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Calf Sex-Associated Variations in Hematological, Biochemical, Thyroid Hormones, and Immune Profiles in Pre-Weaned Egyptian Buffalo Calves

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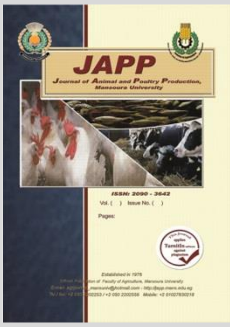

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ABSTRACT

This investigation examined the effects of calf sex on hematological indices, metabolic biomarkers, thyroid hormone levels, and immune response indicators in Egyptian buffalo calves throughout the pre-weaning phase. The study involved fifty healthy neonates (23 males and 27 females), which were followed from birth through three months of age. Serial blood samples were obtained to measure immunoglobulins (IgA, IgM, IgG), complete blood count parameters, glucose, creatinine, total protein and its constituents (albumin, globulin, and the albumin-to-globulin ratio), in addition to triiodothyronine (T_3) and thyroxine (T_4) concentrations. Results indicated significant sex-related differences in several parameters. Male calves showed higher IgM (0.71 vs. 0.64 g/L), IgG (12.46 vs. 12.23 g/L), total protein (6.77 vs. 6.14 g/dL), albumin (3.72 vs. 3.49 g/dL), globulin (3.04 vs. 2.65 g/dL), glucose (83.47 vs. 78.65 mg/dL), T_3 (49.82 vs. 46.32 ng/dL), and T_4 (6.30 vs. 5.55 µg/dL) compared to females. IgA levels were similar between sexes (0.25 g/L). In contrast, females exhibited higher creatinine (1.50 vs. 1.30 mg/dL) and albumin-to-globulin ratio (1.48 vs. 1.33). Hemoglobin and hematocrit values were comparable, although males tended to show slightly higher means (Hb: 13.21 vs. 13.14 g/dL; Ht: 40.61 vs. 40.46%). In conclusion, calf sex significantly affects hematological, metabolic, and immune traits in buffalo calves during early life. These findings highlight the importance of incorporating sex-related differences into neonatal health assessments and management strategies to optimize growth and survival.

Keyword: Egyptian Buffalo Calves, Hematological parameters, Blood metabolites, Thyroid hormones Immunoglobulins, Pre-weaning.

INTRODUCTION

The Egyptian water buffalo (Bubalus bubalis) is a vital component of the country's livestock industry, playing a major role in both the dairy and meat sectors. It is responsible for roughly 81% of Egypt's total milk production and contributes approximately 45% to its national red meat supply (FAO, 2019). Indeed, owing to its remarkable adaptability to harsh climates and exceptional feed conversion efficiency, the Egyptian water buffalo stands as a vital and indispensable asset to the nation's rural economy (Rabie, 2020). The evaluation of hematological and biochemical profiles is a standard approach for evaluating the health status of neonatal calves. Hematological indices such as hemoglobin concentration, hematocrit percentage, erythrocytes and leukocyte counts are essential indicators of physiological status and immune function (Jones and Allison, 2007).

Blood metabolites serve as key indicators of metabolic and renal health; for instance, glucose levels reflect energy status, while creatinine is a primary marker of renal function (Alberghina *et al*., 2015; Otomaru *et al*., 2016). Additionally, thyroid hormones play critical roles in thermoregulation, growth, and metabolic activity during early life (Ingole *et al*., 2012). Moreover, the thyroid hormones peaked at birth and declined markedly by the time of weaning (Habeeb *et al*., 2016). However, age had significant effect on plasma T4. in the Sarabi calves, with values being lower in 1–2-month-old calves and the highest levels of thyroid hormones were seen during the 1st 2-weeks after birth (Eshratkhah *et al*., 2010).

Immunoglobulins, IgG, IgM, and IgA are essential components of passive immunity transferred through colostrum. They are critical for neonatal survival in buffalo calves, which are born agammaglobulinemic (Feitosa *et al*., 2010; Aydogdu and Guzelbektes, 2018;). Meanwhile, Fernández *et al*. (2016) observed no significant effect of calf sex on cytokine, indicating that sex-related influences may be specific to certain immune components.

The absorption and metabolism of immunoglobulins are influenced by multiple factors, including the calf's sex. Sex of the newborn calf had a significant influence ($P<0.01$) on the IgM level. Moreover, higher immunoglobulin absorption was reported in female (25.12 mg/mL) compared to male calves (20.69 mg/mL); Similarly, at weaning, female calves exhibited significantly higher IgM levels ($P<0.01$) than males (Akbulut *et al*., 2003). In contrast, total immunoglobulin levels were higher in male calves than in females (Angulo *et al*., 2015).

Research in cattle and small ruminants has documented sex-specific disparities in hematological profiles and immune function (Gulliksen *et al*., 2008; Rojas *et al*., 2024). Several studies indicate that male calves often present with significantly higher circulating levels of certain immunoglobulins and thyroid hormones than their female counterparts (Akbulut *et al*., 2003; Fayed *et al*., 2014). While such physiological

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distinctions have been well-documented in other species, there is a notable lack of research regarding the impact of calf sex on the early physiological and immune development of buffalo calves. Consequently, this study was conducted to examine how sex influences hematological parameters, blood metabolite levels, thyroid hormone concentrations, and key immune indicators in Egyptian buffalo calves throughout the pre-weaning phase. Elucidating these sex-specific differences is crucial for developing targeted management strategies that enhance calf health, welfare, and overall productivity within buffalo breeding operations.

MATERIALS AND METHODS

The experimental work of this study was conducted at the Animal Production Research Station (ElNattf-ElKadym), affiliated with Animal Production Research Institute, Egypt, located at Mahallet Mousa, Kafrlsheikh governorate (31°.7' N latitude and 30°.51' N longitude), Egypt. The experimental period lasted for five months from November 2017 to March 2018. The experimental design and animal handling procedures adhered to the ethical standards set forth by Menoufia University, for the care and use of animals in scientific research and was approved by the Institutional Animal Care and Use Committee of Menoufia Univ., Egypt [№: MUFAG-F-AP-3-22].

