

PROFILING OF SOME IMMUNITY AND INFLAMMATORY PARAMETERS THROUGHOUT BUFFALO-COWS' PARITIES IN COLOSTRUM AND CALVES' BLOOD

Osama M. El-Malky¹, Helmy A. Abed El-Hady², A.F. Neber², H.A. Ashry², Reem S. Mourad²

1- Animal Production, Agricultural Research Institute Research Centre, Dokki, Giza, Egypt, 2- Animal Production Department, Faculty of Agriculture, Menoufia University, Menoufia, Egypt
E-mail: reem.mourad@agr.menoufia.edu.eg

Submitted: 12/12/2023; Accepted: 21/2/2024; Published: 19/3/2024

SUMMARY

The study investigates the profiling of colostral immunoglobulins (Ig's), and the calves' blood profiling of immunoglobulins, cytokines (Interleukin-L1, and-L6) and acute-phase proteins (APPs) includes Total antioxidant capacity (TAC), Fibrinogen (Fib), Haptoglobin (Hap), and serum amyloid-A (SAA) through dams' parities of the Egyptian buffalo. The experiment lasted for five months from Nov2017 to Mar2018. Fifty buffalo-cows and their calves raised in a herd affiliated with APRI, Egypt. Results showed differences through parities ($P \leq 0.05$) for IgA, and IgG, but IgM has a difference during 1st and 3rd-days only of dam's parity on colostral Ig's in buffalo-cows, and Ig's has a difference during 1st and 3rd-days in blood of newborn calves. Meanwhile, there was a disparity ($P \leq 0.05$) among days for sample started from 0-hour until 1st-day but no significant differences among days of sample were detected from 3rd-day till 3rd-month of the experiment in interleukins. A substantial divergence was noted ($P \leq 0.05$) for days of sample extended from 1st to 3rd-day, and 1st-month, also, Fib was different for 1st, 2nd-day, and for a week till the end of the experiment. Also, Hap induced by IL-6 presented an effect for 2nd-hour, 1st-day, and 1st to 3rd-month, whereas SAA was significant at 2nd and 3rd-day and significant ($P \leq 0.01$) from 1st to 3rd-month. TAC, Fib, and Hap participated in high significant during 1st-month. The parity of dams affects quality of dams' colostral Ig's, active transport of Ig and the immunological responses of newborn calves. Level of cytokines and APPs in calves' blood are also related to Ig's.

Keywords: Egyptian buffalo, immunoglobulin, cytokines, acute phase protein

INTRODUCTION

The Egyptian buffalo (*Bubalus bubalis b.*) is an important livestock species, valued for its milk, meat, and labor in Egypt. However, like all animals, buffalo are susceptible to various infectious diseases that can significantly impact their productivity. Understanding the immune response of buffalo, particularly in relation to parities, can provide valuable information for disease prevention and management strategies (Minervino *et al.*, 2020).

Newborn calves exhibit considerable fluctuations in the concentrations of numerous blood parameters. In addition, the parameters that change either peak immediately after birth and decrease to standard levels within a week, or low levels are noted at birth but increase to standard levels through out the subsequent days or months (Mohri *et al.*, 2007 and Lotito *et al.*, 2023).

Immunoglobulins are proteins produced via immune cells called B-lymphocytes. They play a vital role in recognizing and neutralizing pathogens, such as bacteria and viruses (Ahmad *et al.*, 2024). Cytokines are small proteins secreted by immune cells that regulate immune responses and communication between cells (Kaur *et al.*, 2023).

Furthermore, acute phase proteins (APPs) are a protein that are rapidly produced and released into the bloodstream in response to inflammation or infection. They serve as biomarkers of the acute phase response, which is an early and non-specific immune response to tissue damage or infection (Ali *et al.*, 2023). So, profiling APPs in buffalo can provide valuable information about their immune status and help in early detection and monitoring of diseases.

The health and management of calves are regarded as the most pressing concerns during the colostrum and subsequent growth phases (Seckin *et al.*, 2018). Relationship between mother and fetus has fascinated immunologists for decades (Indra *et al.*, 2012). There are several physiological changes that must occur as the fetus adjusts to live outside the uterus, especially immunity. Buffalo calves' life is very crucial and the heavy buffalo calf mortality is as high as 19.5% particularly during first 90 days of their postnatal (Shivarudrappa *et al.*, 2013). Calf's mortality was occurring in higher rates, reaching up to 17% in Murrah buffalo (Shivahre *et al.*, 2014); the expectation that 20% neonatal mortality rate results in 38% reduction in the profit of farm (Constable *et*

al., 2016). Higher values of examined APP's and proinflammatory cytokines (IL-1 β , and IL-12) in the pneumonic calves (El-Bahr and EL-Deeb, 2013)

Colostrum plays a key role in the survival of buffalo calves (Lotito *et al.*, 2023). Colostrum quality affected by many factors such as age of dam, parity, season of calving, body condition, calf sex and other factors can influence concentration of Ig's in colostrum (Hill *et al.*, 2011). The release of cytokines by innate immune cells is crucial in both the advancement and prevention of infectious diseases, ultimately contributing to the maturation of the acquired immune system (Seckin *et al.*, 2018).

The study's main aim was investigating the profiling of colostrum's immunoglobulins, and the calves' blood profiling of immunoglobulins, cytokines, and acute-phase proteins through dams' parities of the Egyptian buffalo.

MATERIALS AND METHODS

This experiment was conducted at the Animal Prod. Res. Station called El-Nattf El-Kadym, affiliated with APRI (Animal Production Research Institute), Egypt, located at Kafr Al -Sheikh governorate (31°.7' N latitude and 30°.51' N longitude), Egypt. The present study lasted for five months from Nov.2017 to Mar.2018. This research was conducted in accordance with Menoufia University guidelines for dealing with animals in scientific research. The approval of Ethics Committee, The Institutional Animal Care, and Use Committee (IACUC), Menoufia Univ. Ref.№ (MUFAG/F/AP/3/22).

Animals:

The experimental work was preceded by a preliminary observation of all buffalo dams at the late pregnancy period till parturition. Fifty buffalo-cows raised in El-Nattf El-Kadym herd affiliated with APRI. Those animals represent five parities, with 3 to 7-years and their weights about 350 to 675 kg. Buffaloes were kept in the delivery units for observation until time of calving, body condition scoring was recorded by week before parturition process and animals were classified into two categories from (1st is 2.5, and 2nd is > 3.5-5) according to their body condition scoring and classified into three categories according to their live body weight (350-450, 451-550, and 551-700 kg). Twenty buffaloes were giving male calves, and thirty buffaloes give female calves. Fifty newborn male and female buffalo calves on the 1st-day of its birth were with initial live body weight around 43.12±0.19, and 37.56±0.28 kg for male and female calves, respectively. Calves were healthy, birth weight of the newborn calves was recorded immediately after birth and then weekly till weaning period. After parturition, calves were stayed with their mothers and took colostrum through the 1st-day of birth, raised

under the same environmental condition, and kept in semi open boxes partially roved with asbestos, clay land and allowed to graze during the daytime from 9a.m. to 3p.m. after 1-month of parturition.

Feeding and managements of dams:

Buffaloes at late pregnancy period were kept in their open sheds up to time of delivery then transferred to the maternity house, during this period the animals were got their feed allowances according to their live body weight and body condition scoring as recommended by APRI (Sayed-Ahmed and Shaarawy, 2019). They were fed on clover, rice straw and concentrate feed mixture two times daily at 7 a.m. and 5p.m. The compositions of the feed mixture are un-decorticated cotton seeds (33%), corn (22%), wheat bran (21%), rice bran (14%), molasse (3%), limestone (3%), NaCl Salt (1.2%) and Ca (2.8%). Multi-mineral licking blocks were available for animals in the stalls. The breeding stock underwent regular managerial practices as they were relocated to the milking unit. Buffaloes were hand milked twice daily at 6 a.m., and 3 p.m., and the milk production of every buffalo was recorded during each milking session.

