Morphological Study and Determination of M/E Ratio in the Bone Marrow of the Male Adult Ostriches (*Struthio camelus*)

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Abstract: Normal haematopoiesis, cellular components and M/E ratio in the bone marrow of the male adult ostriches (*Struthio camelus*) were studied. Bone marrow samples were collected from the proximal tibiotarsus bone of 10 healthy adult ostriches. The bone marrow smears were stained using the Giemsa stain. The results indicated that the development and formation of blood cells in the bone marrow of partridge were similar to other birds. The morphology of the cells was similar to chickens, ducks, quail, and black-head gull. The mean myeloid/erythroid (M/E) ratio was 1.02, the mean erythroid percentage was 47.38%, the mean myeloid percentage was 48.15%, the mean thrombocytic percentage was 3.52% and the mean percentage of all other cells percentage was 0.99%.

Keywords: Bone marrow, Haematopoietic cells morphology, M/E ratio, Ostrich.

INTRODUCTION

Bone marrow examination provides valuable information about haematopoietic status [1]. The proper evaluation of the haematopoietic system involves careful evaluation of the peripheral blood haemogram obtained on the same day as the bone marrow sample. The usefulness of bone marrow aspiration as a diagnostic tool depends on proper collection and handling of the sample, and a knowledge of normal marrow morphology [2]. Various indications of marrow examination in avian patients includes: non regenerative anaemias, thrombocytopenias, heteropenias, pancytopenias, suspected leukaemia and other unexplained cellular changes in the peripheral blood [3]. Fine structure and haematopoietic cell morphology of bone marrow of chickens and pigeons have been investigated by Campbell [3, 4] and Campbell and Coles [5] Tadjalli *et al.,* [6] and Nazifi *et al.,* [7]. Averbeck [8] and Work [9] reported the hematology and blood chemistry of gulls. Tadjalli *et al.,* [10] reported the normal haematopoiesis, cellular components and M/E ratio in bone marrow of the black-headed gull. However there is little information available on the hematopoietic cells of the male adult ostriches so the object of this study was to determine the bone marrow cell morphology and M/E ratio in male adult ostriches.

EXPERIMENTAL AND RESULTS

Bone marrow aspirations were obtained from 10 healthy male adult ostriches (2-3 years old). All birds were free of haematological abnormalities on peripheral blood examination. The medial aspect of the proximal tibiotarsus bone, just below the femoral-tibiotarsal joint, was aseptically prepared and 14 gauge disposable marrow aspiration needles were used to obtain samples. The area was anaesthetized locally by subcutaneous infiltration of 1-1.5 ml of 2% lignocaine HCl over the periosteum. The aspiration biopsy needle was held perpendicular to the bone and advanced into the marrow space by applying light pressure and using slight rotatory motions. With the needle in the marrow space, the stylet was removed and a syring was locked into the needle. The samples were collected into 5 ml syringes containing EDTA. At least five air-dried wedge slides of bone marrow smears were prepared from each ostrich. Slides were stained with Giemsa and were evaluated for cellularity and classification of erythroid, myeloid and thrombocytic precursors. Each sample was used for a 500-cell differential count to classify the marrow precursors in each cell series and to determine myeloid: erythroid (M/E) ratios for each male adult ostrich. The M/E ratio was determined by dividing the total of all the nucleated cells of the granulocytic series by the total of all the nucleated cells of the erythrocytic series [11]. The classification of the erythroid series included rubriblasts, prorubricytes, basophilic rubricytes, early polychromatophilic rubricytes and late polychromatophilic rubricytes. The classification of the myeloid series included myeloblasts, promyelocytes, metamyelocytes, bands and segmenters. The results were expressed as means ± SEM. All data were done with the Statistical Package for Social Sciences (SPSS 16.0 for windows).

Cellular composition of the bone marrow of the male adult ostriches is shown in Table **1**. The mean

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myeloid/erythroid (M/E) ratio was 1.02, the mean erythroid percentage was 47.38%, the mean myeloid percentage was 48.15%, the mean thrombocytic percentage was 3.52% and the mean percentage of all other cells percentage was 0.99%.

