

Hematological state of ewes injected with some mediators during postpartum and lactation period

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Abstract

The effect of injection of some mediators on the blood values of ewes was studied during postpartum and lactation period. The study was conducted on 30 pregnant ewes aged 3-5 years, presented in the farms of the college of veterinary Medicine/ AL-Fallujah University during the period from February 2014 to August 2015. Ewes were divided into three equal groups (10 each). The first group was injected with prostaglandin F_{2α} (PGF_{2α}) and the second group injected with oxytocin the injection was done after the ewe's birth directly, the third group was treated as a control group. Blood values were measured during the first and third week after birth which included (red blood cell count (RBC), hemoglobin (Hb), Packed cell volume (PCV), erythrocyte sedimentation rate (ESR), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), white blood cell count (WBC), WBC differential count). Results show that RBC (329.8±25.59), PCV (28.75±0.86), MCV (1.22±0.08), were in their high rate in the group of ewes injected with PGF_{2α} than other group also high in oxytocin injected group than control which induce the role of PGF_{2α} in the increasing some blood values than other mediators through increasing utilities of corpus luteum and decreasing the time of uterine involution so that bleeding after birth will stop in a short time, Oxytocin came in the second place after PGF_{2α} in terms of the its effect of raising the blood values after birth and lactation period. The effect of PGF_{2α} and oxytocin was the same on Hb concentration in treated ewes. ESR used in this study to show the difference because it not concerned in the study of blood values in domestic animals. WBC count (6330±241.1) were high in G1 than other groups. No significance in Neutrophil and Lymphocyte values in all groups, while the results of Basophil and Monocyte were the highest in G1, Eosinophil were higher in G2

Key word: hematology, prostaglandin, postpartum, RBC count, WBC count.

INTRODUCTION

Livestock is one of the most important sources of nature, where humans use their meat, skins, wool and milk and are still the source of livelihood for many people. There are several types of livestock, including sheep, goats and many others. Sheep are an economic pillar of livestock in Iraq.

The animal's productive capacity and sexuality were linked to blood parameters (1), the researchers relied on blood image examination as an expression of the physiological state, health and productivity of the animal, study the effect of age, sex, environmental conditions and climate, as well as the effect of parasites and parasitosis. And the distinction between races in the world (2,3,4).

Blood is one of the most important tissues of the body, which when evaluating its contents of cells and fluids give a Prostaglandin F_{2α} or know as (prostanoid), pharmaceutically termed dinoprost (INN), in medicine it used to induce labor and increasing uterine contractions. In domestic mammals, it is produced by the uterus when stimulated by, also it cause the luteolysis of the corpus luteum, forming a corpus albicans and stopping the production of progesterone (6).

Oxytocin acts directly on the myometrium to increase contractions and indirectly by enhancing prostaglandin production, the most important is the prostaglandin F_{2α} (PGF_{2α}). PGF_{2α}, in turn, is produced firstly by the maternal decidua and effect on the myometrium to up-regulate gap junctions and oxytocin receptors, that way promoting uterine contractions. PGF_{2α} is foremost of fetoplacental origin and is likely more significant in promoting cervical ripening (maturation) and spontaneous rupture of the fetal membranes (7,8). There are many factors that may affect the normal blood values, especially RBC count, PCV, Hb. The most important factor is the nature of animal feeding, as well as age, season, physiological status and parasitic infections (9). In studies on blood values in sheep, there was a significant decrease in PCV, Hb during rainfall seasons and also with parasitic infection (10,11). Physiological state and the age of the animal and their effect on the whole picture of blood studied in a number of research (12,13) where the results of these researches showed high blood values in lambs aged 6-12 months, while during pregnancy was found to increase during the prenatal period, but

questionnaire to the health situation of ewe, especially after the birth of and associated bleeding, which may affect the blood values (3).

