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Assessment of erythroblastic cells in the bone marrow of healthy Holstein-Friesian cows

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Summary

The aim of the study was to determine the activity and effectiveness of bone marrow hematopoiesis in healthy, adult cattle, and to compare hematopoietic activity levels in diseased cattle. The experimental material comprised 10 high-yielding, clinically healthy Holstein-Friesian cows aged 2-3 years. Bone marrow aspirate smears were analyzed. Marrow samples were collected with a marrow biopsy needle with a length of 63 mm (13G, from the medullary cavity of the third and fourth rib in the sternal region, and the material was subjected to a smear test. Marrow and peripheral blood smears were stained by a method proposed by May Grünwald-Giemsa (MGG). An increase in the number of orthochromatic erythroblasts with a simultaneous decrease in basophilic and polychromatic erythroblast counts was noted in this study. The results indicate that the nucleoli of bovine proerythroblasts are less visible, and the cytoplasm is stained light blue.

Keywords: bone marrow, cattle, hematopoiesis, proerythroblast

Erythropoesis is a process of the proliferation and differentiation of red blood cells from stem cells in the bone marrow of flat bones and the epiphyses of long bones (5). All cell lines are derived through the development of stem cells, which includes the processes of self-renewal, differentiation, aging, and wearing out. Stem cells have a unique and extraordinary ability to copy themselves. They differentiate into progenitor cells of four lines of development. The first morphologically distinguishable cell of the erythroblastic system is a proerythroblast (6). This cell develops into a reticulocyte, going through the intermediate stages of a basophilic erythroblast, eosinophilic erythroblast, and polychromatic erythroblast. All these cells are characterized by the presence of residual RNA and increasing amounts of haemoglobin. The process of erythropoiesis is regulated by a number of vitamins, minerals, and proteins (3).

The aim of the study was to evaluate erythroblastic cells in the bone marrow of healthy cows.

Material and methods

The experimental material comprised 10 high-yielding, clinically healthy Holstein-Friesian (HF) cows aged 2-3 years. To rule out bone marrow diseases or dysfunctions, blood morphology and biochemical tests were performed by determining the enzymatic activity of aspartate transaminase, alkaline phosphatase, lactate dehydrogenase, creatine kinease, total protein, and iron concentrations.

Bone marrow aspirate smears were analyzed. Marrow samples were collected with a marrow biopsy needle with a length of 63 mm, 13G, from the medullary cavity of the third and fourth rib in the sternal region, and the material was subjected to a smear test (9, 11). Prior to biopsy, hematological analyses were performed, using whole blood sampled from the caudal vein and stored in test-tubes with K_2EDTA as anticoagulant. Serum was separated by centrifuging whole blood samples. It was stored in test-tubes with a coagulation activator, and used in biochemical analyses.

Marrow and peripheral blood smears were stained by a method proposed by May Grünwald-Giemsa (MGG). Marrow smears were stained for 80 seconds in line with the May-Grünwald protocol, and for 5 minutes according to the Giemsa protocol. Peripheral blood staining times were 3 and 12 minutes, respectively. The Giemsa stain was diluted with a phosphate buffer, pH 7.2, at a ratio of 1 : 10. The stained specimens were analyzed by immersion microscopy with 1000 × magnification. Peripheral blood smears were evaluated with an ADVIA 2120i hematology analyzer, capable of determining standard morphological parameters (CBC), performing automatic smear analyses (6 DIFF), and determining reticulocyte parameters. Biochemical tests were carried out with an Accent-200 biochemical analyzer.

