

## **Comparative Study on Electrophoretic Hemoglobin Types and Serum Protein Fractions, Some Biochemical and Hormones Parameters Changes During Transition Period in Iraqi Cows**

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### **Abstract**

This study was conducted to determine the type of blood hemoglobin blood and its effect on biochemical parameters, which include the lipid profile and hormones of local cows in Basrah province by obtaining blood samples for cows from different areas of Basrah province in order to determine the type of hemoglobin and its effect on hormones and lipid parameters. Results show that hemoglobin type (B) achieved highest percentage of two types (AB and A) respectively. A significant elevation hemoglobin type (B) with the highest level of total cholesterol and triglycerides. The lipoprotein results (LDL-C, HDL-C, and VLDL-C) showed in hemoglobin type (B) was superior to that of another hemoglobin which having serum lipoprotein levels in lactation and pregnancy periods. Different hemoglobin phenotypes showed elevation of progesterone and estrogen levels in some physiological periods while hemoglobin type (B) can be seen to have a higher level in control periods and pregnancy periods when compared with other hemoglobin types. As for cortisol, it showed increase for hemoglobin type (B), while the lowest level of hemoglobin type (AB) when compared with physiological conditions (control, estrus, pregnancy, and lactation). It can concluded that type of hemoglobin effect on sex hormones and lipoproteins that using them as indicators in improving economic production and choosing the good type of hemoglobin in breeding, improving and increasing animal production and milk production.

**Key words:** *Hemoglobin phenotype, electrophoresis, hormones.*

## Introduction

Cows are used in a variety of ways across the world. They are raised for meat, milk, and other dairy products and for use as draft animals (that pull carts, plows, and other implements). Cow also used to provide leather for clothes and dung for manure or fuel. Despite, or may be because of, their pervasiveness as consumer goods, most people find it difficult to relate to cows on their terms, that is, without the biases generated by social and economic value [1]. The 3 weeks prior to parturition to 3 weeks following parturition is considered the transition period [2]. However, animal performance is not solely determined by adaptive factors because the reproductive period of pregnancy may results in significant physiological changes in biomarkers, particularly in conditions of high metabolic demand due to the increased blood volume required by the uterus during pregnancy [3][4]. Numerous physiological changes may occur, such as preparation for calving, preparation of the mammary gland for colostrum, and a peak of milk production, the physiological changes generate high energy and protein demand for maternal maintenance and fetal development, increasing the nutrients requirements that promote changes in blood parameters [6][7]. Hemoglobin (Hb)

has been one of the most studied polymorphisms in vertebrate species since the infancy of both population and evolutionary genetics. However, owing to the close relationship between structure and function, this complex protein remains a fascinating subject from all points of view, especially in its molecular, genetic, and adaptive features. Accordingly, Hb has been recently defined as “an evergreen red protein, Many researchers have discovered that there are three different forms of hemoglobin (A,B and AB) [8][16]. Hemoglobin represents about 90% of the dry weight of the red blood cell, and it is a tetrameric metalloprotein composed to two  $\alpha$  and two  $\beta$  globin chains, each bound to one heme group. Each heme group includes one iron atom capable of engaging one oxygen molecule. Hemoglobin is a very sensitive molecule, and can be easily denatured during purification. Many different procedures for isolation and purification of hemoglobin from mammals' erythrocytes exist [9]. Three phenotypes were detected, i.e., a slow-moving (AA) band, a fast-moving (BB) band, and a combination of slow + fast-moving bands (AB). Electrophoresis showed one slow band for HbAA, a fast one for HbBB, and two for the heterozygous HbAB. The HbAA and HbBB from Ogaden cattle were found to have similar electrophoretic mobility to those from Western cattle. The Hb allelic and genotypic

frequency and expected. The observed hemoglobin polymorphism in Ogaden cattle genotypes of the Ogaden cattle population studied is given in There was no sample that showed any abnormal phenotype [10]. The current study aimed to determine the hemoglobin subtypes by using the electrophoresis technique and study the effects on some biochemical, hormonal parameters in the transition period in local Iraqi cows.