Animals:

A cohort of 50 Egyptian buffalo calves was included in this study at ElNattaf-ElKadeem Farm. The calves were stratified by sex into two groups: 20 males (average birth weight: 43.12 ± 0.19 kg) and 30 females (average birth weight: 37.56 ± 0.28 kg). Management protocols were consistent for all animals. Calves received colostrum from their dams for the 1st three days postpartum. They were housed in semi-open pens with concrete floors and asbestos roofing. From one month of age onward, they were provided daily outdoor grazing access between 9:00 a.m. and 3:00 p.m. Body weight was recorded at birth (Day 0) and bi-weekly until weaning.

Management and feeding of calves:

Feeding protocol, immediately postpartum (within 20 minutes), calves suckled colostrum directly from their dams and cohabited with them for the 1st three days. Subsequently, calves were separated and housed in individual rearing pens. From day 4 through week 6, calves were fed whole milk at 10% of their birth weight, divided into two equal meals administered at 07:00 and 16:00 via individual plastic buckets fitted with teat feeders. A gradual weaning process began in week 7 by reducing the milk allowance by approximately 1% of body weight weekly until complete weaning at 15 weeks of age. Solid feed and water, from the 3rd week onward, calves were provided with ad-libitum access to a solid ration (concentrate feed mixture, CFM, and Berseem hay). Clean drinking water was available continuously throughout the study.

Blood sampling and chemical analysis:

Blood samples were collected from all calves at predetermined intervals to assess hematological profiles, biochemical markers, thyroid hormone levels, and specific immune indicators. The sampling schedule was as follows: at birth (0 h), 2 hours post-initial colostrum intake, on days 1, 2, 3, and 7, and at 1, 2, and 3 months of age (days 30, 60, and 90), thereby encompassing the entire pre-weaning and weaning period. All samples were obtained in the early morning prior to feeding. Two blood draws were performed per calf during each sampling event, collected via sterile venipuncture of the external jugular vein. Blood was aliquoted into both heparinized tubes for plasma and plain tubes for serum. A subset of fresh whole blood from heparinized samples was immediately utilized for hematological analysis. The parameters measured included: hemoglobin concentration (Hb, g/dL); hematocrit percentage (Hct, %); red blood cell count (RBCs, $\times 10^6/\mu L$) and white blood cell count (WBCs, $\times 10^3/\mu L$). The remaining blood sample was centrifuged at 3,000 rpm for 20 minutes to separate plasma. The resulting clear plasma was aspirated and stored at –20°C in cryogenic vials until further analysis. Subsequent biochemical and immunological assays were performed on the thawed plasma samples. The concentrations of total protein (TP), albumin (ALB), glucose (GLU), and creatinine (CRT) were determined in blood plasma. Thyroid hormone profiles, including triiodothyronine (T_3) and thyroxine (T_4), along with immune markers interleukin-1 (IL-1) and interleukin-6 (IL-6), were quantified using validated commercial enzyme-linked immunosorbent assay (ELISA) kits.

Statistical analysis:

All statistical analyses were performed with the General Linear Model (GLM) procedure in SAS (SAS, 2002). Mean comparisons were conducted using Duncan's multiple range test, with a significance level established at $p < 0.05$. The following statistical model was applied:

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

Where; **Y_{ijklm} = the observed value of the dependent variable, μ = the overall mean, Pi = the fixed effect of the ith parity (i = 1 to 5), W_j = the fixed effect of the jth live body weight category (j = 1 to 3), C_k = the fixed effect of the kth body condition score (k = 1 to 2), S_l = the fixed effect of the lth calf sex (l = 1 for males, 2 for females), e_{ijklme} = Error term NID (0, σ2e).**

RESULTS AND DISCUSSION

Effect of calf sex on immunoglobulins in blood plasma

Table 1 shows the levels of immunoglobulins (IgA, IgM, and IgG) in the blood of male and female Egyptian buffalo calves at various time points from birth up to 3 months of age. At birth as zero hour and 2 hours after 1st suckling day, no significant differences were observed between males and females in IgA, IgM, or IgG levels. However, starting from the 1stday of age, immunoglobulin concentrations increased markedly in both sexes, indicating effective colostrum absorption.

Male calves exhibited consistently higher levels of IgM and IgG than females across most time points. The overall mean IgM concentration was significantly higher in males (0.71 ± 0.04 g/L) compared to females (0.64 ± 0.04 g/L; $P<0.05$). Similarly, the overall mean IgG concentration was significantly higher in males (12.46 ± 0.26 g/L) than in females (12.23 ± 0.25 g/L; $P<0.05$). In contrast, IgA levels remained relatively constant over time and did not differ significantly between sexes, with both groups having an overall mean of 0.25 g/L.

These findings suggest a sex-based variation in the acquisition and maintenance of passive immunity, with male calves demonstrating slightly higher levels of IgM and IgG during the pre-weaning period.

Regarding impact of calf sex on level of Igs in blood serum, calf sex did not significantly influence level of IgM obtained at various stages of the calf growth except for weaning and female calves had higher IgM level than male ones (Akbulut *et al*., 2003). Also, calf sex hadn't any significant predictors for detection of calf serum IgG (Gulliksen *et al*., 2008). Calf sex did not influence serum levels of IgG in cattle (P>0.05) (Cavirani *et al*., 2024) . Serum IgG level peaked at 24h after birth, these results were agreement with the previous reports in cattle (Feitosa *et al*., 2010; Rocha *et al*., 2012).

Table 1. Immunoglobulin's (g\l) in blood plasma of newborn calves (N= 23male; 27 female).