Modern studies has been focused on many factors that regulate activity and work of the myometrium, regardless of the traditional hormones such as progesterone, LH and FSH. The most important of these factors are the prostaglandins and, oxytocin, corticosteroids, leukotriene, platelet activation agent, Internal thyme and cytokines. Prostaglandin is involved in the levels of regulating the myometrium such as the myometrial gap junction formation, intracellular calcium flux modulation, synchronization of myometrial contraction via interaction with oxytocin thus having stimulatory effects on uterine contractility, as well as cervical maturation (via PGE₂) (5).

decreased after that and during the period of breastfeeding (14,15,16).

According to the study presented by (17), no changes were observed in the values of the period before the birth except for the slight increase during the first two weeks after birth, the differential leukocyte and white blood cell (WBC) counts in goats and sheep are also subject to diversity due to age, parasitic infection and physiologic stage. Consequently, higher lymphocyte and WBC counts are found in kids less than 12 months of age compared with values in adult goats (12,18) moreover, total WBC perhaps elevated in late pregnancy in sheep (19) and goats (20) because of an ACTH-related hormonal stress reaction. Sometimes basophilia and eosinophilia in adult and growing animals probably indicative of an allergic response to contemporary parasitic infection (12).

MATERIALS AND METHODS

The study was carried out on 30 local Iraqi ewes, presented in the farms of College of Veterinary Medicine/ Al-Fallujah University. The age of the animals ranged between 3-5 years with 2-3 parturition. The animals having similar managemental conditions and randomly selected from entire farm, the animals were fed green grasses and concentrated diet and supplied with water *ad libitum*, each animal was given an identification number, the study extended from August 2014 to July 2015. The ewes were pregnant in different stages of pregnancy.

These animals were divided postpartum into three groups having equal (10) number of animals in each group.

Group 1 had given (7.5 mg) of PGF₂α and Group 2 had given 20 IU oxytocin whereas animals of group 3 were assigned to control group had given 2ml normal saline

Blood samples were taken from the jugular vein using disposable plastic syringes after sterilizing the origin with 70% alcohol. 5 mL of each animal was withdrawn and placed in a tube on the EDTA anticoagulant for blood tests.

Blood tests included the total count of red blood cells(RBC count) and white blood cells (WBC count) by using Haemocytometere slide, packed cell volume (PCV) by using Centrifuge Microhaematocr, hemoglobin concentration (Hb) by Spectrophotometer (22) , The MCV, MCH and MCHC were calculated according to the following formulas described by (13,14) ;

$$MCV = PCV / RBC (m) \times 10fl ,$$

$$MCH = Hb / RBC (m) \times 10pg \text{ and}$$

$$MCHC = Hb/PCV \times 100g/dl$$

Differential leucocyte counts (DLC) include neutrophil (N), basophil (B), eosinophil (E), lymphocyte (L) and monocyte (M) (15).

RESULTS

Hematological parameters; PCV % , Hb, RBC, MCV and ESR of this study were decreased during the first week postpartum , no significant difference in MCH and MCHC, WBC count , as shown in table (1).

The results of the statistical analysis of the variables in the blood values in sheep in the three groups under study during the third week postpartum as observed in table (2) which shows the effect of injection on the values of the total count of The RBC, Hb, PCV,ESR, MCV, MCH and MCHC. RBC count in were higher in group injected with PGF₂α (G1) significant differences(329.8±25.59) than the oxytocin injected group (G2) and control (G3) as shown in figure (1).

Figure (2) present the values of PCV, MCV which were higher in (G1) than in (G2) and (G3). Hb were the same in G1 and G2. No significance in the value of MCH and MCHC.

ESR with high value (12.65±0.65) in G1 Followed by G3 then G2. The total WBS counts influenced by PGF₂ injection than oxytocin and control with significant differences (6330±241.1). No significance in Neutrophil and Lymphocyte values in all groups, while the results of Basophil Monocyte were the highest in G1, Eosinophil were higher in G2 as showed in table (2) figure (3).