Table 1: Cellular Composition of the Bone Marrow of Male Adult Ostriches

	Cells (%)
Erythroid series	
Rubriblast	0.81 ± 0.02
Prorubricyte	2.81 ± 0.11
Basophilic rubricyte	4.76 ± 0.07
Early poly chromatophilic rubricyte	23.61 ± 0.13
Late poly chromatophilic rubricyte	15.37 ± 0.22
Total erythroid cells *Myeloid series*	47.38
Myeloblast	1.00 ± 0.03
Promyelocyte	3.39 ± 0.15
Myelocyte, heterophilic	16.64 ± 0.20
Myelocyte, eosinophilic	3.18 ± 0.04
Myelocyte, basophilic	0.70 ± 0.03
Metamyelocyte, heterophilic	8.4 ± 0.23
Metamyelocyte, eosinophilic	6.38 ± 0.07
Metamyelocyte, basophilic	0
Band, heterophilic	6.02 ± 0.05
Band, eosinophilic	0.24 ± 0.02
Band, basophilic	0
Heterophil	1.73 ± 0.08
Eosinophil	0.37 ± 0.06
Basophil	0
Total myeloid cells	48.15
Thrombocytic series	
Thromboblast	0.26 ± 0.02
Prothrombocyte	0.32 ± 0.01
Thrombocyte	2.93 ± 0.04
Total thrombocytic cells	3.52
Other cells	
Mitotic cells	0.25 ± 0.01
Osteoclast	0
Plasma cell	0.21 ± 0.01
Promonocyte	0.06 ± 0
Monocyte	0.08 ± 0
Lymphoblast	0.14 ± 0.02
Prolymphocyte	0.16 ± 0.02
Lymphocyte	0.07 ± 0
Total other cells	0.99
Myeloid: Erythroid ratio (M/E)	1.02

Percentages are given as mean ± SE.

The mean sizes of the erythroid, myeloid and other cells of the bone marrow of the male adult ostriches are shown in Table **2**.

Table 2: Mean Sizes of the Erythroid Series, Myeloid Series and other Cellular Series of the Bone Marrow of the Male Adult Ostriches (n=50) (x ± SE)

Erithroid series	
Rubriblast (diameter)	15 ± 0
Prorubricyte (diameter)	12.5 ± 0.29
Basophilic rubricyte (diameter)	12.2 ± 0.24
Early polychromatophilic rubricyete (diameter)	9.21 ± 0.72
Late polychromatophilic rubricyete (width) Late polychromatophilic rubricyete (lenghth) 12.25 0.48	8.5 ± 0.29
spherical shape late polychromatophilic rubricyete (diameter)	11 ± 0
Myeloid series	
Myeloblast (diameter)	15 ± 0
Promyelocyte (diameter)	15.13 ± 0.89
Eosinophilic myelocyte (diameter)	13 ± 0.84
Heterophilic myelocyte (diameter)	12.4 ± 0.4
Basophilic myelocyte (diameter)	14.25 ± 0.59
Eosinophilic metamyelocyte (diameter)	13 ± 0.41
Heterophilic metamyelocyte (diameter)	11.5 ± 0.87
Eosinophilic band (diameter)	1.07 ± 0.7
Heterophilic band (diameter)	13.2 ± 0.9
Thrombocyte series	
Thromboblast (diameter)	7.7 ± 0.33
Prothrombocyte (diameter)	6.7 ± 0.33
Other cells	
Promonocyte (diameter)	17.4 ± 0.68
Lymphoblast (diameter)	10.5 ± 0.5
Prolymphocyte (diameter)	15 ± 0
Plasmablast (diameter)	12 ± 0

Rubriblasts were round cell with large round central nuclei with granular chromosome and large nucleoli or nucleoli rings. The cytoplasm was basophilic. The N: C ratio was high (Figure **1**). Prorubricytes were round cell with large round central nuclei and two or three nucleoli. The cytoplasm was more extensive and more basophilic than rubriblasts. The cytoplasm contained small vacuoles (Figure **1**). The Basophilic rubricytes were round cells with central round nuclei but they

were smaller than prorubricytes. Their cytoplasm were deeply basophilic (Figure **2**). Early polychromatophilic rubricytes were round cells with round central nuclei with clumped chromatin. The cytoplasm was basophilic to slightly eosinophilic. Early polychromatophilic rubricytes were smaller than basophilic rubricytes (Figure **3**). Late polychromatophilic rubricytes were round to oval in shape with a light nucleus containing reticular chromatin. Cytoplasm is large and light basophilic (Figure **3**). Late polychromatophilic rubricytes change to reticulocytes. The reticulocytes are oval in shape with oval nucleus and were observed in peripheral blood smears (Figure **3**).