Samples of calves' blood, colostrum, and chemical analysis:

Samples were collected from newborn calves at different times of the experiment via jugular vein by sterile needle, included the following periods, immediately after birth at 0-time, 2nd hour after 1st suckling of colostrum; 1st, 2nd, and 3rd day; 1st week and 1st, 2nd, and 3rd month to determined blood Ig's, cytokines, APPs. Samples collected in un-heparinized and heparinized tubes directly from calves in the morning before feeding, the blood sample was centrifuged for 30 min. at 3000 rpm. to collect clear plasma samples. The plasma samples were stored at -20 °C until assaying the concentrations of Ig's. Pro and anti-inflammatory cytokines and APPs were determined by ELISA using the hemoglobin-binding assay (Makimura and Suzuki, 1982). TAC was estimated by antioxidant assay kit (Koracevic *et al.*, 2001). Colostrum samples were taken twice a day during 1st, 2nd, and 3rd day after parturition for immunoglobulins analyzed as described by (Abdel Hady *et al.*, 2022)

Statistical analysis:

The data collected underwent statistical analysis through the utilization of the SAS computer program (SAS, 2002) via General Linear Model (GLM) with Duncan test ($\alpha=0.05$) as follow:

$$Y_{ijklm} = \mu + P_i + W_j + C_k + S_l + e_{ijklm}$$

Where: Y_{ijklm} = is the observed variable; μ = overall mean of the observed variable; P_i = the fixed effect due to the i^{th} parity, $i = 1:5$ parities. ; W_j = the fixed effect due to the j^{th} live body weight, $i = 1:3$ categories.; C_k = the effect due to the k^{th} body

conditions score, $k = 1$, and 2 categories.; S_1 = the effect due to the l^{th} calves' sex, $L = 1$, male, and 2, females.; and e_{ijklm} = Error term NID ($0, \sigma^2_e$).

RESULTS AND DISCUSSION

Colostrum's profiling of immunity parameters.

Table 1 and Fig. 1 clarify the effect of dams' parity on Ig's concentration in dam's colostrum. Results showed increasing in Ig's concentration associated with advanced dams' parity until 5th parity. The overall means of Ig's concentration includes IgA, IgM, and IgG in 1st parity comparable with 5th parity were 0.79 ± 0.03 vs. 1.3 ± 0.06 , 1.4 ± 0.04 vs. 1.7 ± 0.05 , and 9.5 ± 0.49 vs. 13.8 ± 4.31 mg/ml, respectively. It presented marked distinctions ($P \leq 0.05$) all over the experiment for IgA, and IgG, but IgM showed a significant difference during 1st and 3rd-day only. Holstein dairy cows at parities 1, and 2 were lower total production and Ig's level at the 1st milking postpartum than those older cows that has parity ≥ 3 ($P \leq 0.05$) to describe the parity effect on colostrum quality; attributed those results to younger dairy cows would have limited time on the farm for exposure to pathogens and vaccinations. Hence, reduced exposure and a lower number of vaccinations would result in a decline in the level of circulating antibodies, subsequently leading to a decrease in the amount of antibodies transferred into colostrum (Angulo *et al.*, 2015). Similarly, some investigators show that Ig's was lower in the 1st and 2nd lactation of dairy cows than later lactations (Gomes *et al.*, 2011), and colostrum immune quality continued to increase with parity after the 2nd calving

and that older dairy cows have the best colostrum in terms of immune quality in general (Morrill *et al.*, 2012).

Dam's age influences colostrum quality (Downey *et al.*, 2011), and a significant ($P \leq 0.001$) associated between lactation number and colostrum IgG concentration, which older cows tending to have higher IgG concentrations than those younger cows (Mulder *et al.*, 2017). In addition, the colostrum of multiparous dairy cows has 19.5 grams of IgG more than primiparous dairy cows (Tyler *et al.*, 1999). In Murrah buffalos, the parity effect was noted on the colostrum's level of IgG. Results showed that levels of IgG in buffalo's colostrum ranged from 11.22 : 185.1 mg/ml and its mean was 51.71 ± 5.99 mg/ml. Parity presented a remarkable effect on colostrum IgG concentrations at $P \leq 0.01$, and $P \leq 0.05$ levels, respectively (Chaudhary *et al.*, 2017). Many researchers reported significant effect of dam's parity on colostrum IgG levels in cows (Gulliksen *et al.*, 2008, and Kehoe *et al.*, 2011). Meanwhile, no effects were noted by (Agrawal, 2015). Colostrum IgG was significantly lower in 2nd parity cows (Johnsen *et al.*, 2019). The IgG concentration as the predominant colostrum Ig's may vary among cows (Weaver *et al.*, 2000). Cows that have given birth multiple times tend to produce colostrum of higher quality in terms of antigen level because of exposure to different pathogens and antigens for a long time and build up a larger number of antibodies, which are transported to their calves in higher amounts through the maternal colostrum (Godden, 2008).

Table 1. Colostrum Ig's through dams' parities (1st to 5th)

Ig (mg/ml)	Dam's parity	Days of sample			Overall mean
		1 st day	2 nd day	3 rd day	
IgA (mg/ml)	1 st	$0.98^b \pm 0.07$	$0.90^{bc} \pm 0.06$	$0.55^b \pm 0.04$	$0.79^c \pm 0.03$
	2 nd	$0.99^b \pm 0.05$	$0.81^c \pm 0.03$	$0.50^b \pm 0.05$	$0.76^c \pm 0.02$
	3 rd	$1.7^a \pm 0.15$	$1.10^{ab} \pm 0.10$	$0.62^b \pm 0.05$	$1.1^b \pm 0.06$
	4 th	$1.7^a \pm 0.12$	$1.20^a \pm 0.10$	$0.82^a \pm 0.08$	$1.2^{ab} \pm 0.07$
	5 th	$1.8^a \pm 0.11$	$1.30^a \pm 0.08$	$0.95^a \pm 0.07$	$1.3^a \pm 0.06$
IgM (mg/ml)	1 st	$1.7^b \pm 0.12$	$1.4^a \pm 0.06$	$1.2^a \pm 0.05$	$1.4^c \pm 0.04$
	2 nd	$1.8^b \pm 0.13$	$1.6^a \pm 0.14$	$0.72^b \pm 0.07$	$1.3^{bc} \pm 0.05$
	3 rd	$1.9^{ab} \pm 0.13$	$1.7^a \pm 0.18$	$1.4^a \pm 0.18$	$1.6^{ab} \pm 0.04$
	4 th	$2.0^{ab} \pm 0.17$	$1.7^a \pm 0.16$	$1.5^a \pm 0.25$	$1.7^a \pm 0.15$
	5 th	$2.2^a \pm 0.04^a$	$1.7^a \pm 0.11$	$1.5^a \pm 0.07$	$1.7^a \pm 0.05$
IgG (mg/ml)	1 st	$13.0^b \pm 0.87$	$9.1^b \pm 0.49$	$6.4^{cd} \pm 0.65$	$9.5^c \pm 0.49$
	2 nd	$14.1^b \pm 0.76$	$10.7^b \pm 0.45$	$4.9^d \pm 0.6$	$9.9^c \pm 0.34$
	3 rd	$16.8^a \pm 0.55$	$12.7^a \pm 0.62$	$7.9^{bc} \pm 0.85$	$12.4^b \pm 0.28$
	4 th	$16.8^a \pm 0.98$	$13.4^a \pm 0.78$	$9.4^{ab} \pm 0.74$	$13.2^{ab} \pm 0.38$
	5 th	$17.4^a \pm 0.62$	$13.9^a \pm 0.30$	$10.3^a \pm 0.29$	$13.8^a \pm 4.31$

a, b, c, d Means bearing different superscripts in the same row are significantly different ($P \leq 0.05$).