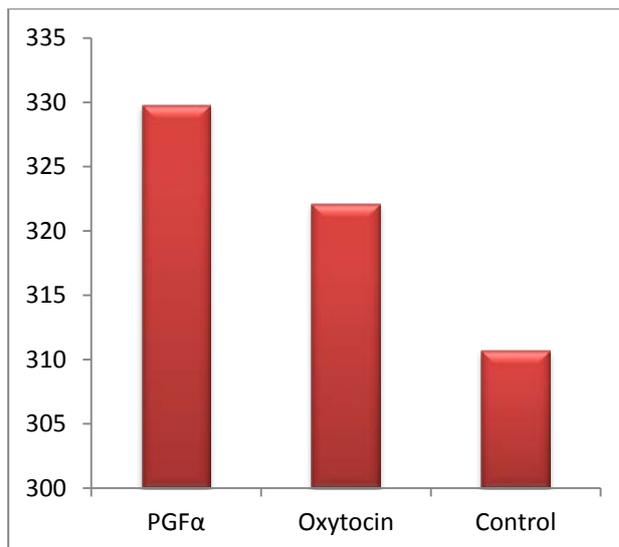


Figure (1) show RBC count in different groups

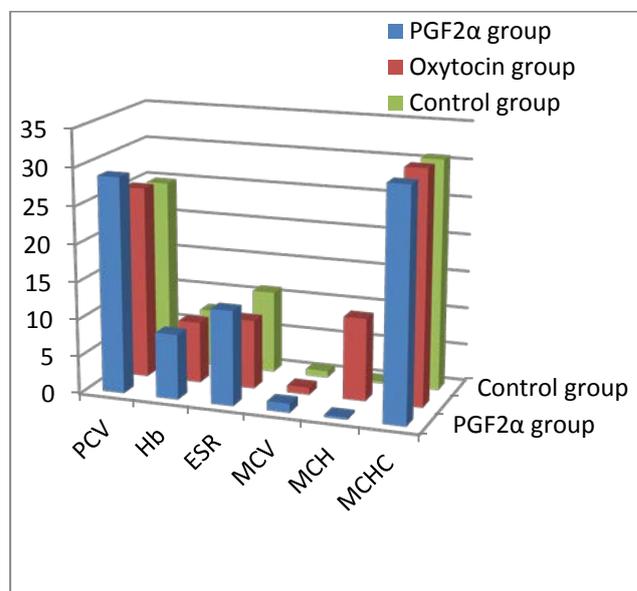


Figure (2) show the values of the RBC, Hb, PCV,ESR, MCV , MCH and MCHC in different groups.

Table (1): show significant difference in PCV % , Hb, RBC count, MCV ESR, MCH and MCHC in different groups.

	G1	G2	G3
PCV(%)	20.15±0.66 A	18.43±0.41 B	19.22±0.52 B
Hb (g/dl)	5.60±0.33 A	5.09±0.15 AB	6.43±0.19 B
RBC (×106/μL)	197.5±16.44 A	185.11±11.32 B	188.56±11.82 B
ESR(mm/24hr)	10.66±0.66 A	8.18±0.22 C	10.05±0.40 B
MCV (fl)	1.15±0.11 A	0.67±0.11 B	0.64±0.05 B
MCH (pg)	0.227±0.022 N.S	0.262±0.022 N.S	0.277±0.019 N.S
MCHC (g/dl)	29.55±0.11 N.S	27.11±0.15 N.S	27.65±0.16 N.S

The different capital letters refer to significant differences between different groups at (p> 0.05)

Table (2) : significant difference in WBC count and differential WBC count, in different groups.