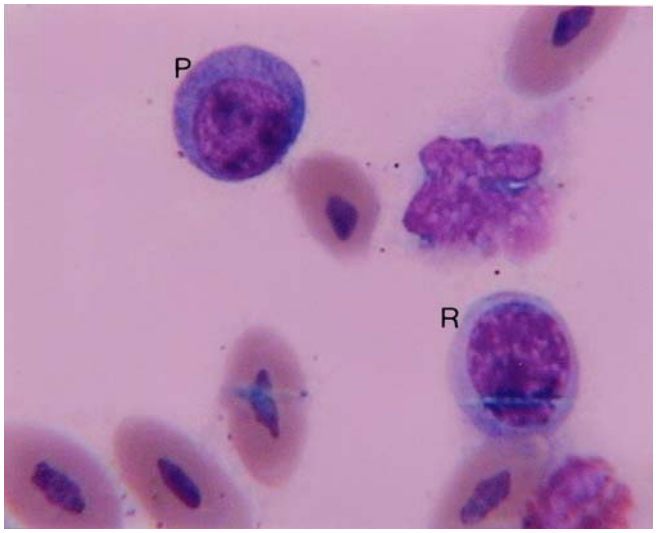

Figure 1: Rubriblast (R), prorubricyte (P), (Giemsa, × 1800).

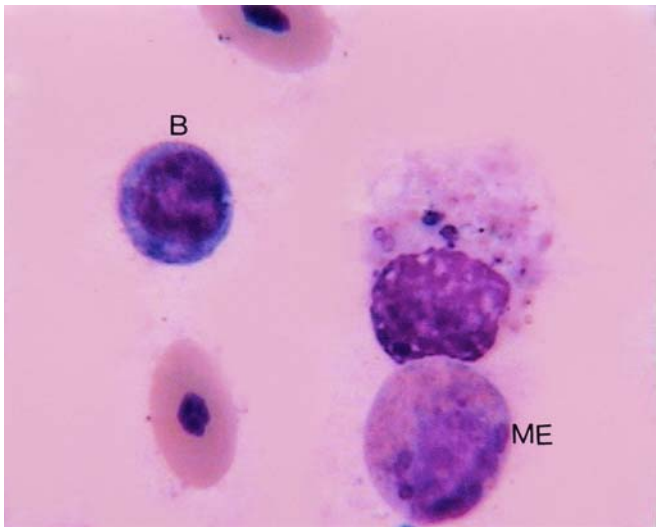

Figure 2: Basophilic rubricyte (B), eosinophilic myelocyte (ME), (Giemsa, × 1800).

Myeloblasts were large and round cells with a narrow rim of blue cytoplasm. The cytoplasm does not contain specific granules. Their nucleus was round with

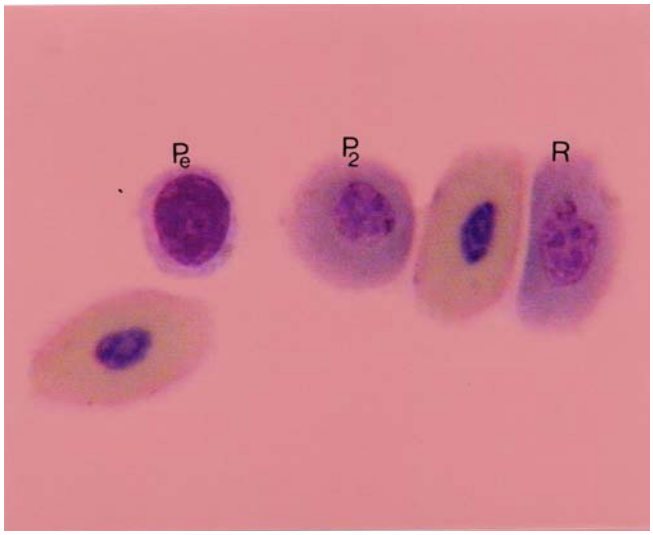

Figure 3: Early polychromatophilic rubricte (Pe), round late polychromatophilic rubricte (P2) and reticulocyte (R), (Giemsa, × 1800).