Time#	IgA		IgM		IgG	
	Male	Female	Male	Female	Male	Female
0 h	0.1±0.0	0.1±0.0	0.3±0.0	0.3±0.0	0.7±0.0	0.6±0.0
2 h	0.1±0.0	0.1±0.0	0.6±0.6	0.5±0.1	3.0±0.2	2.7±0.2
1 day	0.1±0.0	0.1±0.0	0.7±0.1	0.6±0.1	8.4±0.4	9.0±0.3
2 day	0.1±0.0	0.3±0.1	0.7±0.1	0.7±0.1	17.5±0.6	17.4±0.6
3 day	0.4±0.1	0.3±0.1	0.8±0.1	0.8±0.1	18.4±0.5	18.2±0.5
7 day	0.4±0.1	0.4±0.1	0.9±0.1	0.8±0.1	18.8±0.4	18.4±0.4
30day	0.4±0.1	0.4±0.1	0.9±0.1	0.8±0.1	17.7±0.3	17.4±0.4
60day	0.4±0.1	0.3±0.1	0.8±0.0	0.8±0.0	15.5±0.5	14.7±0.5
90day	0.3±0.1	0.3±0.1	0.7±0.0	0.7±0.0	12.1±0.4	11.7±0.5
Mean*	0.25±0.04	0.25±0.05	0.71±0.04^a	0.64±0.04^b	12.46±0.26^a	12.23±0.25^b

^{a,b,c,d} **Means bearing different superscripts in the same row are significantly different (P ≤ .05). #Times of Sampling, * overall mean±SE**

Effect of calf sex on characteristics of its blood traits:

Table 2 shows the hematological profiles of male and female Egyptian buffalo calves from birth up to three months of age, including hemoglobin, hematocrit, RBCs, and WBCs. Overall mean of hemoglobin concentrations showed sex-based variation, with male calves having significantly (P≤0.05) lower value (13.14 ± 0.09 g/dL) than females (13.21 ± 0.12 g/dL), but no significant differences between both sexes were found at different sampling times.

Hematocrit (Ht) levels showed significant differences between both sexes at specific time points. On Day 2, male calves had a significantly higher Ht (41.0 ± 0.4%) than females (39.6 ± 0.5%), while at two months of age, female calves exhibited a higher Ht (41.3 ± 0.2%) than males (40.1 ± 0.4%). The overall mean of Ht was significantly (P≤0.05) higher in males (40.61 ± 0.18%) than in females (40.46 ± 0.26%).

Table 2. Hematological parameters of the newborn calves (N= 23male; 27 female).

Time#	Hb, g/dL		Ht%		RBCs		WBCs	
	Male	Female	Male	Female	Male	Female	Male	Female
0 h	12.6 ±0.2	12.6 ±0.2	40.1 ±0.3	39.6 ±0.4	12.5 ±0.2	12.0 ±0.2	10.4±0.2	10.5 ±0.2
2 h	12.5 ±0.2	12.3 ±0.2	40.7 ±0.2	40.2 ±0.4	12.7 ±0.2	12.3 ±0.2	10.3 ±0.3	10.0 ±0.3
1 day	12.9 ±0.2	13.0 ±0.2	40.6 ±0.4	40.1 ±0.4	12.9 ±0.3	12.4 ±0.3	10.0 ±0.3	9.8 ±0.3
2 day	13.0 ±0.2	13.1 ±0.2	41.0±0.4^a	39.6±0.5^b	12.6 ±0.3	12.4 ±0.3	9.7 ±0.3	9.6 ±0.3
3 day	13.2 ±0.2	13.1 ±0.2	40.5 ±0.4	39.8 ±0.5	13.0 ±0.2	13.0 ±0.2	9.8 ±0.3	9.6 ±0.4
7 day	13.6 ±0.2	13.3 ±0.2	41.0 ±0.4	40.4 ±0.5	9.5 ±0.3	9.9 ±0.4	9.5 ±0.3	9.9 ±0.4
30 day	13.2 ±0.2	13.7±0.3	40.7 ±0.5	41.7 ±0.4	13.5 ±0.3	13.7 ±0.2	9.2 ±0.4	9.4 ±0.4
60 day	13.3 ±0.3	13.9 ±0.3	40.1±0.4^b	41.3±0.2^a	13.5 ±0.3	13.9 ±0.2	9.0 ±0.4	9.4 ±0.4
90 day	14.0 ±0.4	14.0 ±0.3	40.7 ±0.4	41.3 ±0.3	14.2 ±0.3	14.2±0.3	8.6 ±0.4	9.3 ±0.4
Mean*	13.14 ±0.09^b	13.21 ±0.12^a	40.61 ±0.18^a	40.46 ±0.26^b	13.07 ±0.19^a	13.02 ±0.17^b	9.5 ±0.27^b	9.7 ±0.22^a

$^{a\,a,b,c,d}$ **Means bearing different superscripts in the same row are significantly different (P ≤ .05). #Times of Sampling, * overall mean±SE**

Count of RBCs was not affected by sex at different sampling times, with an overall mean being significantly (P≤0.05) higher in males (13.07 ± 0.19×10⁶/mm³) than females (13.02 ± 0.17×10⁶/mm³). Conversely, white blood cell counts were significantly higher in female calves (9.7 ± 0.22×10³/mm³) than in males (9.5 ± 0.27×10³/mm³), suggesting a potential sex-related difference in leukocyte development or immune activity. These results revealed that, male calves tended to show higher overall means of erythrocytic indices (Hb, Ht, and RBCs), while females had higher WBC counts, indicating mild but consistent sex-based variations in hematological parameters during the pre-weaning period.

The neonatal phase represents a critically vulnerable period characterized by heightened susceptibility to morbidity and mortality. These risks stem from factors such as congenital disorders, complications during parturition, and a pronounced vulnerability to infectious diseases. Consequently, comprehensive hematological assessment is indispensable for the timely diagnosis, monitoring, and prognostic evaluation of health in newborn calves (Mee, 2008). Hematological analysis is globally established as an essential diagnostic and monitoring tool within veterinary practice. These evaluations yield critical information regarding an animal's physiological status, nutritional health, and potential pathological conditions (Khan *et al*., 2011) and help distinguish the normal state from the state of stress, which can be nutritional, physical or environmental. Hematological parameters are influenced by the interaction of various factors, including species, breed, age, sex, nutritional status, disease, physical activity, transportation, and seasonal changes. (Farooq *et al*., 2011). Following initial clinical assessment, the complete blood count serves as a fundamental supportive tool for confirming diagnoses and forecasting clinical outcomes in veterinary practice (Jones and Allison, 2007). The determination of whether a measured parameter is normal relies on comparison with reference intervals (RIs) established from a clinically healthy reference population. Consequently, the formulation of RIs must account for potential variations due to genotype, age, sex, management conditions, and laboratory methodologies (Friedrichs *et al*., 2012). Hematocrit (Ht) represents the percentage of total blood volume composed of red blood cells (RBCs). Elevated hematocrit levels are associated with conditions such as dehydration, burns, vomiting, polycythemia, and strenuous physical activity. Conversely, reduced hematocrit values are observed in various forms of anemia, including macrocytic, normocytic, and microcytic anemia (Brooks *et al*., 2022). The reference range for hematocrit in most domestic animal species falls between 38% and 40% (Swenson, 2004). Female calves had higher RBCs count, Ht % and Hb level than males (Mohri *et al*., 2007). In contrast, decreasing RBCs and Ht % values from birth up to 3 or 6 d of age (Knowles *et al*., 2000).