	G1	G2	G3
WBC (c/ μ L)	6330 \pm 241.1 A	5550 \pm 272 \pm 14.53 B	5497 \pm 270.93 B
Neutrophil (c/ μ L)	47.02 \pm 0.49 N.S	45.85 \pm 0.43 N.S	46.98 \pm 0.39 N.S
Lymphocyte(c/ μ L)	35.96 \pm 0.53 N.S	35.73 \pm 0.46 N.S	36.45 \pm .57 N.S
Monocyte(c/ μ L)	5.81 \pm 0.33 B	6.77 \pm 0.24 A	6.43 \pm 0.26 AB
Eosinophil(c/ μ L)	5.74 \pm 0.35 B	6.73 \pm 0.31 A	6.15 \pm 0.24 AB
Basophil (c/ μ L)	5.25 \pm 0.32 A	4.64 \pm 0,25 B	4.33 \pm 0.15 B

The different capital letters refer to significant differences between different groups at (p> 0.05)

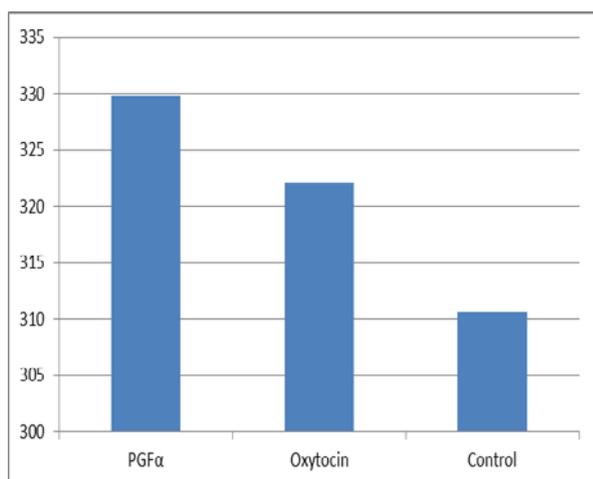


Figure (3) : variables in WBC count in group under study.

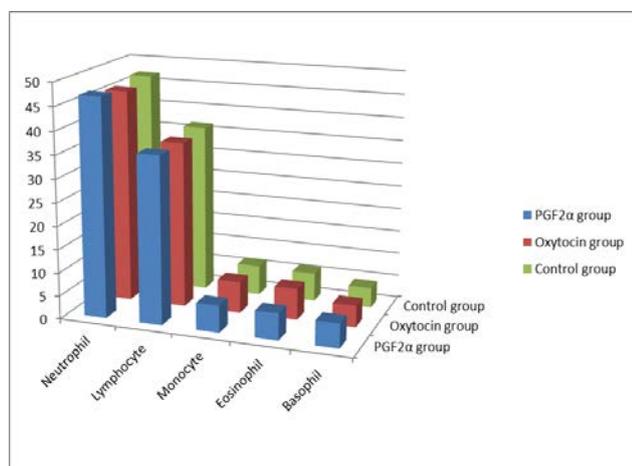


Figure (4) : show available in WBC differential count in groups under treatment

DISCUSSION

The knowledge of the basic blood values of the animal during pregnancy and beyond and during the period of breastfeeding help the veterinarian in diagnosing the health of the animal and assess the severity of the situation and thus describe appropriate treatments that raise blood values, which enhance the reproductive efficiency and productivity of ewes.(16,17).

In early postpartum period RBC, Hb, PCV values They were at the lowest levels, In general, hematological parameters decrease during pregnancy and remain low during early postpartum as show in study in cows, mares, sows, ewes, and bitches (18). Hb and PCV values were not different between late period of pregnancy and early lactating ewes, generally the values Hb and

PCV in treated animals reflect mostly a rise in late pregnancy, decreasing in the postpartum period and declining in early lactation period (19,20). while (21) registered an increase in these parameter in late pregnancy in Egyptian Barki ewes compared with parameter in dry ewes.

As shown in Figure (1), the values of red blood cells in ewes of the G1 injected with PGF2 α were higher than other groups, indicating the effect of PGF2 α as an intermediary in increasing the level of blood corpuscles in postpartum ewes.