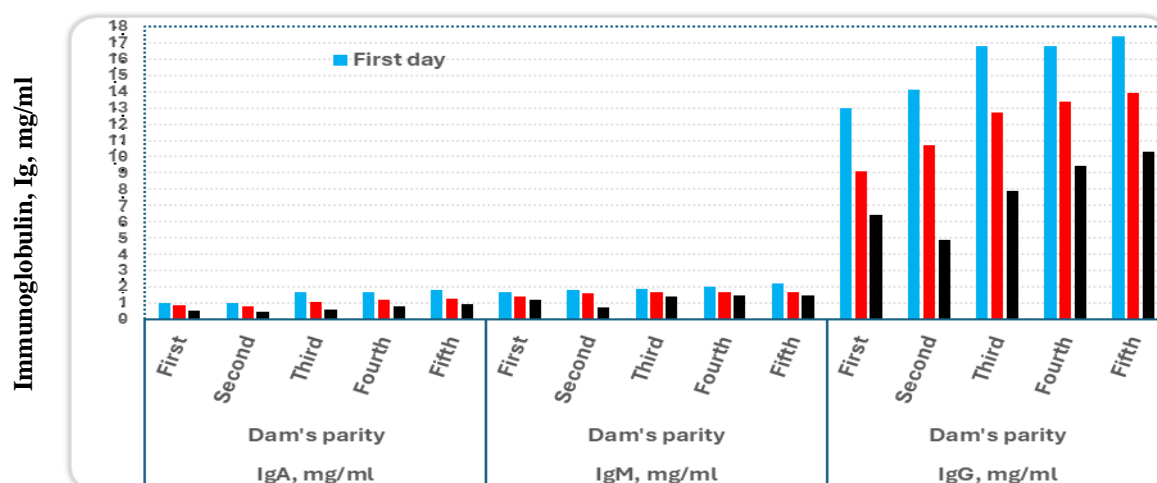


Figure 1. Colostrum Ig's through dams' parities.

On the other side, primiparous ones have not had the time to accumulate and build up resistance to the various herd-specific pathogens. So, significantly differ between primiparous and multiparous cows in Ig's concentration was found for cows which secreting 1.3 to 1.6 times higher antibody concentration. The effect of dam's parity, 1st to 4th parity on immune transfer in calves from birth to 15 weeks of age, showed insignificant effect of dam's parity on serum concentration of Ig's includes IgG, IgM, and IgA. In addition, a noticed reduction in Ig's concentration in calves' serum from the 3rd day after birth in Friesian cow (Wafa, 2017). The parity had no effect on colostrum quality in Holstein dairy cows and confirmed that 1st, and 2nd parity had no significant effects on major components of colostrum, and its quality includes IgG, and IgM concentrations, but there was a trend of higher IgG, and IgM concentrations with advanced parity (Zarei *et al.*, 2017).

The dairy cows' parity has an impact on the Ig levels in the colostrum, with multiparous dairy cows demonstrating a significant effect ($P \leq 0.05$) and exhibiting higher Ig levels in multiparous (117.45 g/l) than primiparous (73.81 g/l) (Aydogdu and Guzelbektes, 2018). Furthermore, the level of IgG and the age/parity of the mother are directly linked to the quality of colostrum (Gokce and Erdogan, 2013).

Calves' blood profiling of immunity and inflammatory parameters:

Several factors affect the colostrum concentration of Ig, including, among others, dry period length, time from calving to milking, dam's vaccination status, parity, late-gestation nutrition, and season of calving (Godden, 2008). Colostrum antibody profiles that are specific to pathogens on a particular farm would be of high value to the young calf. Therefore, it should never be assumed that colostrum quality is solely a factor of IgG content, but is multi-faceted, with antibody profile (i.e. specificity against certain pathogens) being equally as important as actual IgG content (Kassa *et al.*, 2020).

Cytokines are small glycoproteins, released by different cells to facilitate intercellular signaling and communication. Controlling cell proliferation, stimulating immune responses, activating inflammatory responses, and regulating angiogenesis are the few functions of different cytokines (Chung *et al.*, 2019). The release of cytokines and chemokines by innate immune cells, particularly by activated macrophages following the initial pattern recognition, is crucial in both the advancement and prevention of infectious diseases. These molecules play a vital role in modulating the immune response, including the induction of fever and the production of cellular and humoral factors like antibodies, cytokines, APPs, and interferons. Furthermore, they contribute to the development of the acquired immune system (Seckin *et al.*, 2018). IL-1 and IL-6 are two pro-inflammatory cytokines that considered small, secreted proteins which mediate and regulate immunity, inflammation, and hematopoiesis (Balaram *et al.*, 2009).

Acute phase proteins (APPs) play a crucial role in herd health, aiding in the diagnosis and prognosis of different diseases, as well as monitoring treatment progress during infections in veterinary medicine. A surge in interest has been highlighted by a researcher regarding the exploration of the connections between the expression of APPs, the endocrine system, and immunomodulation. Various stressors, such as inadequate nutrition, weaning shock, extreme temperature fluctuations, injuries, and infections, can compromise the immune function in calves. Therefore, APPs have been utilized as biomarkers to assess the stress levels of ruminants in both experimental and field studies (Yun *et al.*, 2014).

Immunoglobulins (Ig's) levels in calves' blood throughout dams' parities

Table 2 and Fig. 2 clarifies the blood profiling of Ig's concentration in newborn calves throughout dams' parities. Blood's Ig concentration in calves increases with dams' parity until 4th parity then it stills at the same level with 5th parity. The overall mean of Calves' blood Ig values for five parities that

Table 2: Immunoglobulins concentration in calves' blood throughout dams' parities (1st to 5th)