Effect of calf sex on concentrations of blood protein fractions:

Table 3 presents the serum protein fractions, total protein (TP), albumin (ALB), globulin (GLB), and albumin/globulin (ALB/GLB) ratio, in male and female Egyptian buffalo calves during the pre-weaning period. Male calves showed significantly higher concentrations of TP, albumin, and globulin compared to females at most time points. Notably, at birth (0 h), males had significantly higher TP (5.6 ± 0.1 vs. 5.0 ± 0.1 g/dL), ALB (3.2 ± 0.1 vs. 3.0 ± 0.1 g/dL), and GLB (2.6 ± 0.1 vs. 2.0 ± 0.1 g/dL) levels than females (P<0.05). This trend persisted throughout the study, with the overall mean TP, ALB, and GLB concentrations significantly greater in males (6.77 ± 0.11, 3.72 ± 0.06, and 3.04 ± 0.10 g/dL, respectively) than females (6.14 ± 0.10, 3.49 ± 0.08, and 2.65 ± 0.10 g/dL, respectively; P<0.05). In contrast, female calves consistently exhibited a significantly higher ALB/GLB ratio than males across several sampling points. At birth, the ALB/GLB ratio was 1.7 ± 0.2 in females compared to 1.3 ± 0.1 in males (P<0.05), and the overall mean ratio remained higher in females (1.48 ± 0.08) than males (1.33 ± 0.05; P<0.05). This suggests that although male calves had higher absolute protein levels, females maintained a more balanced serum protein profile.

These findings indicate a clear sex-based difference in protein metabolism or colostrum absorption efficiency, with male calves showing enhanced protein synthesis or uptake, while females displayed a relatively higher ALB dominance. The present findings showed that males calves had significantly (P<0.05) higher level of TP, ALB and GLB than females. In contrast, values of plasma TP didn't differ between male and female (Abdel-Fattah *et al*., 2013). In neonate's dairy calves, ALB is an important carrier protein for maintaining homeostasis and bind to multiple molecules including long-chain fatty acid and steroid adding that plasma GLB is primarily influenced by changes in IgG level which serves an important role in passive transfer of immunity to the neonate (Kaneko *et al*., 2008). Also**,** GLU level increased during the 1st 48 h of life, due to colostrum intake (Duncan, 2018; Larson, 2016). Also, there was no clear effect of age on TP. Which, plasma TP remained within normal range. Peak plasma TP levels were observed at 30 days of age, while the lowest measurements were recorded at 90 days. This reduction in TP levels corresponded temporally with the weaning process at 90 days of age. These findings align with previously reported values in early-weaned lambs, where pre-weaning plasma TP levels remained within the range of 6.0–6.2 g/dL and demonstrated no significant differences between male and female animals(Abdel-Fattah *et al*., 2013).

Table 3. Protein fractions in the blood of newborn calves (N= 23male; 27 female).

Time#	Total protein (TP, g/dl)		Albumin (ALB, g/dl)		Globulin (GLB, g/dl)		ALB/GLB ratio	
	Male	Female	Male	Female	Male	Female	Male	Female
0 h	5.6 ±0.1[a]	5.0 ±0.1[b]	3.2 ±0.1[a]	3.0 ±0.1[b]	2.6 ±0.1[a]	2.0 ±0.1[b]	1.3 ±0.1[b]	1.7 ±0.2[a]
2 h	5.6 ±0.1[a]	5.1 ±0.1[b]	3.3 ±0.1	3.1 ±0.1	2.6 ±0.1[a]	2.0 ±0.1[b]	1.3 ±0.1[b]	1.6 ±0.1[a]
1-day	6.0 ±0.1[a]	5.3 ±0.1[b]	3.4 ±0.1	3.2 ±0.1	2.7 ±0.1[a]	2.1 ±0.1[b]	1.3 ±0.1[b]	1.6 ±0.1[a]
2-day	6.4 ±0.1[a]	5.7 ±0.1[b]	3.5 ±0.1[a]	3.3 ±0.1[b]	2.9 ±0.1[a]	2.4 ±0.1[b]	1.3 ±0.1	1.5 ±0.1
3 day	6.8 ±0.1[a]	6.1 ±0.1[b]	3.6 ±0.1	3.4 ±0.1	3.2 ±0.1[a]	2.7 ±0.1[b]	1.2 ±0.1	1.4 ±0.1
7 day	7.3 ±0.2[a]	6.7 ±0.2[b]	3.9 ±0.1	3.6 ±0.1	3.4 ±0.2	3.1 ±0.1	1.2 ±0.1	1.2 ±0.1
30 day	7.5 ±0.2	7.0 ±0.2	4.1 ±0.1	3.9 ±0.1	3.4 ±0.2	3.1 ±0.1	1.3 ±0.1	1.3 ±0.1
60 day	7.8 ±0.3	7.4 ±0.2	4.2 ±0.1	4.0 ±0.1	3.6 ±0.2	3.4 ±0.2	1.3 ±0.1	1.3 ±0.1
90 day	7.6 ±0.3	7.1 ±0.3	4.4 ±0.1[a]	4.1 ±0.1[b]	3.2 ±0.3	3.0 ±0.2	1.9 ±0.3	1.7 ±0.2
Mean*	6.77 ±0.11[a]	6.14 ±0.10[b]	3.72 ±0.06[a]	3.49 ±0.08[b]	3.04 ±0.10[a]	2.65 ±0.10[b]	1.33 ±0.05[b]	1.48 ±0.08[a]