a reticular chromatin and prominent nucleoli. Promyelocytes were round cells with eccentric round nuclei that contained reticular chromatin. Their cytoplasm was light blue and contained large round azurophilic primary granules that the granules covered the nucleus (Figure **4**). Myelocytes were smaller than Promyelocyteas. They were round cells with an eccentric oval shape nucleus. The cytoplasm was blue and contained secondary or specific granules, which could be classified as the heterophilic, eosiniphilic or basophilic series (Figures **2**, **5**, **7**, **9**). Metamyelocytes were smaller than their precursor cells. They were round cells with an eccentric nucleus. Their nucleus was oval or slightly indented in one side or bean shape. The cytoplasm contained specific granules (Figure **5**). Band cells or immature heterophils were round with an

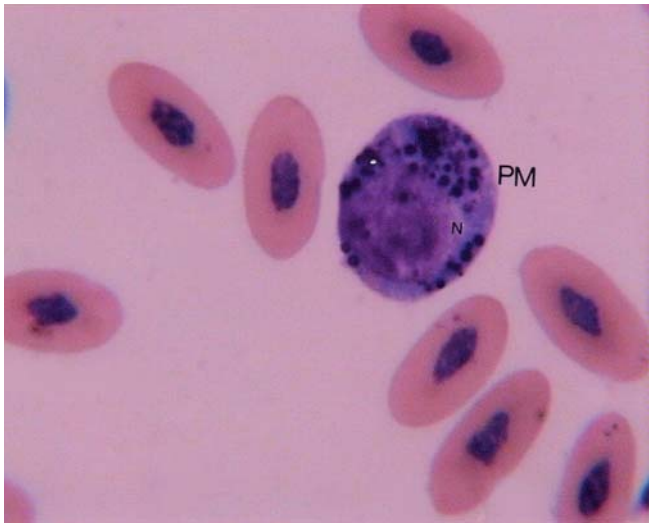

Figure 4: Promyelocyte (PM), (Giemsa, × 1800).

eccentric nucleus. The nucleus is horseshoe-shaped. The cytoplasm contained specific granules (Figure **6**).

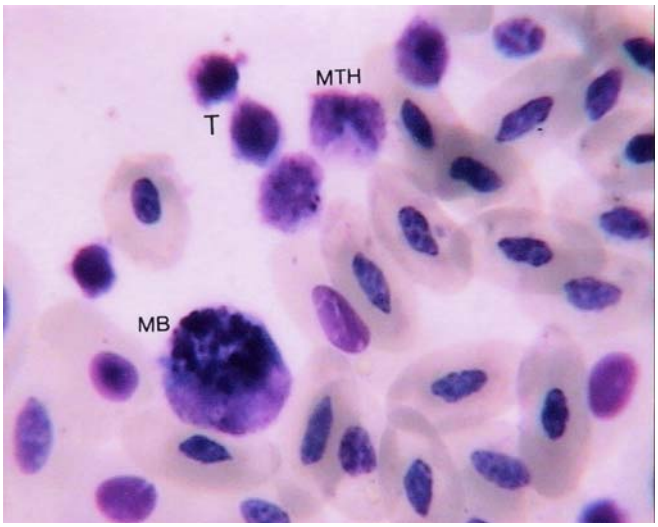

Figure 5: Heterophilic meta myelocyte (MTH), thrombocyte (T) and basophilic myelocyte (MB), (Giemsa, × 1800).