This varies with the fact that the blood values in postpartum ewes are reduced as a result of the stress associated with parturition and lactation added to the bleeding that accompanies the complications of childbirth and the length of the period in which the uterus returns to its normal state, this corresponds to what it found by (15,22). Also reduce blood volume and Hb may related to dietary condition and effect of climate and season, this supported by studies in pregnant and lactating ewes, anemia found was also probably related to an plasma volume increasing in late pregnancy and to milk production changes in early lactation (15,23) Also, blood problems observed in a low percentage of sheep maybe an occasional finding noticed in sheep or may be dietary related. (24) .

The reproductive performance of the group treated with PGF2 α (G1) was significantly improved when compared to oxytocin treated group (G2) and control (G3), this supports studies of the role of PGF2 α and oxytocin in the restoration the reproductive efficiency of ewes postpartum, where (25) found that PGF2 α and oxytocin when injected into ewes after parturition at three periods, Prostaglandin F2 α and oxytocin might be helpful to reduce the time uterine involution and induce earlier expulsion of fetal membranes in local Iraqi ewes. reproductive functions could be regulated by interactions of some mediators of Inflammation such as prostaglandins PG, chemokines, cytokines .

There was a slight increase in values of MCV in the first group compared to other groups while the values of MCH and MCHC remaining within the normal value. (2) found that the fall of hemoglobin in the blood of goats in lactation, While the MCV, MCH and MCHC remain constant during investigation. ESR with high value (12.65 \pm 0.65) in G1Followed by G3 then G2. A consistent measurement of ESR is not important in blood tests in animals in general.

In the study of goats the results were different from the current study, (19) found Declining in the number of RBC and WBC in goats after 3 weeks postpartum and lactation period.

However, In Iraq many studies have been conducted on the factors that affected the blood values in ewes because of the economic importance of this kind of farm animals to the livestock in Iraq, (26) found in study in Baghdad governorate on Awassi ewes that PCV, RBC, Hb, significantly decrease (p<0.05), during lactation period, while MCV, MCH, MCHC and ESR were with no significance and stay with normal ranges compared with normal ewes. Also (27) noticed in a study in the north region of

Iraq that blood parameters of ewes were normal according to sex, breed, physiological statue and other factors affecting blood parameters. In a study in Salah al-ddin governorate which included ewes with mastitis and in lactation period he found that there were a significance increase in PCV, Hb, RBC and Platelets, while decreasing of ESR and no significance in MCV, MCH, MCHC.(28)

The role of white blood cells in healing and inflammation is known especially during postpartum and uterine involution, Total WBCs count increased significantly ($p < 0.05$) during early postpartum and lactation in (G1) compared to other groups, our result were in accordance with a study on Tsigai ewes by (29) suggesting that the low number of total WBCs count during pregnancy and the increase at parturition and early lactation is probably a response to uterine involution. Our results may support the theory that gestation is associated with changes in immunity within the uterus. According to (30), there were significant increases in N: L ratio during late pregnancy and early postpartum periods compared with non pregnant ewes. No significance differences in Neutrophils and lymphocyte values in al studied groups, as compared with other researches there were a significance increase in Neutrophils in ewes with mastitis compared with lactating with no significance in the values of Lymphocyte according to (28), while there a significance decrease in L / N ratio in lactating compared to pregnant ewes according to (26).

The increases in the number of neutrophils in ewes after birth and during lactation period may reflect the effect of udder in infection that stimulate the immune system to defense against the infection factors, injection of PGF₂ α and Oxytocin play an important role in decreasing the infection during the period of the current study. Our data showed that Monocyte and Eosinophil were significant higher in G2 than G1 group and normal lactating ewes (G3), while Basophil was significant higher in G1 than other groups, these results disagree with the results of (26) in lactating ewes and agree with (28) in the results of Eosinophil numbers in ewes during lactation period. According to our result we suggested that the increase the number of Eosinophil in G1 during the postpartum period reflect the role of these cell in the immune/inflammatory process. Quantitation of Eosinophil were detected in cells of luteal tissue obtained from ewes treated with prostaglandin (PG) F₂ α that have a luteolytic effect. Increased numbers of cells were detected before the onset of either functional (decline in sera or tissue concentrations of progesterone) or morphological regression. A specific chemo attractant were produced by Luteal tissue for eosinophil as measured by a linear under-agarose migration assay. Because eosinophil are capable of mediating tissue damage in immune/inflammatory conditions, it is suggested that these cells could play a similar role in the mechanics of luteolysis (31). Decrease number of WBC during late pregnancy and the increase at parturition and early lactation may responsive to uterine involution. These result were agree with (23) in a study in goat.