[a,b,c,d] Means bearing different superscripts in the same row are significantly different (P ≤ .05). #Times of Sampling, * overall mean±SE

TP values in the Egyptian buffalo calves were nearly the same at birth, weaning and after 24 months of age. The highest GLB level was at birth while GLB level was not changed from weaning to 24 months of age (Habeeb *et al*., 2016). However, age related changes had significant effect on TP, ALB and GLB levels and approximately similar changes were seen in both of male and female Holstein calves (Mohri *et al*., 2007). In beef breed, ALB level at 3 d of age was higher in Charolais calves (28.8 g/l) than in Hereford calves (25.1 g/l), while GLB decreased in level during the 1st month of life (Seppä-Lassila, 2018). The level of ALB starts increasing toward adult levels after birth with a slightly steeper rate. They noticed a marked decrease in ALB after the 1st d of colostrum intake followed by subsequent significant increase of level until the end of the 1st month of age (P<0.001) (Tothova *et al*., 2015). Levels of TP and γ-globulins were correlated with colostrum intake and IgG level, as demonstrated with small ruminants (Loste *et al*., 2008)**.** Serum Igs, TP and GLB in calves were increased following colostrum feeding (Aydogdu and Guzelbektes, 2018). However, serum Igs, TP and GLB levels in calves from multiparous cows were higher than those of calves from primiparous cows; so, there was a positive correlation among serum components, which could be used to determine the passive transfer status of calves. TP and GLB increased significantly one d after colostrum intake (P<0.001) then significantly decreased gradually till the end of the 1st month of calf age. An opposite trend was observed in level of ALB (P<0.001), where the initial decline of ALB level occurred at 1st 2-d after birth, followed by gradual increase in values till the end of the 1st month of life. Then the above-mentioned changes are also reflected in changes of ALB/GLB ratio (P<0.001). Also, they cleared that serum TP profile in calves was significantly influenced by colostrum intake and the age of calves (Tóthová *et al*., 2016).

In the same manner, at birth, the serum TP level in calves was quite low due to the minimal quantities of Igs and the increase in its values during the 1st 24-h of life reflects the intestinal absorption of proteins (particularly Igs) from colostrum due to enhanced intestinal permeability (Hammon *et al*., 2002). The highest TP level on the 1std of calf life (Piccione *et al*., 2010). However, from the 2nd d of calf life gradually decreasing values of TP were observed until the end of the 1st month and then TP level was lower almost by 20 g/l at the end of the study due to degradation of colostral Igs (Mohri *et al*., 2007). Longitudinal analyses have indicated a steady rise in ALB level in calves throughout the neonatal period, extending through the first 80 days of life.

(Ježek *et al*., 2006; Piccione *et al*., 2009)**.** Changes in ALB level throughout growth are inversely correlated with changes in GLB levels. This is characterized by a disproportionately high ALB/GLB ratio in neonates, which gradually equilibrates with maturity (Thomas, 2000).

Calves showed higher level of GLB on d 5 compared with values measured on d 30 of life. However, higher levels of GLB in calves shortly after birth are not necessarily a sign of the activation of inflammatory processes or a sign of a disease (Szewczuk *et al*., 2011). ALB/GLB ratio must be interpreted cautiously with attention paid to which part of the ratio has changed since it allows systematic classification and identification of dysproteinemias (abnormal Ig) (Alberghina *et al*., 2015).

The TP level increased significantly (p<0.05) after colostrum feeding in buffalo neonates, while ALB was the lowest on d 0 then increased significantly (p<0.05) after colostrum feeding on d-1 and d-2 after birth (Lone *et al*., 2003). TP level increased in calf's serum after 12 h of colostrum consumption by 80.5% dependent on the quantity and quality of colostrum provided to calves in the 1st 2-h after birth then, a gradual decrease was observed from 72 to 168 h. Also, ALB level was the highest in calves 24 h after birth then decreased by reaching 72– 168 h (Quezada-Tristán *et al*., 2014). ALB functions as a transport protein absorbed through the intestine of calves, its levels decreased 3 d after birth and the level of ALB and TP reflect the immunity of calves and can be used diagnostically (Kehoe *et al*., 2007). TP is absorbed by the intestine of newborn calves between 12–24 h post-partum, denoting that TP level can be used diagnostically to reflect the immunity of a calf. A level of TP in serum is lower than 6 g/dL indicates a deficiency in passive immunity (Güngör *et al*., 2004). However, serum TP level in calves at 72 h post- partum was 60 g/L (Singh *et al*., 2011). Levels of TP and GLB of newborn calves were 57.75 and 14.4g/L respectively (Stojić *et al*., 2017). In buffalo calves, TP and GLB exhibited an abrupt increase between the time before colostrum intake and 24h after birth (Souza *et al*., 2019).

The higher TP level to the enhanced intestinal absorption of Igs of colostrum, indicating an overall improved immune and protein status of the calves (Knowles *et al*., 2000). Increased intake of nutrients consumed through liquid feeding resulted in greater deposition of fat and protein in calves. Moreover, TP is a reliable criterion for assessment of passive transfer status in newborn calves. Even though, detection slight differences in the passive transfer group regarding the levels of ALB, GLU or IgG (Diaz *et al*., 2001). TP and ALB levels in the infected calves were insignificantly higher than that in the healthy group (Akgul *et al*., 2019). The significant increases in TP level from birth to 24- and 48-h samples in 2 different breeds may reflect not only colostrum intake but also the maturation of the protein synthesis (Probo *et al*., 2019). Blood plasma protein changes within short intervals until the 1st d of calf life. The results indicate that blood proteins undergo dynamic changes during the 1st week of life in Holstein calves (Herosimczyk *et al*., 2013).