Days of sample	Immunoglobulin-A (IgA), g/l					Immunoglobulin-M (IgM), g/l					Immunoglobulin-G (IgG), g/l				
	1 st	2 nd	3 rd	4 th	5 th	1 st	2 nd	3 rd	4 th	5 th	1 st	2 nd	3 rd	4 th	5 th
0	0.03 ^c	0.03 ^c	0.05 ^b	0.08 ^a	0.08 ^a	0.11 ^c	0.18 ^c	0.37 ^b	0.41 ^{ab}	0.46 ^a	0.39 ^c	0.46 ^c	0.72 ^b	0.88 ^a	0.87 ^a
hour	±0.0	±0.0	±0.01	±0.0	±0.0	±0.01	±0.01	±0.03	±0.04	±0.03	±0.05	±0.04	±0.03	±0.03	±0.03
2 nd hour	0.03 ^c	0.03 ^c	0.06 ^b	0.08 ^a	0.08 ^a	0.17 ^d	0.25 ^c	0.61 ^b	0.64 ^b	0.88 ^a	2.42 ^b	2.53 ^b	2.51 ^b	2.76 ^b	4.06 ^a
hour	±0.0	±0.0	±0.01	±0.01	±0.0	±0.02	±0.0	±0.03	±0.03	±0.04	±34	±0.17	±0.16	±0.20	±0.34
1 st day	0.03 ^b	0.06 ^{ab}	0.07 ^{ab}	0.18 ^{ab}	0.27 ^a	0.23 ^c	0.29 ^c	0.79 ^b	0.81 ^b	1 ^a	7.31 ^d	8.6 ^{bc}	8.1 ^{cd}	10.12 ^a	9.58 ^{ab}
day	±0.0	±0.01	±0.01	±0.09	±0.12	±0.01	±0.02	±0.03	±0.02	±0.0	±0.52	±0.38	±0.31	±0.51	±0.31
2 nd day	0.04 ^b	0.06 ^b	0.08 ^b	0.36 ^a	0.46 ^a	0.32 ^d	0.4 ^c	0.85 ^b	0.87 ^b	1.05 ^a	13.51 ^d	16.67 ^c	18.17 ^{bc}	18.93 ^{ab}	20.15 ^a
day	±0.01	±0.01	±0.00	±0.14	±0.15	±0.02	±0.02	±0.03	±0.02	±0.0	±0.72	±0.60	±0.48	±0.34	±0.57
3 rd day	0.05 ^b	0.06 ^b	0.08 ^b	0.64 ^a	0.83 ^a	0.4 ^e	0.61 ^d	0.86 ^c	0.98 ^b	1.11 ^a	15.44 ^c	16.8 ^c	18.29 ^b	19.77 ^a	20.91 ^a
day	±0.01	±0.01	±0.00	±0.15	±0.12	±0.04	±0.02	±0.02	±0.01	±0.02	±0.76	±0.37	±0.48	±0.37	±0.39
Week	0.06 ^c	0.07 ^c	0.08 ^c	0.73 ^b	1.02 ^a	0.55 ^e	0.65 ^d	0.88 ^c	0.98 ^b	1.22 ^a	16.65 ^c	17 ^c	18.58 ^b	19.97 ^a	20.67 ^a
	±0.01	±0.00	±0.00	±0.14	±0.01	±0.03	±0.04	±0.02	±0.03	±0.02	±0.52	±0.38	±0.42	±0.41	±0.39
1 st month	0.07 ^c	0.08 ^c	0.17 ^c	0.69 ^b	1.03 ^a	0.58 ^c	0.63 ^c	0.8 ^b	0.99 ^b	1.28 ^a	16.44 ^c	16.7 ^c	17.26 ^{bc}	18.47 ^{ab}	18.78 ^a
month	±0.01	±0.01	±0.09	±0.14	±0.01	±0.03	±0.05	±0.04	±0.03	±0.04	±0.58	±0.30	±0.40	±0.58	±0.36
2 nd month	0.07 ^b	0.07 ^b	0.17 ^b	0.72 ^a	0.87 ^a	0.61 ^c	0.65 ^c	0.82 ^b	0.85 ^b	1.01 ^a	14.07 ^c	14.39 ^{bc}	13.33 ^c	17.49 ^a	16.03 ^{ab}
month	±0.01	±0.02	±0.09	±0.14	±0.09	±0.03	±0.03	±0.05	±0.05	±0.04	±0.40	±0.71	±0.63	±0.64	±0.72
3 rd month	0.05 ^c	0.06 ^c	0.07 ^c	0.54 ^b	0.76 ^a	0.54 ^b	0.55 ^b	0.71 ^a	0.77 ^a	0.81 ^a	10.88 ^b	11.72 ^{ab}	11.07 ^b	12.2 ^{ab}	13.45 ^a
month	±0.01	±0.01	±0.04	±0.15	±0.06	±0.04	±0.04	±0.05	±0.03	±0.04	±0.58	±0.75	±0.44	±1.09	±0.68
Overall mean	0.04 ^c	0.05 ^c	0.09 ^c	0.47 ^b	0.6 ^a	0.39 ^e	0.47 ^d	0.74 ^c	0.8 ^b	0.98 ^a	10.78 ^c	11.65 ^b	12 ^b	13.41 ^a	13.83 ^a
	±0.00	±0.00	±0.01	±0.04	±0.03	±0.01	±0.01	±0.02	±0.01	±0.01	±0.24	±0.10	±0.20	±0.24	±0.13

a,b,c,d Means bearing different superscripts in the same row are significantly different ($P \leq 0.05$)

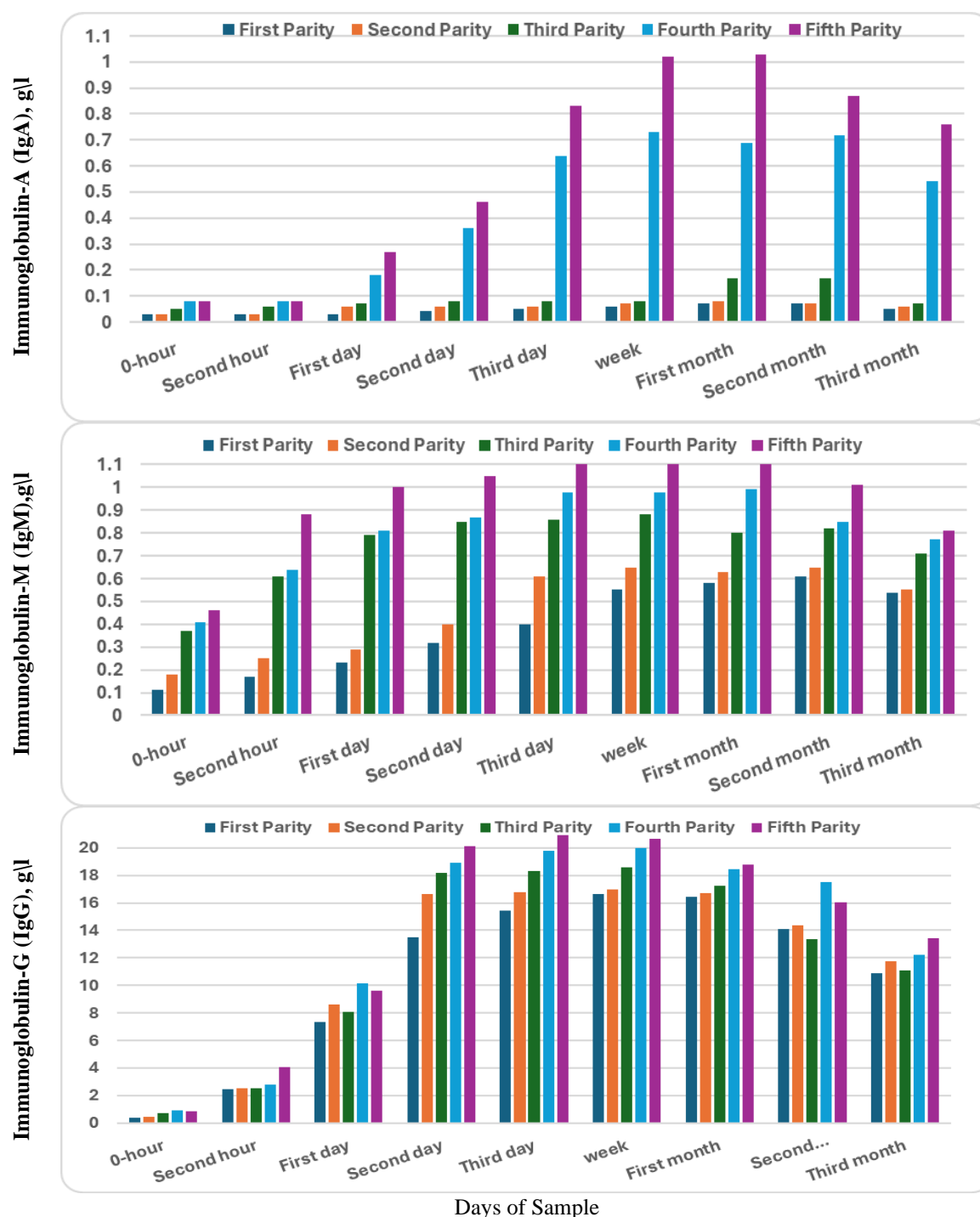


Figure 2. Immunoglobulins in calves' blood throughout dams' parities.

describe the period from 0-time after calving to 3 months of age (weaning) were 0.04, 0.05, 0.09, 0.47, and 0.60 g/l for IgA; 0.39, 0.47, 0.74, 0.80, and 0.98 g/l for IgM, and 10.78, 11.65, 12.0, 13.41, and 13.83 g/l for IgG, respectively.