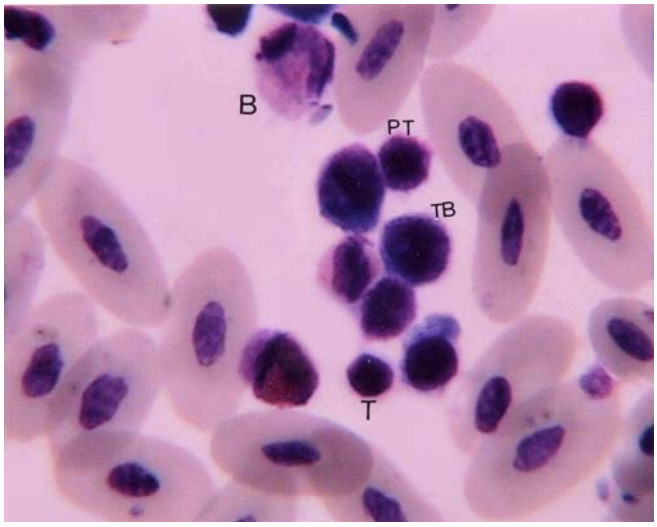

Figure 6: Band cell (B), Prothrombocyte (PT), throbmoblast (TB) and thrombocyte (T), (Giemsa, × 1800).

Thromboblast were large round cell with a narrow rim of basophilic cytoplasm. The nucleus is round with dense chromatin (Figures **6**, **9**). Prothrombocytes resembled to thromboblasts but smaller than it. The nucleus contained dense chromatin (Figure **6**).

Promonocytes were large round cell with an eccentric round or oval shaped nucleus. The cytoplasm is light blue and vacuolated (Figure **7**). The lymphoblasts were large round cell with a narrow rim of basophilic cytoplasm. The nucleus was round with two or three nucleolus and contained reticular chromatin. Prolymphocytes were similar to lymphoblaste, but did not show nucleoli (Figure **8**). Plasmablasts were round cell with an eccentric round nucleus. The cytoplasm is light blue and the N: C ratio was high (Figure **9**).

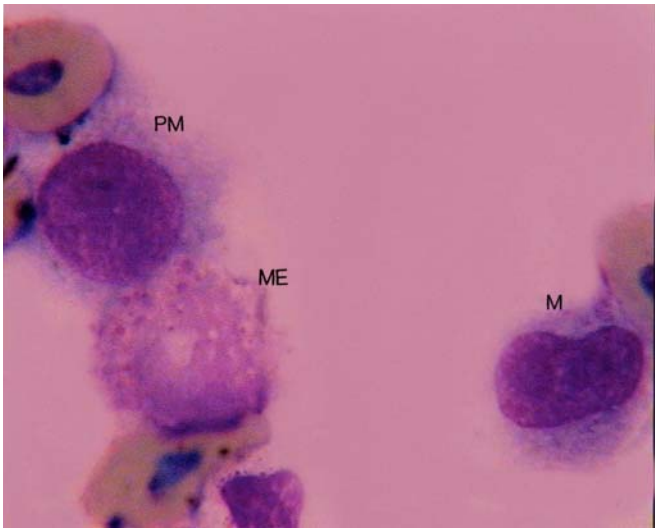

Figure 7: Promonocyte (PM), eosinophilic myelocyte (ME) and monocyte (M), (Giemsa, × 1800).

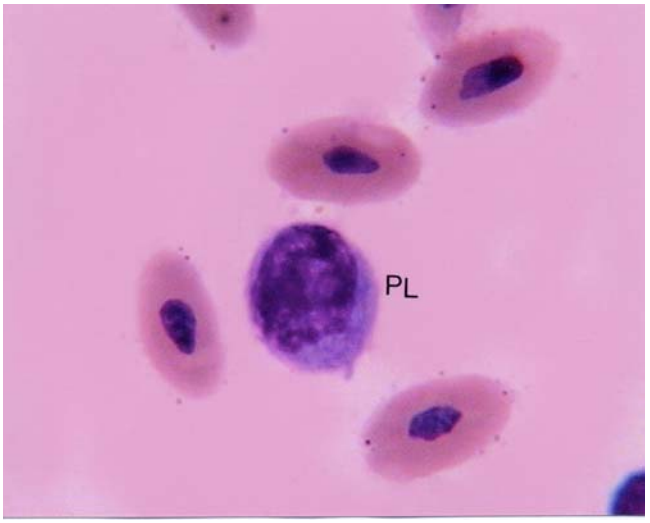

Figure 8: Prolymphocyte (P), (Giemsa, × 1800).