4. Effect of calf sex on Glucose (GLU):

Table 4 shows the blood glucose concentration (mg/dL) in male and female Egyptian buffalo calves from birth to three months of age. Throughout the early postnatal period (0 h to 1stweek), female calves consistently exhibited significantly higher GLU levels compared to males (*P*<0.05). At birth, GLU concentration was 82.26 ± 1.59 mg/dL in females vs 76.41 ± 1.46 mg/dL in males. This significant difference persisted through the 1stday (84.47 ± 1.59 vs. 78.60 ± 1.46 mg/dL), 2ndday (85.78 ± 1.59 vs. 80.27 ± 1.47 mg/dL), 3rdday (87.60 ± 1.56 vs. 81.59 ± 1.44 mg/dL), and the end of the 1st week (89.53 ± 1.64 vs. 84.00 ± 1.51 mg/dL). From the 1st month onward, GLU began to decline gradually in both sexes. Although differences were not statistically significant during the 1st, 2nd, and 3rdmonths, females still maintained numerically higher GLU levels than males. The overall mean GLU across the experimental period remained significantly higher in females (83.47 ± 1.53 mg/dL) compared to males (78.65 ± 1.41 mg/dL; *P*<0.05). These findings suggest a sex-related variation in GLU metabolism or energy regulation during the pre-weaning phase, with females potentially exhibiting greater metabolic activity or efficiency during early life.

Table 4. Glucose and Creatinine in the blood of newborn calves (N= 23male; 27 female).

Time#	Glucose, GLU (mg/dl)		Creatinine, CRT (mg/dl)	
	Male	Female	Male	Female
0 h	76.41±1.46 ^{b}	82.26±1.59^a	1.3±0.01^b	1.5±0.03^a
2 h	77.48±1.43 ^{b}	83.19±1.55 ^{a}	1.3±0.03^b	1.4±0.01^a
1 day	78.60±1.46 ^{b}	84.47±1.59 ^{a}	1.2±0.01^b	1.4±0.02^a
2 day	80.27±1.47 ^{b}	85.78±1.59 ^{a}	1.2±0.02^b	1.3±0.01^a
3 day	81.59±1.44 ^{b}	87.60±1.56^a	1.2±0.01^b	1.3±0.03^a
7 day	84.00±1.51 ^{b}	89.53±1.64^a	1.1±0.03^b	1.2±0.02^a
30 day	85.51±1.80	89.96±1.95	0.9±0.01^b	1.1±0.01^a
60 day	75.42±1.22	78.27±1.32	0.9±0.03	1.0±0.01
90 day	68.58±0.92	70.15±1.00	0.9±0.01	0.9±0.02
Mean*	78.65±1.41^b	83.47±1.53^a	1.3±0.01^b	1.5±0.01^a

a,b,c,d Means bearing different superscripts in the same row are significantly different (P ≤ .05). #Times of Sampling, * overall mean±SE

These findings suggest that from birth to weaning, calves primarily rely on glucose (GLU) derived from lactose in maternal milk as their main energy source. A significant effect of calf sex on glucose levels was observed, underscoring the importance of fetal sex as a determinant of maternal metabolic adaptation during the early transition period in dairy cows (Alberghina *et al*., 2015). Sex of the calf was significantly affected its GLU level that changed during a few weeks after birth, indicating that the physiological state in calves greatly changed referring these developmental changes in metabolite levels to ruminal maturation with increment of grain intake in Holstein calves (Sasaki *et al*., 2002). Sex of lambs had a significant effect on blood metabolism, which male lambs showed higher plasma level of GLU than female ones (p<0.05) (Pesántez-Pacheco *et al*., 2019). However, both of dam parity and gender did not affect GLU homeostasis in calves (Bossaert *et al*., 2014).

5. Effect of sex of calf on creatinine (CRT):

Table 4 shows the blood creatinine concentrations (mg/dL) in male and female Egyptian buffalo calves during the pre-weaning period. Female calves consistently exhibited higher creatinine levels than males across all sampling times, with statistically significant differences observed from birth through the end of the 1st month (*P*<0.05). At birth (0 h), the mean creatinine concentration was significantly higher in females (1.5 ± 0.03 mg/dL) than in males (1.3 ± 0.01 mg/dL). This trend continued Day 1 (1.4 ± 0.02 vs. 1.2 ± 0.01 mg/dL), Day 3 (1.3 ± 0.03 vs. 1.2 ± 0.01 mg/dL), and at one month of age (1.1 ± 0.01 vs. 0.9 ± 0.01 mg/dL).

While creatinine levels gradually declined over time in both sexes, the values remained slightly higher in females up to two months of age. By the 3rd month, concentrations became equal in both groups (0.9 mg/dL). The overall mean creatinine throughout the study was significantly higher in females (1.5 ± 0.01 mg/dL) compared to males (1.3 ± 0.01 mg/dL). These results suggest a sex-related difference in renal metabolism or muscle turnover during the early postnatal period. **C**reatinine is often used to indicate renal function and can be a diagnostic tool for neonatal morbidity (Pirrone *et al*., 2014). Significant decrease of **c**reatinine at 24 and/or 48 h compared with 30 min for in 2 breeds of calves (Probo *et al*., 2019).

No significant differences in CRT level between the different passive transfer groups of Holstein calves, and no trend of CRT elevation was found (Pekcan *et al*., 2013). The observed decrease in CRT levels supports the hypothesis that the elevated serum CRT levels detected in newborns immediately after birth primarily reflect maternal levels (Weintraub *et al*., 2015). In calves a significant effect (P<0.05) on CRT (Piccione *et al*., 2010). The observed decline in creatinine (CRT) levels from birth through the 1st week and 1st month of life reflects key physiological adaptations in neonates. This reduction is indicative of the calf's successful renal maturation and metabolic adjustment, supporting the maintenance of homeostasis during this critical developmental period (Steinhardt and Thielscher, 1999). The mean level of CRT between 14 and 90 d was (0.94 mg/dl) during the suckling stage of age (Otomaru *et al*., 2016). Levels of TP, GLU and CRT showed significant (p<0.05) variation with advanced calf age (Patel *et al*., 2016); but the variations were not significant between sex of calves, which agreed with the findings in Jaffar Abadi buffalo calves (Jacob, 2012).