There was a significant effect of Dams' parity ($P \leq 0.05$) on plasma IgA, IgM, and IgG of calves. blood IgA, IgM, and IgG increase with the increasing of parity. The lowest value of IgM concentration was

in 1st lactation and tended to increase ($P \leq 0.05$). The highest levels of plasma IgG ($P \leq 0.05$) and IgM ($P \leq 0.05$) were in the neonates from the 4th to 5th lactation. The lowest values noted at 1st and 2nd lactations, comparable results are conducted by (Abd El-Hady *et al.*, 2006).

Serum IgG levels were higher at 0-day at birth after suckling colostrum than at 14 days after birth in buffalo calves (Arthington *et al.*, 2000). Calves' IgG levels gradually reduced significantly ($P < 0.05$) by

progress of age, the highest at initial age, moderate at 15-week of age (weaning) and the lowest at 18-week of age (post-weaning). Meanwhile, calves' IgM and IgA levels conducted insignificant differences among ages (Shahin *et al.*, 2018).

The observed reduction in IgG level by age progressing might be attributed to that calves are born without fully mature immune system and presence of ≥ 10 mg IgG/ml in blood of calves is necessary for successful immunity response in the first few days of age (Logue and Mayne, 2014). In Dairy calves' blood, reduction in IgG level starting after birth up to 4 weeks of age. Meanwhile, IgM and IgA levels were decreased from birth till 1st-month of age (Ježek *et al.*, 2012).

Cytokines levels in calves' blood throughout dams' parities:

Table 3 and Fig. 3 shows a significant effect among five parities at $P \leq (0.05)$ in overall means of examined proinflammatory cytokine (IL-1) for calves especially that born to dams at the 1st parity that has a higher value compared with other parities. The overall means for five parities were 5.4 ± 0.18 ,

4.9 ± 0.21 , 5.0 ± 0.07 , 4.7 ± 0.11 , and 4.7 ± 0.11 pg/mL, respectively. Also, plasma concentrations of IL-1 for calves born to 1st dam's parity maintained the highest value from birth to 3rd month. The statistical analysis for IL-1 showed that no significant differences among days of sample from 0 time to 1st week but there are significant differences were reported during the period extended from 1st to 3rd month of the experiment.

Moreover, it illustrates that those calves born to dams in 5th-parity had higher interleukin-6 level than the other ones. Also, these calves were kept with the highest IL-6 values from birth to 3rd-month. Whereas calves born to dams in the 2nd-parity showed a lower overall mean of IL-6. On the other hand, they showed that IL-6 had a significant difference at $P \leq 0.05$ among days of sampling from 0-time to 1st-day but no significant disparities among days of sampling were detected from 3rd-day to 3rd-month.

Approximately 30 distinct cytokines have been identified, with the tumor necrosis factor- α and interleukins (IL-1, IL-6, IL-8, and IL-12) being the most prominent proinflammatory cytokines (Eckersall and Bell, 2010).

Table 3. Interleukins (IL1 and IL6) in calves' blood throughout dams' parities (1st to 5th)

Days of sample	Interleukin-1 (IL-1), pg/mL					Interleukin-6 (IL-6), pg/mL				
	1 st	2 nd	3 rd	4 th	5 th	1 st	2 nd	3 rd	4 th	5 th
0 hour	4.8 ^a ± 0.3	4.7 ^a ± 0.2	4.6 ^a ± 0.1	4.6 ^a ± 0.1	4.6 ^a ± 0.2	65.7 ^{abc} ± 1.4	61.7 ^c ± 1.7	64.4 ^{bc} ± 1.6	68.5 ^{ab} ± 1.3	69.2 ^a ± 0.9
2nd hour	4.9 ^a ± 0.3	4.8 ^a ± 0.2	4.6 ^a ± 0.1	4.6 ^a ± 0.1	4.6 ^a ± 0.2	65.7 ^{abc} ± 1.3	61.8 ^c ± 1.5	64.5 ^{bc} ± 1.6	68.5 ^{ab} ± 1.3	69.3 ^a ± 0.9
1st day	4.9 ^a ± 0.3	4.8 ^a ± 0.2	4.7 ^a ± 0.1	4.7 ^a ± 0.1	4.6 ^a ± 0.2	65.8 ^a ± 1.6	64.9 ^a ± 1.5	65.1 ^a ± 1.5	68.8 ^a ± 1.3	69.3 ^a ± 1.2
2nd day	4.9 ^a ± 0.3	4.8 ^a ± 0.2	5.1 ^a ± 0.1	4.6 ^a ± 0.1	4.6 ^a ± 0.2	66.8 ^a ± 1.4	64.9 ^a ± 1.5	65.6 ^a ± 1.5	68.9 ^a ± 1.3	68.6 ^a ± 1.3
3rd day	5.1 ^a ± 0.2	4.7 ^a ± 0.3	5.0 ^a ± 0.1	4.9 ^a ± 0.1	4.8 ^a ± 0.2	66.8 ^a ± 1.2	65 ^a ± 1.6	65.9 ^a ± 1.5	68.2 ^a ± 1.0	68.7 ^a ± 1.3
Week	5.1 ^a ± 0.2	5.1 ^{ab} ± 0.3	5.4 ^a ± 0.1	4.8 ^a ± 0.2	4.7 ^a ± 0.2	66.8 ^a ± 1.2	65.6 ^a ± 1.3	66.4 ^a ± 1.6	69.2 ^a ± 1.1	69.4 ^a ± 1.1
1st month	6.1 ^a ± 0.2	5.2 ^{bc} ± 0.3	5.4 ^b ± 0.1	4.8 ^{cb} ± 0.2	4.7 ^c ± 0.2	68.8 ^{ab} ± 1.4	65.8 ^b ± 1.5	66.4 ^{ab} ± 1.7	70.5 ^a ± 1.2	69 ^{ab} ± 1.1
2nd month	6.6 ^a ± 0.1	5.2 ^b ± 0.3	5.2 ^b ± 0.1	4.9 ^{bc} ± 0.2	4.6 ^a ± 0.2	70.1 ^a ± 1.4	66.2 ^a ± 1.3	67.7 ^a ± 1.6	68.8 ^a ± 1.7	69.8 ^a ± 1.4
3rd month	6.4 ^a ± 0.2	5.2 ^b ± 0.2	5.0 ^{bc} ± 0.2	4.6 ^c ± 0.2	4.5 ^a ± 0.2	69.6 ^a ± 1.7	66.6 ^a ± 1.2	67.8 ^a ± 1.7	68.8 ^a ± 1.9	70.8 ^a ± 1.1
Overall mean	5.4 ^a ± 0.18	4.9 ^{ab} ± 0.21	5.0 ^{ab} ± 0.07	4.7 ^b ± 0.11	4.6 ^b ± 0.19	67.3 ^{ab} ± 1.18	64.7 ^b ± 1.37	65.97 ^{ab} ± 1.5	68.9 ^a ± 1.1	69.3 ^a ± 1.0

a, b, c, d Means bearing different superscripts in the same row are significantly different ($P \leq 0.05$)