### **Materials and methods**

The present study was conducted in Basrah province, where blood samples of local cows were collected from four different areas of Basrah province, the period of blood collection samples during January and February of 2020. A total of 80 samples were divided into four groups (control group, estrus, pregnant and lactating cows) each group contain 20 samples 10 ml of blood were collected and divided in to two tube 5 ml were placed in a test tube containing an anticoagulant used to determine the type of hemoglobin by using vertical electrophoresis device as hemoglobin, type A is faster than hemoglobin type B. While, hemoglobin type AB is intermediate between types A and B, by using polyacrylamide gel electrophoresis and preparing solutions for hemoglobin and gel by modified method (Tucker *et al.*, 1980) . Other tube does not

contain anticoagulant samples were used to examine biochemical and hormones tests. By the kit was used for each test according to instructions. The results were then read and compared (Friedewald *et al.*,1972).

### **Results**

According to electrophoresis analysis of hemoglobin sample that collected from different cattle in different physiological status (non-pregnant, estrus , lactation, pregnancy) to recognize the dominance genotype of hemoglobin in the area experiment (Basra province ) and due to it hard environment such as heating and humidity the result (Table 1) showed that the hemoglobin genotype (B) was the dominance type than other types with frequency ( 0.587 ). While, the other hemoglobin types was (AB = 0.212 ),( HbA=0.2). When comparing the result total cholesterol and triglyceride levels in each physiological status groups according to the different in the hemoglobin phenotypes the result showed significant ( $p \leq 0.05$ ) change due to these types of hemoglobin which appeared that the hemoglobin type (B) has the highest level to total cholesterol and triglyceride in all physiological periods when compared with hemoglobin type (A, AB) which appear have lowest value from hemoglobin type (B), (Table2).

**Table (1): Genotype frequency of local cattle (n=80).**

Genotype frequency	A	AB	B
Local cattle	0.20	0.212	0.587

**Table ( 2 ): Effects of different hemoglobin types in physiological statuses on total cholesterol and triglyceride levels(n=80)**

parameters	Total cholesterol (mg/dl)				Triglyceride (mg/dl)			
	Hb types				Hb types			
Groups	A	AB	B	LSD	A	AB	B	LSD
Control	110.27	101.33	119.33		14.42	15.33	15.60	
	±13.20	±24.96	±18.08	8.4	±0.88	±1.29	±0.56	0.85
Estrus	B	C	A		B	A	A	
	134.02	132.55	145.65		9.85	10.14B	11.26	
Lactation	±20.68	±14.47	±16.23	9.45	±0.11	±0.25	±0.90d	1.00
	B	B	A		B		A	
Pregnancy	176.35	186.55	192.81		17.29	18.27	19.71	
	±8.813	±6.51	±14.01	10.4	±1.98	±0.70	±0.15	0.85
y	B	A	A	5	C	B	A	
	176.57	179.74	190.86		18.80	20.04	21.48	
	±17.68	±14.66	±8.13	12.8	±0.14	±1.02	±1.33	1.35
	B	A	A		C	B	A	

**Different letters denote significant (p≤0.05) changes between groups**

Table (3) denote the results of lipoprotein levels (HDL-C , LDL-C and VLDL-C) in local cattle of Basrah, the result appeared highly significant increased (p≤0.05) HDL-C, LDL-C and VLDL in Hb type (B) in all groups as compared to other types (A and

AB). Table (4): hemoglobin different phenotypes showed significant (p<0.05) effects on the progesterone and estrogen levels in some physiological period as we can see that the hemoglobin type (B) has higher level in the control and pregnant periods

when compared with other hemoglobin types while not showed any significant effects in estrus and lactating period. Also the effects of hemoglobin type appear significantly ( $p < 0.05$ ) on the estrogen levels showed elevation in the estrus period and the hemoglobin type (B) when compare with other physiological periods. Table (5)

showed the effects of hemoglobin polymorphism (A, B and AB) on the level of cortisol hormone, the result showed a significant decrease ( $p \leq 0.05$ ) in hormone cortisol through lactation and pregnant periods in B hemoglobin compared with other groups.

