbay Ben-Ziv R, *et al.,* 2016. The effect of labor and delivery on white blood cell count. J Matern Fetal Neonatal Med 2918:2904-8.

Bazzano M, Giannetto C, Fazio F, *et al.,* 2014. Physiological adjustments of haematological profile during the last trimester of pregnancy and the early post partum period in mares. Anim Reprod Sci 4:199-203.

Camacho-Fernandez C, Hervas D, Rivas-Sendra A, *et al.,* 2018. Comparison of six different methods to calculate cell densities. Plant Methods 14:30.

Caroprese M, Ciliberti MG, Albenzio M, *et al.,* 2015. Dietary polyunsaturated fatty acids from flaxseed affect immune responses of dairy sheep around parturition. Vet Immunol Immunopathol 2:56-60.

Eger M, Hussen J, Drong C, *et al.,* 2015. Impacts of parturition and body condition score on glucose uptake capacity of bovine monocyte subsets. Vet Immunol Immunopathol 2:33-42.

Eger M, Hussen J, Koy M, *et al.,* 2016. Glucose transporter expression differs between bovine monocyte and macrophage subsets and is influenced by milk production. J Dairy Sci 993:2276-87.

Gat R, Hadar E, Orbach-Zinger S, *et al.,* 2019. Distribution of extreme vital signs and complete blood count values of healthy parturients: A retrospective database analysis and review of the literature. Anesth Analg 1296:1595-606.

Ge C, Monk IR, Monard SC, *et al.,* 2020. Neutrophils play an ongoing role in preventing bacterial pneumonia by blocking the dissemination of Staphylococcus aureus from the upper to the lower airways. Immunol Cell Biol 987:577-94.

Hernandez-Castellano LE, Moreno-Indias I, Sanchez-Macias D, *et al.,* 2019. Sheep and goats raised in mixed flocks have diverse immune status around parturition. J Dairy Sci 1029:8478-85.

Hussen J, Koy M, Petzl W, *et al.,* 2016. Neutrophil degranulation differentially modulates phenotype and function of bovine monocyte subsets. Innate Immun 222:124-37.

Hussen J, Shawaf T, Al-herz Al, *et al.,* 2018. Expression patterns of cell adhesion molecules on CD4+ T Cells and WC1+ T cells in the peripheral blood of dromedary camels. Pak V J 38:231-6.

Hussen J, Shawaf T, Al-Herz Al, *et al.,* 2017. Reactivity of commercially available monoclonal antibodies to human CD antigens with peripheral blood leucocytes of dromedary camels (Camelus dromedarius). Open Vet J 72:150-3.

Hussen J, Shawaf T, Al-Mubarak AIA, *et al.,* 2020. Leukocytes immunophenotype and phagocytosis activity in pregnant and nonpregnant dromedary she camels. Pak Vet J 40:239-43.

Hussen J, Shawaf T, Al-Mubarak AIA, *et al.,* 2020. Dromedary camel CD14 (high) MHCII(high) monocytes display inflammatory properties and are reduced in newborn camel calves. BMC Vet Res 161:62.

Jongh RD, Jorens P, Student I, *et al.,* 1996. The contribution of the immune system to parturition. Mediators Inflamm 53:173-82.

Jonsson NN, Fortes MRS, Piper EK, *et al.,* 2013. Comparison of metabolic, hematological, and peripheral blood leukocyte cytokine profiles of dairy cows and heifers during the periparturient period. J Dairy Sci 964:2283-92.

Kourtzelis I, Mitroulis I, von Renesse J, *et al.,* 2017. From leukocyte recruitment to resolution of inflammation: the cardinal role of integrins. J Leukoc Biol 1023:677-83.

Lentini G, Fama A, Biondo C, *et al.,* 2020. Neutrophils enhance their own influx to sites of bacterial infection via endosomal TLR-Dependent Cxcl2 production. J Immunol 2043:660-70.

Lessard M, Gagnon N, Godson DL, *et al.,* 2004. Influence of parturition and diets enriched in n-3 or n-6 polyunsaturated fatty acids on immune response of dairy cows during the transition period. J Dairy Sci 877:2197-210.

Ley K, Laudanna C, Cybulsky MI, *et al.,* 2007. Getting to the site of inflammation: the leukocyte adhesion cascade updated. Nat Rev Immunol 79:678-89.

Meglia GE, Johannisson A, Petersson L, *et al.,* 2001. Changes in some blood micronutrients, leukocytes and neutrophil expression of adhesion molecules in periparturient dairy cows. Acta Vet Scand 421:139-50.

Mitroulis I, Alexaki VI, Kourtzelis I, *et al.,* 2015. Leukocyte integrins: role in leukocyte recruitment and as therapeutic targets in inflammatory disease. Pharmacol Ther 147:123-35.

Muller WA 2013. Getting leukocytes to the site of inflammation. Vet Pathol 501:7-22.

Nowak J, Borkowska B and Pawlowski B 2016. Leukocyte changes across menstruation, ovulation, and mid-luteal phase and association with sex hormone variation. Am J Hum Biol 285:721-8.

Ohtsuka H, Koiwa M, Fukuda S, *et al.,* 2004. Changes in peripheral leukocyte subsets in dairy cows with inflammatory diseases after calving. J Vet Med Sci 668:905-9.

Roos D and Law SK 2001. Hematologically important mutations: leukocyte adhesion deficiency. Blood Cells Mol Dis 276:1000-4.

Schalk C, Pfaffinger B, Schmucker S, *et al.,* 2019. Pregnancy-Associated alterations of peripheral blood immune cell numbers in domestic sows are modified by social rank. Animals (Basel) 93.

Soehnlein O and Lindbom L 2010. Phagocyte partnership during the onset and resolution of inflammation. Nat Rev Immunol 106:427-39.

van de Vijver E, Maddalena A, Sanal O, *et al.,* 2012. Hematologically important mutations: leukocyte adhesion deficiency (first update). Blood Cells Mol Dis 481:53-61.

Xie F, Zan Y, Zhang X, *et al.,* 2020. Differential abilities of mammalian cathelicidins to inhibit bacterial biofilm formation and promote multifaceted immune functions of neutrophils. Int J Mol Sci pp:215.