Formation and development of blood cells (WBC, RBC and thrombocytes), in male adult ostrich, similar to other birds take place in bone marrow [3, 5]. The cells which are formed in erythropoiesis are rubriblasts, prorubricytes, basophilic rubricytes, early polychoromatophiilic rubricytes and late polychromatophiilic rubricytes. The morphology of rubriblasts was similar to those in, Iranian green-head ducks, adult quail and black headed gull [6, 7, 10]. The number of rubriblasts was 0.81 ± 0.02 percent of the entire bone marrow cells in male adult ostrich although it was, 0.84 ± 0.09 in Iranian green-head ducks, 2.97 ± 1.03 and 3.05 ± 0.73 in male and female adult quail and 0.72 ± 0.46 in black headed gull [6, 7, 10].

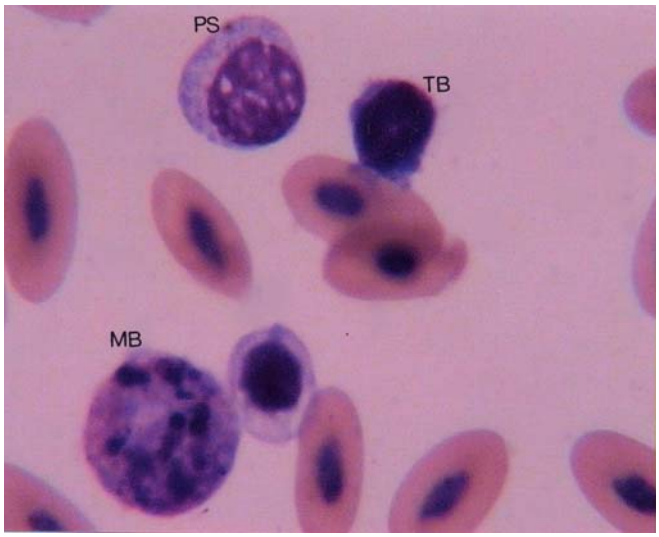

Figure 9: Plasmablast (PS), thromboblast (TB) and basophilic myelocyte (MB), (Giemsa, × 1800).

The morphology of Prorubricytes except it's distinct nucleolus were similar to those in north Iranian green-head ducks, adult quail and black headed gull [6, 7, 10]. The number of prorubricytes was 2.81 ± 0.11 percent of the entire bone marrow cells in male adult ostrich; it was 3.54 ± 0.047 in Iranian green-head ducks, 5.22 ± 1.56 and 4.89 ± 1.13 in male and female adult quail and 3.34 ± 1.49 in black headed gull [6, 7, 10].

The basophilic rubricytes in male adult ostrich were similar to those in north Iranian green-head ducks, adult quail and black headed gull [6, 7,10]. The number of basophilic rubricytes was 4.76 ± 0.07 percent of the entire bone marrow cells in male adult ostrich; it was 6.18 ± 0.59 in Iranian green-head ducks, 0.66 ± 0.65 and 0.93 ± 0.59 in male and female adult quail and 4.17 ± 2.43 in black headed gull [6, 7, 10].

Early polychoromatophiilic rubricytes in male adult ostrich were similar to those in north Iranian green-head ducks, adult quail and black headed gull [6, 7, 10]. They were smaller than basophilic rubricyte and its nucleolous were round and smaller. Their light basophilic cytoplasm (grayish) was indicated hemoglobin formation. The number of early polychoromatophiilic rubricytes was 23.61 ± 0.13 percent of the entire bone marrow cells in male adult ostrich; it was 9.08 ± 0.55 in north Iranian green-head ducks, 36.68 ± 4.79 and 34.76 ± 3.41 in male and female adult quail and 19.49 ± 4.87 in black headed gull [6, 7, 10]. This study revealed that the highest percentage of cells were early polychoromatophiilic rubricytes.