**Table (3): Effects of different hemoglobin types in physiological statuses on lipoprotein levels**

paramet ers	HDL-C(mg/dl)				LDL-C (mg/dl)				VLDL-C (mg/dl)			
	Hb types											
Groups	A	AB	B	LS D	A	AB	B	LS D	A	AB	B	LSD
Control	65.38	66.20	68.51	1.9 7	24.3	25.3	26.5	0.93	2.21	2.89	3.85	0.95
	$\pm 3.57$ B	$\pm 2.25$ B	$\pm 2.17$ A		$\pm 2.5$ 0	$\pm 5.1$ 2	$\pm 5.9$ 2		$\pm 0.6$ 5	$\pm 0.7$ 2	$\pm 0.6$ A	
Estrus	64.82	67.23	69.97	2.5 1	25.8	27.5	30.6	3.00	5.09	5.28	6.16	0.84
	$\pm 2.01$ C	$\pm 3.45$ B	$\pm 1.56$ A		$\pm 1.2$ 3	$\pm 1.8$ 7	$\pm 1.1$ 0		$\pm 0.4$ 8	$\pm 1.0$ 5	$\pm 0.4$ 3	
Lactatio n	110.7	112.06	120.38	4.8 9	81.6	82.1	85.0	2.74	5.21	5.87	6.76	0.63
	$7 \pm 6.6$ B	$\pm 17.82$ B	$\pm 10.2$ 8 A		$\pm 1.1$ 4	$\pm 1.5$ 1	$\pm 2.7$ 4		$\pm 0.3$ 6	$\pm 0.6$ 5	$\pm 0.6$ 1	
Pregnan cy	77.01	78.82	79.78 $\pm 2$	0.7 4	44.7	45.6	47.1	1.2	1.61	2.10	2.84	0.45
	$\pm 3.50$ B	$\pm 2.41$ B	$.36$ A		$\pm 2.4$ 3	$\pm 2.4$ 0	$\pm 1.4$ b		$\pm 0.2$ 2	$\pm 0.5$ 3	$\pm 0.8$ 2	

Different letters denote significant ( $p \leq 0.05$ ) changes between groups

**Table (4): Effects of different hemoglobin types in physiological statuses on progesterone and estrogen levels**

Parameters	Progesterone (ng/ml)				Estrogen (ng/ml)			
	Hb types							
Groups	A	AB	B	LSD	A	AB	B	LSD
<b>Control</b>	1.30	1.41	1.6 ±0.30	0.17	34.92	29.00	43.00	9.19
	±0.17	±0.24	A		±8.18	±4.00	±6.17	
	B	B			B	B	A	
<b>Estrus</b>	1.67	1.63	1.65	N.S	58.25	58.63	60.25	1.5
	±0.86	±0.37	±0.57		±2.06	±2.42	±2.77	
					B	B	A	
<b>Lactation</b>	1.75	1.96	2.13	N.S	26.16	27.58	30.50	2.1
	±0.10	±0.65	±0.04		±3.43	±5.28	±3.70	
					B	B	A	
<b>Pregnancy</b>	6.91	7.15	9.36	0.797	15.16	15.86	17.00	0.9
	±0.38	±4.29	±1.33		±1.60	±0.70	±1.76	
	B	B	A		B	B	A	

Different letters denote significant ( $p \leq 0.05$ ) changes between groups.

**Table (5): Effects of different hemoglobin types in physiological statuses on cortisol levels.**

parameters	Cortisol (mg/dl)			
	Hb types			
Groups	A	AB	B	L.S.D
<b>Control</b>	19.33	19.50	19.77	N.S
	±1.52	±0.50	±1.72	
<b>Estrus</b>	23.52	22.91	24.12	N.S
	±0.59	±0.81	±0.79	
<b>Lactation</b>	19.23	17.70	19.49	0.93
	±2.029	±1.047	±0.42	
<b>Pregnancy</b>	20.30	20.50	22.25	1.22
	±1.12	±3.67	±1.120	
	B	B	A	