Tab. 1. Erythrocyte percentage in bone marrow evaluations of healthy HF cows

Number of animal	Proerythroblasts	Basophilic erythroblasts	Polychromatic erythroblasts	Orthochromatic erythroblasts	Total normoblast counts
1	1.3	5.1	15.2	20.0	41.6
2	2.3	5.3	12.1	18.0	37.7
3	1.4	4.9	14.4	19.2	39.9
4	1.9	5.5	13.2	20.1	40.7
5	2.5	5.4	11.8	18.4	38.1
6	3.1	4.8	12.5	21.3	41.7
7	2.9	4.6	12.8	18.9	39.2
8	1.8	3.9	14.5	23.2	43.4
9	3.0	4.2	13.6	21.1	41.9
10	2.8	3.5	13.1	19.6	39.0
Mean	2.3	4.72	13.32	19.98	36.32

Results and discussion

The percentage share of erythroblast counts at various growth stages in healthy cows is presented in Tab. 1. A comparison of average normoblast counts yielded normal values for the species. Minor variations were observed in the number of basophilic, polychromatic, and orthochromatic erythroblasts relative to the norm given by Jain (8). An increase in the number of orthochromatic erythroblasts with a simultaneous decrease in basophilic and polychromatic erythroblast counts was noted in this study (Fig. 1).

The results of all tests were consistent with the reference values for the species.

The proerythroblast is the earliest morphologically identifiable stage in the development of red blood cells. Proerythroblasts are large, nucleated cells with 1-2 nucleoli (11). The results of our work indicate that the nucleoli of bovine proerythroblasts are less visible, and the cytoplasm is stained light blue. In other animal species, including cats and dogs, proerythroblast nucleoli are stained a darker color, and they form more compact structures in comparison with the surrounding cytoplasm (5, 14). In cattle, the proerythroblast membrane is stained dark blue, and it is visibly separated from the small amount of cytoplasm.

The following developmental stage is marked by the appearance of basophilic erythroblasts. In adult cattle, the nucleus of a basophilic erythroblast is often observed near the edge of the cytoplasm (2). The cell is somewhat smaller, with a compact nucleus, without nucleoli. The cytoplasm is stained light blue with a purple hue (24). In cats and dogs, erythroblast nuclei are rarely adjacent to the cell membrane. Basophilic erythrocytes are transformed into polychromatic erythrocytes with much smaller nuclei, which are stained with both base and acid dyes. The following stage of development is the orthochromatic erythrocyte. Its nucleus is stained dark blue, and the cytoplasm takes on a clearly pink hue. Every developmental form is characterized by a progressive decrease in nucleus size and greater amounts of cytoplasm. Orthochromatic erythrocytes are transformed into reticulocytes, that is, immature red blood cells. Reticulocytes are generally larger than erythrocytes. Reticulocytes cannot be differentiated from erythrocytes (normocytes) in peripheral blood specimens stained with MGG. The claim that the two cell types can be distinguished by their size and the degree of staining is false, because megalocytes are similarly sized. The cells under discussion are commonly identified by in vivo staining with the use of, for example, methylene blue to expose residual RNA after the loss of the cell nucleus.

Hematological tests are performed to diagnose anemia and identify its type on the basis of red blood cell parameters (15). One of the most frequent causes of anemia is iron deficiency, in particular in young cattle (21). Anemia generally affects the entire herd, and it is caused by feeding iron-deficient milk replacers to suckling calves. The above is a prime example of sideropenic anemia that affects mostly young animals of various species, including calves and piglets. Hematological tests of calves affected by an iron deficiency