6. The effect of calf sex on thyroid hormones:

Table 5 presents the concentrations of thyroid hormones, triiodothyronine (T_3) and thyroxine (T_4), in the blood of male and female Egyptian buffalo calves from birth to three months of age. Across all time points, male calves consistently exhibited significantly higher levels of thyroid hormones compared to females (*P* < 0.05). At birth (0 h), the mean T_3 concentration in males was 42.6 ± 0.5 ng/dL vs 39.1 ± 0.4 ng/dL in females, while T_4 levels were 4.0 ± 0.2 µg/dL and 3.2 ± 0.1 µg/dL, respectively. These differences remained statistically significant throughout the early postnatal period.

The highest levels were observed during the 3rd month of life, when T_3 reached 66.6 ± 2.7 ng/dL in males compared to 58.5 ± 2.9 ng/dL in females, while T_4 peaked at 9.6 ± 0.3 µg/dL in males and 8.9 ± 0.3 µg/dL in females, though this final difference in T_4 was not statistically significant. The overall mean concentrations across the study period were significantly higher in males for both hormones, with T_3 averaging 49.82 ± 0.93 ng/dL vs 46.32 ± 0.89 ng/dL in females, and T_4 averaging 6.30 ± 0.18 µg/dL in males vs 5.55 ± 0.13 µg/dL in females. These findings indicate a clear sex-related variation in thyroid hormone profiles during the pre-weaning period, suggesting that male calves may have higher metabolic activity or endocrine responsiveness in early life.

Table 5. Thyroid hormones in blood of newborn calves (N= 23male; 27 female).

Time#	T3 (ng/dl)		T4 (µg/dl)	
	Male	Female	Male	Female
0 h	42.6±0.5^a	39.1±0.4^b	4.0±0.2^a	3.2±0.1^b
2 h	43.1±0.5^a	39.8±0.4^b	4.3±0.2^a	3.5±0.1^b
1 day	43.8±0.5^a	40.8±0.5^b	4.8±0.2^a	4.0±0.1^b
2 day	45.7±0.6^a	42.8±0.6^b	5.2±0.2^a	4.5±0.1^b
3 day	46.9±0.6^a	44.0±0.7^b	5.7±0.2^a	5.1±0.1^b
7 day	49.1±0.7^a	46.2±0.8^b	6.7±0.2^a	5.9±0.2^b
30 day	53.7±1.2 ^{a}	50.6±1.2 ^{b}	8.0±0.3^a	7.1±0.2^b
60 day	56.9±2.4	55.1±2.1	8.6±0.3^a	7.9±0.2^b
90 day	66.6±2.7^a	58.5±2.9^b	9.6±0.3	8.9±0.3
Mean*	49.82±0.93^a	46.32±0.89^b	6.30±0.18^a	5.55±0.13^b

^{a,b,c,d} Means bearing different superscripts in the same row are significantly different (P ≤ .05). #Times of Sampling, * overall mean±SE

In Egyptian buffalo calves, age of calf had no significant effect on thyroid hormones. Adding that, T3 was higher at birth then decreased progressively and reached the lowest value (1.91 ng/ml) at 120 d of age (Fayed *et al*., 2014). These findings are consistent with observations that T4 levels exhibited a trend like that of T3, with no significant differences detected throughout the growth period until puberty. The elevated T3 concentrations observed in newborn calves are considered part of an adaptive physiological response to cope with the stress associated with birth. The subsequent decline in thyroid hormone levels may reflect the activation of a negative feedback mechanism. High concentrations of thyroid hormones at birth are essential for facilitating the calf's adaptation to the extrauterine environment and mitigating stress during this critical transition (Ingole *et al*., 2012). Levels of thyroid hormones have a negative significant correlation with age and BWG of buffalo calves. The highest levels of thyroid hormones were found at birth and decreased significantly at weaning (Habeeb *et al*., 2016). However, age had significant effect on plasma T4 level in the Sarabi calves, with values being lower in 1- to 2-month-old calves and the highest levels of thyroid hormones were seen during the 1st 2-weeks after birth (Eshratkhah *et al*., 2010).

7. The effect of calf sex on Interleukins (IL-1 and IL-6):

Table 6 shows IL-1 rose slightly after birth but were nearly identical in males and females (5.0 vs 4.9 pg/mL). This lack of a strong sex bias is consistent with IL-1's role as a general pro-inflammatory mediator. By contrast, IL-6 was consistently higher in males at all ages (69.6 vs 65.3 pg/mL; P<0.05). The observed elevation in IL-6 among male calves indicates a heightened innate inflammatory response compared to females. This sex-dependent pattern is consistent with findings in other species; for instance, pathogen-challenged beef calves demonstrated more pronounced IL-6 production in steers than in heifers. Further supporting this trend, stimulated peripheral blood mononuclear cells (PBMCs) from male calves secreted higher levels of IL-6, suggesting a generalized malebiased enhancement of IL-6 reactivity under immunogenic conditions. These results align with broader evidence across human and animal studies indicating sexual dimorphism in neonatal innate immunity, wherein males often exhibit stronger IL-6 responses than females following immune challenge (Burdick Sanchez *et al*., 2022).

Table 6. Interleukins in blood of newborn calves (N= 23male; 27 female).