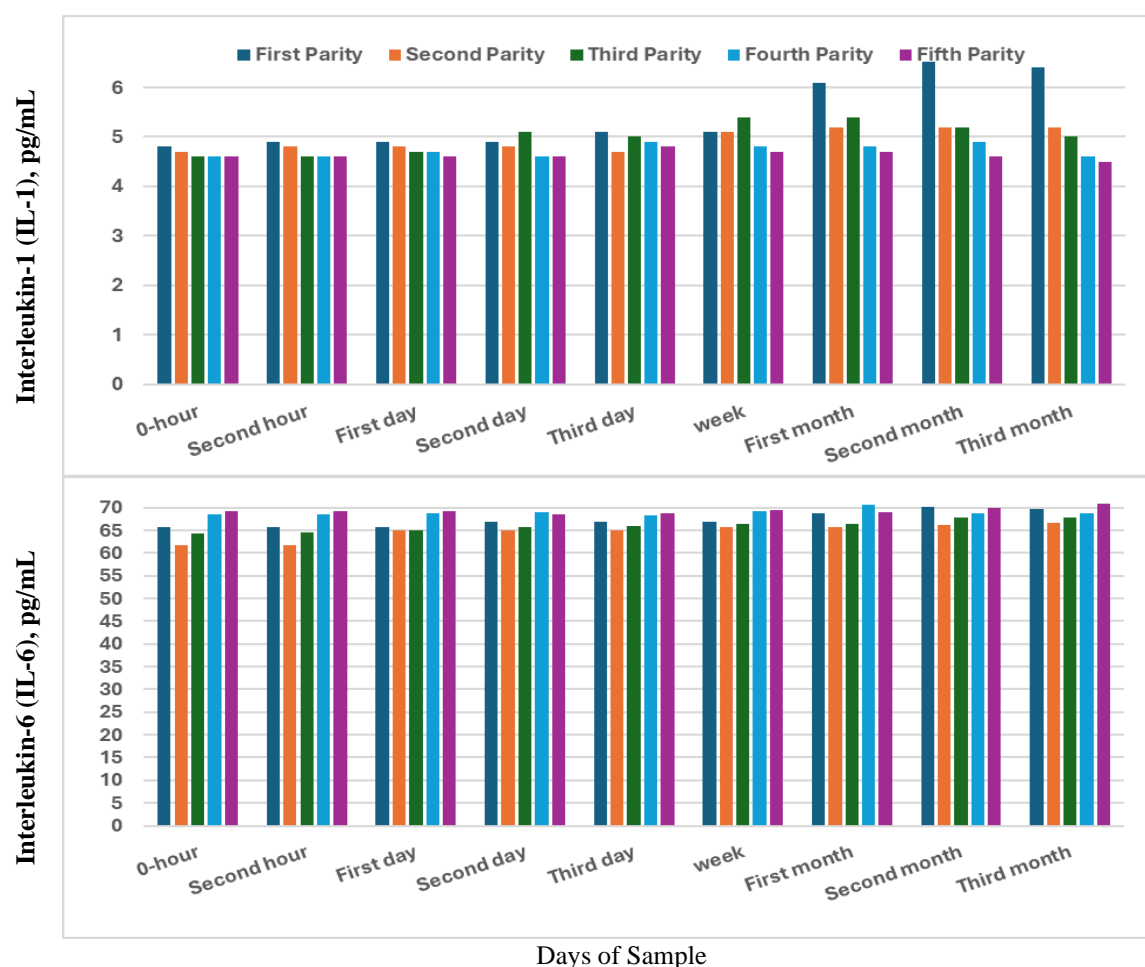


Figure 3. Interleukins in calves' blood throughout dams' parities.

Acute phase proteins (APPs) levels in calves' blood throughout dams' parities:

The effect of dams' parity on APPs concentrations includes Total antioxidant capacity (TAC, mM/l), Fibrinogen (Fib, g/l), Haptoglobin (Hap, mg/ml), and Serum amyloid A (SAA, µg/ml) in the newborn calves' blood. APPs are serum proteins produced by both hepatocytes and peripheral tissues (Eckersall and Bell, 2010). APPs are motivated by pro-inflammatory cytokines which increases in concentration during the APPs responses to inflammation or infection making them the 1st line of immune defense mechanism (Mohammed *et al.*, 2013). The fluctuation of Fibrinogen (Fib, g/l) after birth lacks a consistent pattern of change. Haptoglobin (Hap, mg/ml) presents a certain level of growth or variation following birth, which stabilizes within a span of two weeks. Also, levels of Serum amyloid A (SAA, µg/ml) peak rapidly after birth and remain elevated for the first 2 or 3 weeks of the calf's life (Tóthová *et al.*, 2015). APPs may either increase or decrease in cases of infection and inflammation (Jesse *et al.*, 2013), acting as messengers between the local site of injury and the hepatocytes (Petersen *et al.*, 2004). The response is present in all animals; however, it can vary significantly among distinct

species in terms of individual protein reactions. On the other hand, Haptoglobin (Hap, mg/ml) induced by IL-6 and SAA (Skinner *et al.*, 1991). Moreover, (Eckersall and Bell, 2010) conducted that APPs (primarily by IL-1) are the major APPs in ruminants which show a negligible circulating level in normal animals but increases over 100-fold on stimulation. APPs are serum proteins produced by both hepatocytes and peripheral tissues (Eckersall and Bell, 2010).

Table 4, 5 and Fig. 4, and 5 showed those concentrations of APPs during the early three months of calves' life. Significant changes at $P \leq 0.01$ were found in the overall mean levels of TAC, Fib, and SAA among dams' parity, whereas Hap concentrations did not show any significant changes. The lowest concentrations of TAC, and SAA were noted for calves born to dams at the 1st parity and for Fib in calves born to dams at 5th parity. Also, calves born to dams at the 4th parity showed the highest overall mean for SAA, Fib, Hap, and TAC. Also, until the end of the evaluated period the mean values of TAC, Fib, Hap, and SAA were higher than the 1st days after birth.

Table 4. APPs in calves' blood throughout dams' parities (1st to 5th)

Days of sample	Total antioxidant capacity (TAC), mM/l					Fibrinogen (Fib), g/l				
	1 st	2 nd	3 rd	4 th	5 th	1 st	2 nd	3 rd	4 th	5 th
0	1.3 ^a	1.3 ^a	1.3 ^a	1.3 ^a	1.3 ^a	1.7 ^a	1.8 ^a	1.6 ^{ab}	1.7 ^{ab}	1.5 ^b
hour	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1
2 nd	1.3 ^a	1.3 ^a	1.3 ^a	1.3 ^a	1.3 ^a	1.8 ^a	1.8 ^a	1.7 ^a	1.7 ^{ab}	1.5 ^b
hour	±0.1	±0.1	±0.1	±0.1	±0.1	±0.0	±0.1	±0.1	±0.1	±0.1
1 st	1.3 ^b	1.3 ^b	1.4 ^{ab}	1.5 ^a	1.5 ^a	1.9 ^a	2 ^a	1.9 ^a	1.8 ^a	1.6 ^b
day	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.01	±0.1	±0.1
2 nd	1.4 ^b	1.4 ^{ab}	1.5 ^a	1.6 ^a	1.6 ^a	2 ^a	2.2 ^a	2.1 ^a	2.2 ^a	2.1 ^a
day	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1
3 rd	1.4 ^b	1.4 ^{ab}	1.5 ^a	1.6 ^a	1.6 ^a	2.1 ^b	2.2 ^{ab}	2.3 ^{ab}	2.3 ^{ab}	2.4 ^a
day	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.0	±0.1	±0.1	±0.2
Week	1.6 ^a	1.7 ^a	1.7 ^a	1.7 ^a	1.7 ^a	2.3 ^b	2.4 ^b	2.5 ^{ab}	2.6 ^{ab}	2.7 ^a
	±0.1	±0.1	±0.1	±0.1	±0.1	±0.01	±0.01	±0.1	±0.1	±0.2
1 st	1.8 ^b	1.8 ^b	1.8 ^{ab}	1.8 ^{ab}	1.9 ^a	2.6 ^b	2.5 ^b	2.6 ^b	3.2 ^a	3.4 ^a
month	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1
2 nd	1.9 ^a	2.0 ^a	2.0 ^a	2.1 ^a	2.1 ^a	3.1 ^{ab}	3.0 ^b	3.2 ^{ab}	3.3 ^a	3.0 ^b
month	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1 ^a	±0.1
3 rd	2.0 ^a	2.0 ^a	2.1 ^a	2.1 ^a	2.1 ^a	2.6 ^a	2.6 ^{ab}	2.3 ^{bc}	2.2 ^c	2.3 ^c
month	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1	±0.1
Overall mean	1.5 ^c	1.5 ^{bc}	1.6 ^{ab}	1.6 ^{ab}	1.7 ^a	2.2 ^a	2.3 ^a	2.2 ^a	2.3 ^a	2.1 ^b
	±0.03	±0.01	±0.02	±0.02	±0.02	±0.03	±0.03	±0.05	±0.07	±0.04

a, b, c, d Means bearing different superscripts in the same row are significantly different ($P \leq 0.05$).