Capital letters denote significant ( $p \leq 0.05$ ) changes between groups

## Discussion

The result of the present study viewed the effects of hemoglobin phenotypes on some biochemical and hormonal parameters (Table 1,2,3,4 and 5) which appear that the dominance hemoglobin phenotype in this area is Hb B, effects significantly on almost parameters. This finding is in agreement with some studies focused on the effects of the phenotype, this may due to efficacy of this genotype in adaptation to the hot and hard environment in this area (Basrah province) and give it this dominance and give the best parameters levels against the other hemoglobin genotype. This finding is in agreement with Sengupta [12] who offered evidence about cattle. Hb-B was discovered to be more prevalent in hot dry climates than in warm humid climates. While, Hb-A appeared to be less frequent in hot arid climates than in warm humid climates. Different oxygen levels were recommended by Sun et al. [13][17] in their experiments (Hb-B), in comparison to sheep with Hb-A, they discovered that sheep with Hb-B were unable to endure the stress of acute hypoxia. Al-Murrani and Timimi [14][18] discovered that the respiration rates of Sharabi cattle were ranked BB, AB, and AA as the lowest. However the differences were not significant. The BB type had a higher body temperature than the AA and AB types ( $p \leq 0.05$ ). The AB

type has a considerably better heat tolerance coefficient ( $p \leq 0.05$ ) than the AA and BB varieties. Al-Murrani and Al-Samarrae [15][19] discovered that the Awassi native breed of sheep is practically fixed for Hb-B, with a frequency of (0.98) in the study group. The high B frequency of the random breed Awassi group, which was obtained from several geographical locations, is the same (0.98). This suggests that long-term selection for fertility and body weight in Awassi, albeit moderate, is unrelated to Hb-type. There was no link between Hb-type and fertility or mortality in the study indicated above; the only significant link was discovered between Hb-B and HK (high potassium) genotype and high lambing percentage. The majority of investigations on various species and breeds found that blood chemistry variations exist (including Hb-type). However, the mechanism of such polymorphism is not well understood or consistent [14].

## Conclusions

On the basis of the present results, the following conclusions can be made: Electrophoresis determination hemoglobin phenotype-B more significant dominant than other types in cows of Basrah province. Recognize the dominance genotype-B of hemoglobin in Basrah province. Elevation of all biochemical parameters in phenotypes-B in cows of Basrah province, and the sexual

hormones increased in hemoglobin type-B in all groups of different physiological status.

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**Conflict of Interest:** The authors state that there is no conflict of interest..



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دراسة مقارنة لأنواع الهيموغلوبين (الترحيل الكهربائي) وأجزاء بروتين المصل وبعض التغيرات البايوكيميائية والهرمونية

خلال الفترة الانتقالية في الأبقار العراقية

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**المستخلص** أجريت هذه الدراسة لتحديد نوع هيموغلوبين الدم وتأثيره على المعايير البايوكيميائية والتي تشمل الدهون وهرمونات الأبقار المحلية في محافظة البصرة من خلال الحصول على عينات دم لأبقار من مناطق مختلفة من محافظة البصرة لتحديد نوع الدهن. الهيموجلوبين (AB و) حقق أعلى نسبة معنوية من النوعين (B) وقد أظهرت النتائج أن الهيموجلوبين من النوع ( ) وتأثيره على الهرمونات ومعايير الدهون ( مع أعلى مستوى من الكوليسترول الكلي والدهون الثلاثية عند مقارنته بنوع (B) على التوالي. ارتفاع ملحوظ في نوع الهيموجلوبين (A) ( الذي يحتوي (B) الهيموجلوبين الظاهري (VLDL-C و HDL-C و LDL-C). أظهرت نتائج البروتين الدهني (A) ، AB الهيموجلوبين ( على مستويات البروتين الدهني في الدم في فترات الرضاعة والحمل. أظهرت أنماط ظاهرية مختلفة للهيموجلوبين ارتفاعاً في مستويات ( بمستوى أعلى في فترات التحكم B هرمون البروجسترون والأستروجين في بعض الفترات الفسيولوجية بينما يمكن رؤية نوع الهيموجلوبين ( بينما كان أقل B وفترات الحمل عند مقارنتها بأنواع الهيموجلوبين الأخرى. أما بالنسبة للكورتيزول فقد أظهر زيادة في نوع الهيموجلوبين ( ويمكن الاستنتاج أن تأثير ( عند مقارنته بالظروف الفسيولوجية (السيطرة ، الشيق ، الحمل ، الإرضاع). AB مستوى من نوع الهيموجلوبين ( نوع الهيموجلوبين على الهرمونات الجنسية والبروتينات الدهنية يستخدم كمؤشرات في تحسين الإنتاج الاقتصادي واختيار النوع الجيد من الهيموجلوبين في التربية وتحسين وزيادة الإنتاج الحيواني وإنتاج الحليب.