Time[#]	IL-1 (pg/mL)		IL-6 (pg/mL)	
	Male	Female	Male	Female
0 h	4.8±0.1	4.6±0.1	68.7±0.9[a]	63.5±0.9[b]
2 h	4.8±0.1	4.6±0.1	68.7±0.9[a]	63.6±0.8[b]
1 day	4.9±0.1	4.6±0.1	69.5±1.0[a]	64.5±0.6[b]
2 day	4.9±0.1	4.7±0.1	69.9±0.9[a]	64.5±0.7[b]
3 day	5.0±0.1	4.9±0.1	69.2±0.8[a]	65.0±0.7[b]
7 day	5.1±0.1	5.0±0.1	69.7±0.8[a]	65.6±0.7[b]
30 day	5.3±0.2	5.2±0.2	70.1±0.9[a]	66.4±0.8[b]
60 day	5.3±0.2	5.3±0.2	70.4±0.9[a]	66.9±0.8[b]
90 day	5.0±0.2	5.2±0.2	70.3±1.1[a]	67.4±0.8[b]
Mean[*]	5.0±0.1[a]	4.9±0.1[b]	69.6±0.8[a]	65.3±0.7[b]

[a,b,c,d] Means bearing different superscripts in the same row are significantly different (P ≤ .05). [#]Times of Sampling, * overall mean±SE

Gender had no effect on cytokine concentration, but BW at birth had a significant effect (P<0.05) on the cytokine level which was higher in heavier lambs than that determined the lighter weight animal. However, in normal and low BW lambs at birth a statistically significant difference (P<0.05) for IL-1 and IL-6 cytokine levels were detected at d18 (Fernández *et al*., 2016). IL-1 and IL-6 are principal pro-inflammatory cytokines secreted predominantly by macrophages and monocytes during innate immune activation. Following inflammatory stimuli, these mediators contribute to the acute-phase response, along with TNF-α. IL-1 primarily induces localized inflammatory activity at sites of tissue damage or infection, whereas IL-6 exerts systemic effects, including the induction of acute-phase protein synthesis and modulation of genes related to cellular survival, proliferation, and differentiation. Consequently, both cytokines are rapidly upregulated following birth or pathogenic challenge, playing a critical role in orchestrating early immune defense mechanisms (Ciliberti *et al*., 2025).

CONCLUSION

The present study demonstrated clear sex-related differences in hematological, biochemical, hormonal, and immune parameters of Egyptian buffalo calves during the pre-weaning period. Male calves showed higher levels of immunoglobulins, total protein, thyroid hormones, and IL-6, indicating stronger immune and metabolic activity. Conversely, female calves had higher glucose, creatinine, and WBC counts, suggesting possible variations in immune development and hematopoietic activity between sexes.. These findings highlight the importance of considering calf sex in early health evaluations and management practices to optimize growth and immunity in buffalo herds.

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تأثير جنس المولود على صورة الدم والمقاييس البيوكيميائية وهرمونات الغدة الدرقية والاستجابة المناعية في عجول الجاموس المصري قبل الفطام

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الملخص

يهدف البحث إلى دراسة تأثير جنس العجل المولود على صورة الدم، وميتابولزم الدم، وهرمونات الغدة الدرقية، وبعض مقاييس المناعة في عجول الجاموس المصري خلال فترة ما قبل الفطام. تشتمل الدراسة على ٥٠ عجل جاموس حديث الولادة (٢٣ ذكر و٢٧ أنثى) منذ الولادة وحتى عمر ٣ أشهر. تم جمع عينات الدم على فترات محددة لتقدير تركيز الجلوبيولينات المناعية(IgA, IgM, IgG) ، وعدّ الدم الكامل(CBC) ، والجلوكوز، والكرياتينين، والبروتين الكلي وأجزاؤه (الألبومين، الجلوبيولين، ونسبة الألبومين/الجلوبيولين)، وهرمونات الغدة الدرقية (T_3 ,T_4). لقد أظهرت النتائج وجود فروق معنوية مرتبطة بالجنس عبر عدة مؤشرات. أظهرت العجول الذكور تركيزات أعلى من IgM (٠,٧١ ± ٠,٠٤ مقابل ٠,٦٤ ± ٠,٠٤ جم/لتر)، IgG (١٢,٤٦ ± ٠,٢٦ مقابل ١٢,٢٣ ± ٠,٢٥ جم/لتر)، البروتين الكلي (٦,٧٧ ± ٠,١١ مقابل ٦,١٤ ± ٠,١٠ جم/ ديسيلتر)، الألبومين (٣,٧٢ ± ٠,٠٦ مقابل ٣,٤٩ ± ٠,٠٨ جم/ ديسيلتر)، الجلوبيولين (٣,٠٤ ± ٠,١٠ مقابل ٢,٦٥ ± ٠,١٠ جم/ ديسيلتر)، الجلوكوز (٨٣,٤٧ ± ١,٥٣ مقابل ٧٨,٦٥ ± ١,٤١ ملجم/ ديسيلتر)، T_3 (٤٩,٨٢ ± ٠,٩٣ مقابل ٤٦,٣٢ ± ٠,٨٩ نانوجرام/ ديسيلتر)، و T_4 (٦,٣٠ ± ٠,١٨ مقابل ٥,٥٥ ± ٠,١٣ ميكروجرام/ ديسيلتر) مقارنة بالإناث. وعلى العكس من ذلك، أظهر كل من الذكور والإناث فروقًا طفيفة في IgA (٠,٢٥ ± ٠,٠٥ مقابل ٠,٢٥ ± ٠,٠٤ جم/لتر)، لكن الإناث سجلت قيم أعلى من الذكور في الكرياتينين (١,٥ ± ٠,٠١ مقابل ١,٣ ± ٠,٠١ ملجم/ ديسيلتر)، ونسبة الألبومين/الجلوبيولين (١,٤٨ ± ٠,٠٨ مقابل ١,٣٣ ± ٠,٠٥). على الرغم من أن مستويات الهيموجلوبين والهيماتوكريت أظهرت اختلافات طفيفة بين الجنسين، إلا أن العجول الذكور كانت لديها قيم أعلى قليلًا (Hb: 13.14 ± 0.09 vs. 13.21 ± 0.12 g/dL) و (Ht: 40.61 ± 0.18 vs. 40.46 ± 0.26%).نستخلص مما سبق أن جنس العجل يؤثر بشكل ملحوظ على المؤشرات الأيضية والدموية والمناعية في عجول الجاموس المصري خلال المراحل المبكرة من العمر. وتؤكد هذه النتائج على أهمية أخذ الفروق بين الجنسين في الاعتبار عند تقييم صحة العجول حديثة الولادة وإدارة رعايتها.

الكلمات الدالة **:** عجول الجاموس المصري، مقاييس الدم، ميتابولزم الدم، هرمونات درقية، الجلوبيولينات المناعية، ما قبل الفطام.