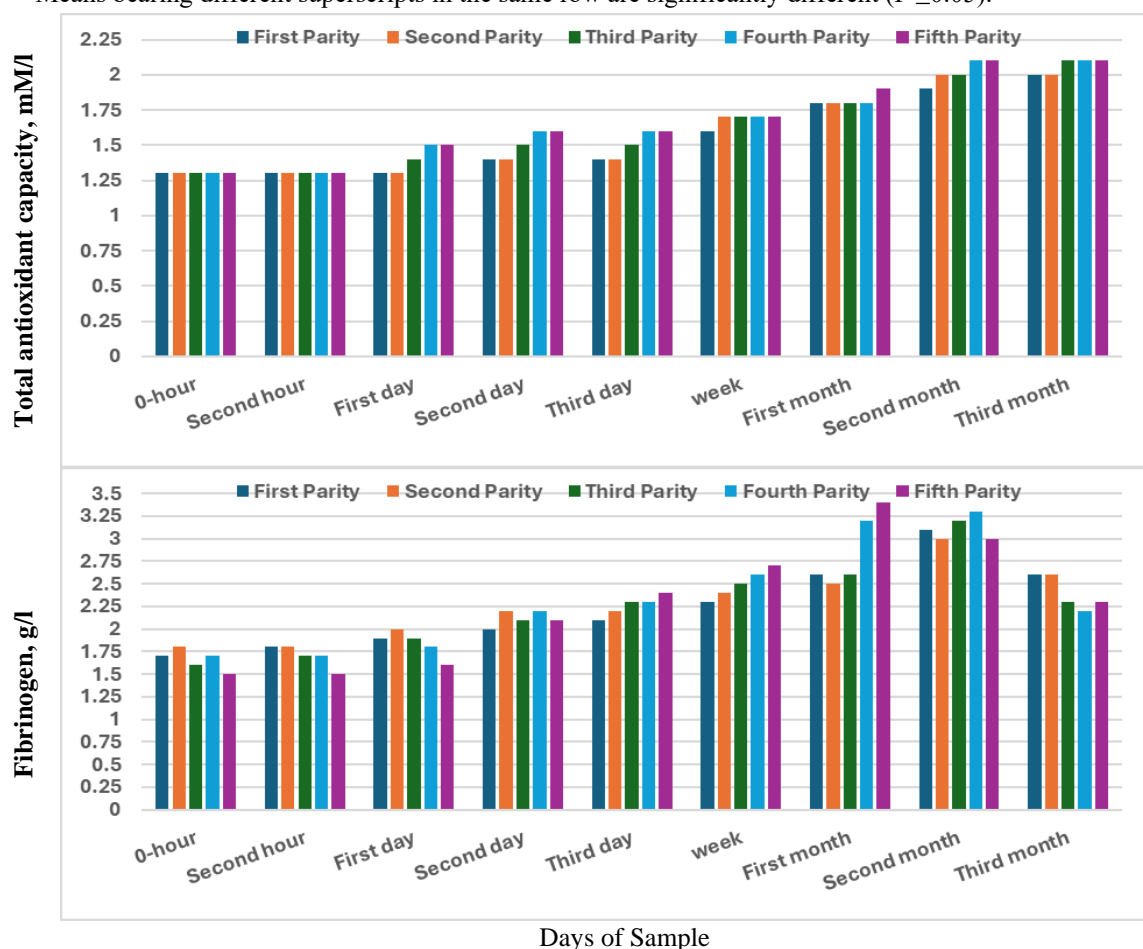


Figure 4. TAC and Fib in calves' blood throughout dams' parities.

Table 4, and 5 illustrated a significant difference at $P \leq 0.05$ for days of sampling extended from 1st to 3rd day and 1 month, whereas Fib was significant during 1st, 2nd day and the period from 1 week to 3rd month. Also, Hap was Presented a remarkable effect for 2nd hour, 1st day and 1st to 3rd month, whereas SAA was

significant at 2nd and 3rd day and highly significant ($P \leq 0.01$) during the period from 1st to 3rd month of the experiment. Both TAC, Fib and Hap participated in high significant during the 1st month of the trial.

Table 5. APPs in calves' blood through dams' parities (1st to 5th)

Days of Sample	Haptoglobin (Hap), mg/ml					Serum amyloid A (SAA), µg/ml				
	1 st	2 nd	3 rd	4 th	5 th	1 st	2 nd	3 rd	4 th	5 th
0	0.1 ^a	0.1 ^a	0.1 ^a	0.1 ^a	0.1 ^a	16.8 ^a	16.9 ^a	17.0 ^a	17.1 ^a	17.1 ^a
Hour	±0.0	±0.0	±0.0	±0.0	±0.0	±0.5	±0.7	±0.7	±0.7	±0.4
2nd	0.1 ^b	0.1 ^{ab}	0.1 ^a	0.1 ^a	0.1 ^a	17 ^a	17.2 ^a	17.2 ^a	17.3 ^a	17.2 ^a
Hour	±0.0	±0.0	±0.0	±0.0	±0.0	±0.6	±0.7	±0.7	±0.7	±0.4
1st	0.1 ^a	0.1 ^b	0.1 ^b	0.1 ^b	0.1 ^b	117.1 ^b	117.6 ^{ab}	120.8 ^{ab}	122 ^a	119.6 ^{ab}
Day	±0.0	±0.0	±0.0	±0.0	±0.0	±1.4	±1.9	±1.3	±1.5	±1.6
2nd	0.2 ^a	0.2 ^a	0.2 ^a	0.2 ^a	0.2 ^a	119.1 ^b	119 ^b	123.1 ^{ab}	123 ^{ab}	126.2 ^a
Day	±0.0	±0.0	±0.0	±0.0	±0.0	±1.4	±1.9	±1.5	±1.6	±1.6
3rd	0.2 ^a	0.2 ^a	0.2 ^a	0.3 ^a	0.3 ^a	121.4 ^b	121.3 ^b	124 ^{ab}	124 ^{ab}	127.1 ^a
Day	±0.0	±0.0	±0.0	±0.0	±0.0	±1.6	±1.7	±1.4	±1.4	±1.5
Week	0.3 ^a	0.3 ^a	0.3 ^a	0.3 ^a	0.3 ^a	128.7 ^a	128.4 ^a	128.6 ^a	127 ^a	128.5 ^a
	±0.1	±0.0	±0.0	±0.0	±0.0	±2.6	±2.2	±2.0	±1.2	±1.2
1st	0.3 ^b	0.3 ^b	0.3 ^b	0.3 ^{ab}	0.4 ^a	105.7 ^c	111.3 ^b	119.5 ^{ab}	130 ^a	120.8 ^{ab}
Month	±0.0	±0.0	±0.0	±0.0	±0.0	±6.6	±3.5	±1.9	±1.1	±1.6
2nd	0.2 ^{ab}	0.3 ^a	0.2 ^b	0.2 ^{ab}	0.3 ^a	96.6 ^b	97.3 ^b	97.8 ^b	120 ^a	102.4 ^b
Month	±0.0	±0.0	±0.0	±0.0	±0.0	±6.2	±4.3	±3.8	±1.4	±3.0
3rd	0.2 ^a	0.2 ^a	0.2 ^a	0.2 ^a	0.2 ^a	90.6 ^{ab}	90.9 ^{ab}	75.6 ^c	98.6 ^a	81.6 ^{bc}
Month	±0.0	±0.0	±0.0	±0.0	±0.0	±5.4	±4.1	±2.6	±3.1	±2.8
Overall	0.19 ^a	0.19 ^a	0.2 ^a	0.21 ^a	0.2 ^a	90.3 ^b	91.75 ^b	91.5 ^b	97.7 ^a	93.38 ^{ab}
Mean	±.00	±.00	±.00	±.00	±.00	±2.66	±2.05	±1.27	±1.0	±1.17

a, b,c,d Means bearing different superscripts in the same row are significantly different ($P \leq 0.05$).