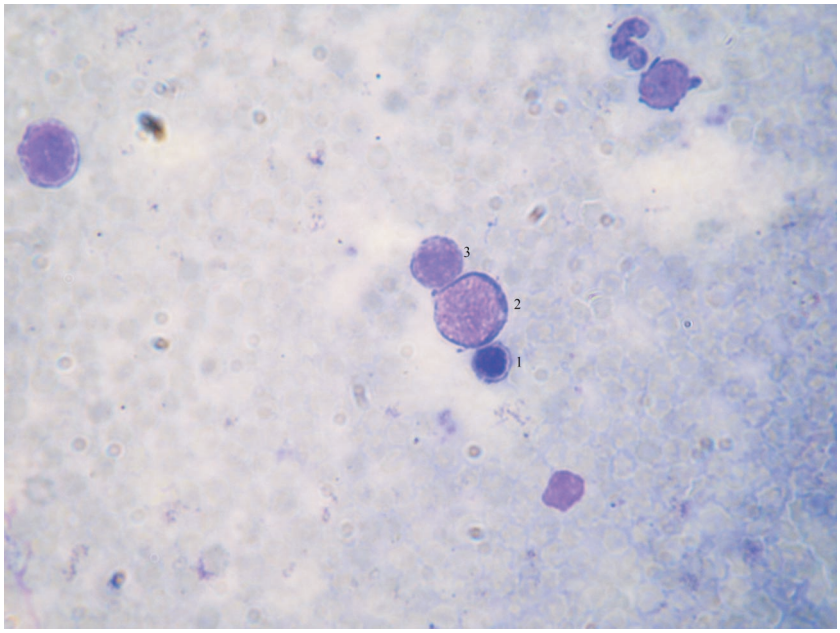

Fig. 1. 1 – Basophilic erythroblast; 2 – Proerythroblast; 3 – Polychromatic erythroblast

reveal a decrease in erythrocyte counts, an increase in reticulocyte and erythroblast counts, a decrease in hematocrit levels, normochromic to hypochromic microcytic anemia with anisocytosis and poikilocytosis (4). The presence of Howell-Jolly bodies, erythroblast nuclear remnants in the form of pink or red, single or double spots, is indicative of enhanced erythropoiesis. An increase in erythroblast counts of every type and a higher number of erythrocyte vacuoles are observed in bone marrow smears (16). Iron is often accumulated around erythroblast nuclei to form ringed sideroblasts. Secondary sideropenia affects all age groups. It is generally caused by iron deficiencies in feed, the use of iron antagonists in feed supplements, and inflammations of the gastrointestinal tract. Changes in the peripheral blood and bone marrow of adult animals are similar to those noted in calves (17). Iron deficiencies are often masked by a copper deficiency (10).

Abnormalities in hematological and bone marrow parameters could also result from a cobalt deficiency that affects cattle administered feed with low levels of this element. Cobalt is a part of the vitamin B12 molecule. In ruminants, cobalt deficiencies lead to metabolic disorders by impairing bacterial flora functions, enzymatic reactions, and bone marrow erythropoiesis. Hematological tests of affected cattle reveal a decrease in erythrocyte counts, hematocrit levels, hemoglobin concentrations, and red blood cells, as well as normocytic or microcytic hypoplastic anemia. A decrease in erythroblast counts and in the number of precursor cells in the white blood cell system, elevated megakaryocyte counts without an increase in peripheral blood thrombocytes are noted in bone marrow.

Pregnancy and birth are accompanied by physiological changes that result from a higher steroid hormone activity (12). Hematological examinations of pregnant and periparturient cows reveal elevated erythrocyte and leukocyte counts, and an increase in the number of precursor cells of both developmental lines is noted in bone marrow smears. In this type of anemia, pancytopenia is observed in hematological tests, whereas a decrease in both red and white blood cell counts is noted in bone marrow analyses.

It can be concluded that bone marrow sampling in cattle and other animal species is an effective technique for diagnosing acute hematopoietic disorders such as anemia, leukocytosis, and coagulopathy (1, 20). Pathological processes impair hematopoiesis and cause irreversible damage to bone marrow (18, 19). The above contributes to anemia and rapid health deterioration, which lower milk yield and calving rates, and lead to massive production losses (23). Biopsy material supports an evaluation of individual cells and their structure (13). If cytomorphological analyses do not support a comprehensive diagnosis, more specific tests are performed, involving cytochemical, cytogenetic, and immunological methods to precisely determine cell type and the level of cell maturity (7). The above changes are visualized by the results of hematological, biochemical, coagulation, and bone marrow aspiration tests (22).

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