This study showed that the morphology of late polychromatophiilic rubricytes is similar to those in Iranian green-head ducks [6]. The number of late polychoromatophiilic rubricytes was 15.37 ± 0.22 percent of the entire bone marrow cells in male adult ostrich; it was 20.53 ± 0.90 in Iranian green-head ducks, 24.8 ± 0.59 and 27.8 ± 2.25 in male and female adult quail and 12.19 ± 2.73 in black headed gull [6, 7, 10]. Tadjalli *et al.,* [6, 10] reported that highest percentage of bone marrow cell were late polychromatophiilic rubricytes .

The granulocytic series in male adult ostriches was similar to those of other birds and mammals [3, 12, 13].

Myeloblast in male adult ostriches, resembled to those in north Iranian green-head ducks [6], adult quail [7] and black headed gull [10]. It is the first recognizable cell in granulopoiesis. Myeloblast in male adult ostriches had the same size as rubriblast and prolymphocyte. The number of Myeloblast was 1.00 ± 0.003 percent of the entire bone marrow cells in male adult ostrich; it was 2.33 ± 1.09 and 1.88 ± 0.64 in male and female adult quail and 1.89 ± 0.72 in black headed gull [7, 10].

The morphology of promyelocyetes in male adult ostriches were similar to those in north Iranian green-head ducks [6], adult quail [7] and black headed gull [10]. Campbell [4] reported that promyelocyetes is the first recognizable cell in leukopoiesis in pigeons and chickens. These cells were 3.39 ± 0.15 percent of the entire bone marrow cells in male adult ostrich; it was 4.90 ± 0.54 in north Iranian green-head ducks, 0.98 ± 0.92 and 1.16 ± 0.77 in male and female adult quail and 4.08 ± 1.93 in black headed gull [6, 7, 10].

Cell characteristics in male adult ostrich myeloctye were similar to those in Iranian green-head ducks [6], adult quail [7] and black headed gull [10]. Myelocytes in male adult ostriches similar to other birds can be divided to heterophilic, basophilic and eosinophilic series. This study showed that the number of heterophilic, basophilic and eosinophilic myelocyte was 16.64 ± 0.2, 3.18 ± 0.04 and 0.70 ± 0.03 percent of the entire bone marrow cells in male adult ostrich; they were 14.82 ± 1.47, 3.64 ± 0.37 and 2.46 ± 0.41 in Iranian green-head duck 15.68 ± 3.62, 5.52 ± 2.41 and 1.76 ± 0.42 in black headed gull [6, 10]. This study showed that the myelocye had the highest percentage of the bone marrow cells in myeloid series, it resembled to those in Iranian green-head duck and black headed gull [6, 10].

The metamyelocytes in male adult ostrich contained specific granules; they were similar to those in Iranian green-head ducks [6], adult quail [7] and black headed gull [10]. Campbell [4] reported that most of metamyelocyte granules in chicken and pigeon are spindle shape. The number of heterophilic, basophilic and eosinophilic metamyelocyte was 8.45 ± 0.23, 6.38 ± 0.07 and 0 percent of the entire bone marrow cells in male adult ostrich; they were 7.42 ± 0.66 in Iranian green-head duck [6], 9.78 ± 2.24, 0.13 ± 0.06 and zero percent in black headed gull [10] The number of heterophilic metamyelocyte in male and female adult quail were 5.54 ± 3.58 and 6.42 ± 2.74 and they were [7]. The band cell in male adult ostriches, similar to those in other birds was the last cell in myeloid series. The number of heterophilic and eosinophilic band cell in male adult ostriches were 6.02 ± 0.05 and 0.24 ± 0.02 but basophilic band were not seen. Tadjalli *et al.,* [6] reported that the number of band cell in north Iranian green head duck were 5.26 ± 0.76 percent of entire of bone marrow cell. The number of heterophilic band cell in male and female adult quail were 1.97 ± 0.77 and 2.2 ± 0.64 percent but the basophilic and eosinophilic band cell were not observed [7]. The number of heterophilic, eosinophilic and basophilic band cell in black headed gull was 6.72 ± 1.47, 0.09 ± 0.03 and 0.03 ± 0.01 [10].