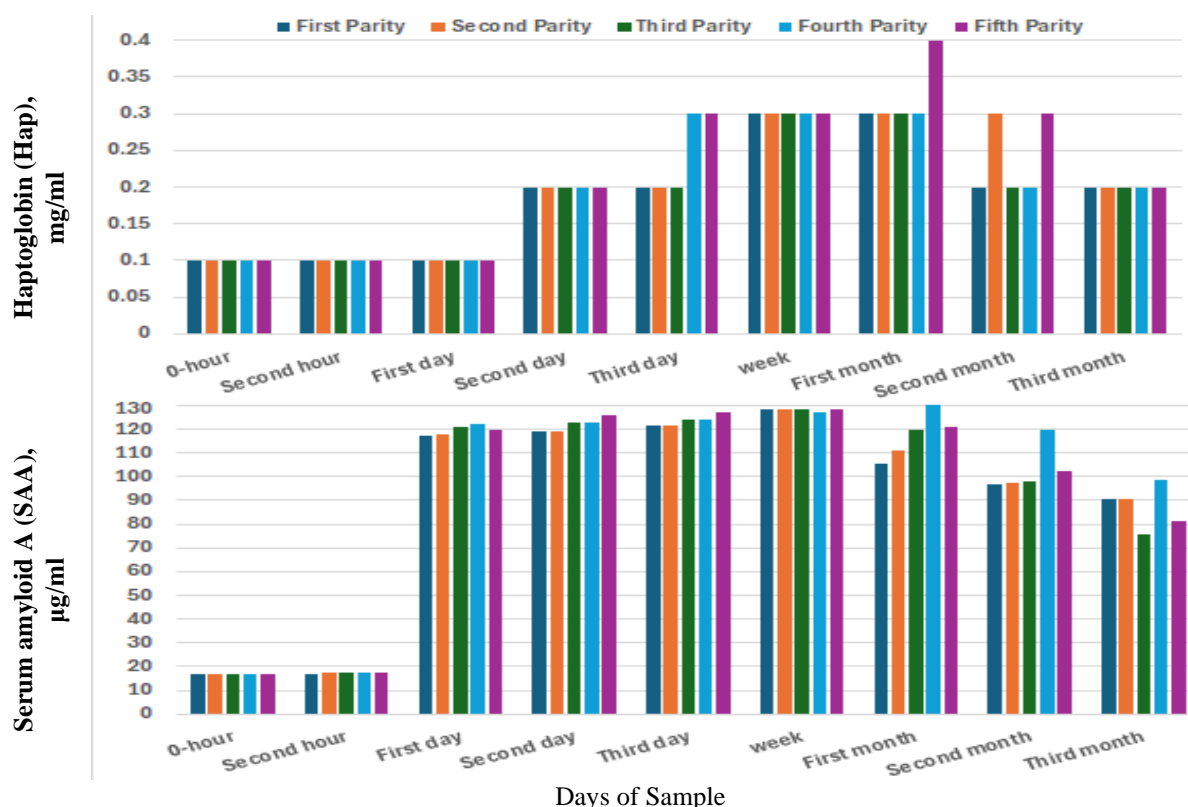


Figure 5. Hap and ASS in calves' blood throughout dams' parities.

CONCLUSION

There are several metabolic and physiological adjustments that fetus must undergo when transitioning from the uterus to the outside world, and the immune system is merely one of the systems impacted. During the 1st year of calves' life, a substantial number were died from newborn calves. Numerous factors can impact the quality of Ig including parity of dams. Additionally, the active transport of Ig and the immunological responses of newborn calves are influenced by parity of dams. The level of cytokines (Interleukin, L1, and L6) and acute phase protein in calves' blood are also related to Ig.

ACKNOWLEDGMENTS

The authors thanks Prof. Dr. Elmalky, O.M. the Chief researcher at APRI, Egypt; to its collaborate and make available the animals and lab required to conduct the research.

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توصيف بعض المقاييس المناعية والالتهابية في الدم خلال المواسم الانتاجية في الجاموس المصري

أسامة المالكي^١، حلمي عبد الهادي^٢، عبدا لله نبير^٢؛ حنان العشري^٢، ريم صابر مراد^٢

١-معهد أبحاث الإنتاج الحيواني، معهد البحوث الزراعية، الدقي، الجيزة، مصر، ٢- قسم الإنتاج الحيواني، كلية الزراعة، جامعة المنوفية، المنوفية، مصر

البريد الإلكتروني: agr.menofia.edu.eg@reem.mourad

أوضحت الدراسة توصيف الجلوبيولين المناعي في لبن السرسوب والجلوبيولين المناعي والسيتوكينات وبروتينات المرحلة الحادة الذي يشمل السعة الكلية لمضادات الأكسدة والفيبرينوجين والهبتوجلوبيولين وسيرم الأميلويد-A والاستجابات المناعية لعجول الجاموس حديثة الولادة خلال المواسم المختلفة للأمهات. تمت التجربة باستخدام ٥٠ جاموسه من قطيع تابع لمركز البحوث الزراعية وكانت مدة التجربة خمسة أشهر من (نوفمبر ٢٠١٧ - مارس ٢٠١٨). وقد أوضحت النتائج وجود فروق معنوية بين مواسم الولادة بالنسبة لكل من IgG و IgA بينما كان هناك فروق معنوية بالنسبة لـ IgM بين اليوم الأول والثالث فقط في المواسم المختلفة على الجلوبيولين المناعي في لبن السرسوب ووجود فروق معنوية بين مواسم الولادة بالنسبة لكل من IgG و IgA بينما كان هناك فروق معنوية بالنسبة لـ IgM بين اليوم الأول والثالث لتركيزها في العجول حديثة الولادة. كما كان هناك فروق معنوية على مستوى (٠.٠٥) بين أيام اخذ العينات بداية من ساعة الصفر وحتى أول يوم بينما لم يكن هناك فروق معنوية بين أيام اخذ العينات بداية من اليوم الثالث حتى الشهر الثالث من التجربة بالنسبة للانترلوكينات. كما استمرت الاختلافات المعنوية على نفس المستوى منذ أول وثالث يوم وكذلك الشهر الأول بعد الولادة بينما كان الفبرينوجين مختلف معنويًا في اليوم الأول والثاني وكذلك الفترة بداية من اسبوع حتى نهاية التجربة. كما ان الهبتوجلوبيولين والانترلوكين-٦ كان لهم تأثير ملحوظ في الساعة ٢:٠٣ وكذلك في اليوم الأول والشهر الأول حتى نهاية الشهر الثالث. وقد وجد ان هناك فروق معنوية في الـ SAA في اليوم الثاني والثالث على مستوى معنوية (٠.٠١) وخلال الفترة من الشهر الأول وحتى الشهر الثالث وكذلك الحال بالنسبة للـ TAC والفبرينوجين والهبتوجلوبيولين خلال الشهر الأول من الولادة. يؤثر الموسم الإنتاجي للأمهات على جودة الجلوبيولين المناعي السرسوب، والنقل النشط للجلوبيولين المناعي والاستجابات المناعية للعجول حديثي الولادة. يرتبط مستوى السيتوكينات وبروتينات المرحلة الحادة في دم العجول أيضًا بالجلوبيولين المناعي.