Oxytocin came in the second place after PGF₂ α in terms of the it's effect of raising the blood values after birth and lactation period. As it has the effect of increasing the receptors PGF₂ α in uterus, which leads to increased contractions and increase the effectiveness of PGF₂ α and reduce the period of uterine involution, which increases the reproductive efficiency of the ewes and reduces the period between births, thus enhancing economic and Livestock.

REFERENCES

1. Abdel-Fattah, M.S., Hashem, A.L.S., Shaker., Ellammei, A.M., and Amer, Z. 2013. Effect of weaning age on productive performance and some plasma biochemical parameters of Barki lambs in Siwa Oasis, Egypt. *Glo. Vet.* 10(2):189-202.
2. Azab, M. & Abdel-Maksoud, H. A. 1999. Changes in some hematological and biochemical parameters during pre-partum and post-partum periods in female Baladi goats. *Small Rum. Res.*, 34: 77-85.
3. Egbe-Nwiyi, T. N.; Nwaosu, S. & Salami, H. 2000. Hematological Values of apparently healthy sheep and Goats as influenced by age and sex in arid Zone of Nigeria. *Afr. J. Biomed Res.*, 13: 109-115.
4. Kadime, I.; Mahgoub, O.; Al-Ajmi- Maabaly, R.; Al-Saqri, N. & Ritchie, A. 2003. An Evaluation of the growth, carcass and Meat quality characteristics of Omani goat breeds. *Meat Sci.*, 66: 203-210.
5. Gillin A. G. 1994. Maintenance of High Risk Pregnancies: Role of Prostaglandins and Other Mediators. 10.1111/j.1479-828X.tb01088.
6. Sharma, I. Dhaliwal, L. Saha, S. Sangwan, S. and Dhawan, V. (2010). Role of 8-iso-prostaglandin F₂ α and 25-hydroxycholesterol in the pathophysiology of endometriosis. *Ferti. and Steril.* 94 (1): 63–70.
7. Wilson, T. Liggins, G.C. and Whittaker, D.J. (1988). Oxytocin stimulates the release of arachidonic acid and prostaglandin F₂ α from human decidual cells. *Prostaglandins* 35:771–780.
8. Sunil K. Kota, Kotni Gayatri, Sruti Jammula, Siva K. Kota, S. V. S. Krishna, Lalit K. Meher, and Kirtikummar D. Modi. (2013). Endocrinology of parturition. *Indian J Endocrinol Metab.*; 17(1): 50–59
9. Morris JS, Dunn JK. *Haematology. In Practice.* 1992;14(2):67–72.
10. Adewuyi AA, Adu IF. Seasonal variation in the levels of some blood components of indigenous and crossbred sheep. *Tropical Animal Production.* 1984;3:223–230.
11. Lutu WZ. Internal parasitism in milk goats in Kenya. *Tropical Animal Health and Production.* 1984;16(3):153–157. [PubMed]
12. Somvanshi R, Biswas JC, Sharma B, Koul GL. Haematological studies on Indian pashmina goats. *Research in Veterinary Science.* 1987;42(1):124–126.
13. Jain N. C. (1986). *Schal's Veterinary Hematology.* 4th. ed. Lea and Febiger Philadelphia
14. GreGG. L. V. 2000 *Hematology Techniques and Concepts for Veterinary Techniques.* 1st. ed. Iowa State Univ. Press. pp. 97-100.
15. Weiss DJ, Wardrop KJ, editors. 2010. *Schlam's Veterinary Haematology Edition.* 6th edition. Wiley Blackwell.