Thrombocytopoiesis in male adult ostriches similar to other birds has some cellular developmental stages: cellular maturation begins with thromboblast and after passing a short stage a cell called prothrombocyte ends to mature thrombocyte.

Thromboblast in male adult ostriches is not similar to megakaryocytes in mammals [14, 15]. The thrombocytes were round cell, they were smaller than primitive cells in erythroid and myeloid series. The morphology of thrombocyte in male adult ostriches was similar to those in Iranian green-head ducks [6], adult quail [7] and black headed gull [10]. These cells were 0.26 ± 0.02 percent of the entire bone marrow cells in male adult ostrich; it was 2.18 ± 0.37 in Iranian green-head ducks, 0.96 ± 0.23 and 1.30 ± 0.92 in male and female adult quail and 1.17 ± 0.48 in black headed gull [6, 7, 10].

Prothrombocyte is the second cell in thrombocyte series, in male adult ostriches it is similar to thromboblast but smaller and the nucleous in more dens than thromboblast. The morphology of prothrombocyte in male adult ostriches is similar to those in Iranian green-head ducks [6], adult quail [7] and black headed gull [10]. The number of thrombocyte in male adult ostriches was 0.32 ± 0.01 percent of the entire bone marrow cells; it was 13.78 ± 1.04 in Iranian green-head ducks, 1.24 ± 0.67 and 1.28 ± 0.38 in male and female adult quail and 1.34 ± 0.39 in black headed gull [6, 7, 10].

Promonocyet is in monocytopoiesis series, it is similar to monoblast, it is the largest cell in bone marrow and the lowest percentage of bone marrow cells, and it is similar to those in north Iranian green-head ducks [6], adult quail [7] and black headed gull [10]. The number of promoncyte in male adult ostriches was 0.06 ± 0 percent of the entire bone marrow cells it was 0.55 ± 0.32 in Iranian green-head ducks, 2.68 ± 1.30 and 2.30 ± 1.05 in male and female adult quail and it is 0.53 ± 0.31 in black headed gull [6, 7, 10].

Lymphoblast and prolymphocyte are in lymphoid series, their morphology were similar but the prolymphocyte did not have nucleous. These cells were similar to those in other birds [6, 7, 10]. The number of lymphoblast and prolymphocyte in male adult ostriches were 0.14 ± 0.02 and 0.16 ± 0.02 percent of the entire bone marrow cells; they were 1.48 ± 1.42 and 1.61 ± 1.1 in male and 1.12 ± 0.63 in female adult quail and 1.47 ± 0.84 and 1.29 ± 0.47 in black headed gull [7, 10]. Tadjalli *et al.,* [6] reported that lymphoblast and prolymphocyte were not observed in bone marrow of north Iranian green-headed duck.

The mitotic cells were observed in bone marrow smear of male adult ostriches similar to Iranian green headed duck, adult quail, black headed gull and other birds [3, 6, 7, 10]. The number of mitotic cells in male adult ostriches was 0.25 ± 0.01 percent of the entire bone marrow cells; it is 3.09 ± 0.02 in Iranian green-head ducks, 0.49 ± 0.44 and 0.41 ± 0.28 in male and female adult quail and 0.56 ± 0.19 in black headed gull [6, 7, 10].

The mean value for M/E ratio in the bone marrow of male adult ostriches was 1.02, which was approximately similar to that in Iranian green headed duck [6]. The M/E ratio was 0.42 ± 0.16 in male adult quail and 0.41 ± 0.03 in female adult quail and it was 1.23 ± 0.17 black headed gulls [7, 10].

In conclusion, the results indicated that the morphological characteristics of haematopoietic cells in the bone marrow of male adult ostriches were similar to chickens, ducks, quail, and black-head gull. The mean myeloid/erythroid (M/E) ratio was 1.02, the mean

erythroid percentage was 47.38%, the mean myeloid percentage was 48.15%, and the mean percentage of all other cells percentage was 0.99 %.

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