16. Roubies N., Panousis. N., Fytianou . A., Katsoulos .P.D., Giadinis. N., Karatzias. H. 2006. Effects of age and reproductive stage on certain serum biochemical parameters of Chios sheep under Greek rearing conditions. *J. Vet. Med. A Physiol. Pathol. Clin. Med.* 53: 277-281.
17. Yokus .B., Cakir. D.U., Kanay. Z., Gulten .T., Uysal. E. 2006. Effects of seasonal and physiological variations on the serum chemistry, vitamins and thyroid hormone concentrations in sheep. *J. Vet. Med. A Physiol. Pathol. Clin. Med.* 53: 271- 276.
18. Jain, N.C. 1993. *Essentials of Veterinary Hematology.* 1st ed. Wiley, Philadelphia. p1-18.
19. Iriadam M. 2007. Variation in certain hematological and biochemical parameters during the peri-partum period in Kilis does. *Small Ruminant Research.* ;73(1-3):54–57.
20. Tanvi D. Manat, Sandhya S. Chaudhary, Virendra Kumar Singh, Sanjay B. Patel and Gopal Puri. 2016. Hematobiochemical profile in Surti goats during post-partum period. *Veterinary World.* EISSN: 2231-0916.
21. El-Sherif and Assad, 2001. Change in some blood constituents of Barki ewes during pregnancy and lactation under semi-arid conditions. *Small Ruminant Research.* 40(3):269-277.
22. AL-jaumeili H. Ali, 2015. Study of hematology according to the morphology classification and Some Biochemical Values Changes Associated With Morphological Anemia. *Tikrit Journal of Pure Science* 1(20). ISSN:1813 – 1662.
23. Mbassa GK, Poulsen JSD. 1992. The comparative haematology of cross-bred and indigenous East African goats of Tanzania and breeds reared in Denmark. *Veterinary Research Communications*; 16(3):221–229.
24. Thrall MA, Weiser G, Allison R, Campbell TW, editors. 2012. *Veterinary Haematology and Clinical Chemistry.* 2nd edition. Wiley Blackwell.
25. Ahmed.M.N. 2016. Effect of PGF₂ α and Oxytocin on Uterine Involution in Iraqi local Ewes. P.H.D thesis Biology/ Zoology , College of Science/ University of Baghdad .
26. Badawi N.M. and AL-Hadithy H.A.H. 2014. The Hematological Parameters in Clinically Healthy Iraqi Awassi Sheep. *World's Vet. J.* 4(1): 01-05.
27. Oramari Rabea A.S., Bamerny Araz O. and Zebari .Hawar M.H. 2014. Factors Affecting Some Hematology and Serum Biochemical Parameters in Three Indigenous Sheep Breeds *Advances in Life Science and Technology* www.ISSN 2224-7181 (Paper) ISSN 2225-062X .Vol.21.
28. AL-Hadithy H.A.H.; Suleiman, J.M. 2014. The hematological parameters in clinically normal lactating and ewes affected with mastitis. *Kufa Journal For Veterinary Medical Sciences.* Vol. (5) No. (2).
29. Antunovic, Z.; J. Novoselec; H. Sauerwein; M. Speranda; M. Vegara and V. Pavic 2011a. Blood metabolic profile and some of hormones concentration in ewes during different physiological status. *Bulg. J. Agric. Sci.*, 17: 687-695
30. Bamerny, A.O. 2013. Changes in Some Haemato-Biochemical and Electrolytes Parameters In Female Meriz Goats during Pregnancy and After Parturition. *J. Anim. Sci.*, 2: 11-14.
31. Murdoch WJ 1987. Treatment of sheep with prostaglandin F₂ α enhances production of a luteal chemoattractant for eosinophils. *Am J Reprod Immunol Microbiol.* 15(2):52-6.