Porcine Research

Association of the maternal colony-stimulating factors with the pig embryos normoblastic erythropoiesis

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Abstract. In the given study the maternal macrophage colony-stimulating factors (CSF) serum level changes in the pigs gestation period were investigated. It is shown, that increase in the maternal macrophage CSF levels precedes the yolk sac erythropoiesis transfer into the liver erythropoiesis, and then coincides with erythropoiesis in the embryos bone marrow. We have not revealed any correlation between the CSF serum levels and the erythropoietin level in blood. The obtained data testify to the maternal macrophage CSF association with the most important stages of the embryo erythropoiesis in pigs.
Key Words: granulocyte macrophage colony-stimulating factor (GM-CSF), macrophage colony-stimulating factor (M-CSF), erythropoietin (Epo), embryo erythropoiesis in pigs.

Абстракт. В работе исследовались изменения сывороточных уровней материнских макрофагальных колониестимулирующих факторов (КСФ) во время беременнсти, у свиней. Показано, что повышение уровней материнских макрофагальных КСФ предшествует переходу от эритропоэза в желточном мешке к печеночному эритропоэзу, а затем совпадает с переходом эритропоэза в костный мозг зародыша. При этом нами не выявлено связи сывороточных уровней КСФ с уровнями эритропоетина. Полученные данные свидетельствуют об ассоциации материнских макрофагальных КСФ с важнейшими этапами эмбрионального эритропоеза у свиней.
Ключевые слова: гранулоцитарно - макрофагальный колониестимулирующий фактор (GM-CSF), макрофагальный колониестимулирующий фактор (MCSF), эритропоэтин (Epo), эмбриональный эритропоез свиней.

Rezumat. În studiul de față am investigat modificările concentrației serice a factorilor de stimulare a coloniilor macrofage materne (FSC) în perioadă de gestație. S-a demonstrat faptul că, creșterea nivelurilor materne a FSC macrofage precede transferul eritropoiezei sacului vitelin în eritropoieza de ficat, iar apoi coincide cu eritropoieza din măduva osoasă embrionară. Studiul întreprins de către colectivul nostru de cercetare nu a evidențiat nici o corelație între nivelurile serice a FSC și nivelul de eritropoietină din sânge. Datele obținute atestă asocierea CSF macrofage materne cu cele mai importante etape ale eritropoiezei embrionare la suine.
Cuvinte cheie: factor de stimulare a coloniilor granulocit macrofage (FSC-GM), factor de stimulare a coloniilor macrofage (FSCM), eritropoietină (Epo), eritropoeză fetală la suine.

Introduction. Macrophages are of crucial importance in the hemopoietic cells functional activity regulation. The leading role of macrophages in the erythroblastic islets (EI) formation, which provide proliferation and differentiation of the erythroid cells, is well known. The EI formation process in the embryonal period coincides with the erythropoiesis hepatic stage onset (Palis 2014). The hemopoiesis maintaining factors are called colony-stimulating factors (CSF).

Monocytar-macrophage line cells production is under the control of a whole group of growth factors: Il-3 and the colony-stimulating factors (GM-CSF, M-CSF) stimulate mitotic activity of the monocytes precursors. The pivotal significance in the macrophages proliferation and differentiation is for the granulocyte macrophage colony-stimulating factor (GM-CSF) and the macrophage colony-stimulating factor (M-CSF-1). Il-3 is a

polycolony-stimulating factor, it stimulates all the hemopoietic bursts of hemopoiesis, and the GM-CSF stimulates a production of granulocytes and macrophages, as well as activates the macrophages function. A specific growth factor for the mononuclear macrophages is M-CSF, which is produced by the bone marrow stromal cells, monocytes, tissue macrophages, T-helpers, fibroblasts, epithelial cells of endometrium and some others (Smith 1990).

The GM-CSF is a unique protein from the cytokines family that takes part in the hemopoietic homeostasis. It stimulates the myeloid line cells differentiation. It influences on the macrophages and neutrophils function, as well as the processes of phagocytosis, migration and metabolism (Bugress & Metcalf 1980; Hübel et al 2002).

In pregnancy the GM-CSF is expressed in the sexual tract cells. It strengthens the cells proliferation, activates the embryos development, formation of blastocyst and the embryos implantation (Azuma et al 1991; Jiwakanon et al 2011). Hence the goal of the present research is to investigate the sows GM-CSF, M-CSF and erythropoietin levels influence onto the normoblasric erythropoiesis in pigs embryos.

An interconnection of the maternal GM-CSF and M-CSF to the erythropoiesis transfer from megaloblastic to the normoblastic type in mammals is not fully investigated and particularly in the pig embryo is not investigated at all.

Material and Method

Animals. 7 sows (of 14 months of gestation) were used in the experiment. Blood samples were collected from the ophthalmic venous sinus as described previously (Stier & Leucht 1980).

Determination of the CSF serum levels. For detection of serum levels of macrophage colony-stimulating factor (M-CSF) and the granulocyte macrophage colony-stimulating factor (GM-CSF) the commercial ELISA kits (Elabscience Biotechnology Co, Ltd) were used. The levels of cytokines (pg/mL) were measured using a colorimetric reader (Stat Fax 303 Plus) and calculated according to the cytokine standard curve supplied in the kits. All samples were tested in duplicate according to manufacturer instructions.

Determination of the EPO plasma levels. The levels of EPO (ng/mL) in plasma samples were measured by commercial ELISA kits (Elabscience Biotechnology Co., Ltd).

Statistical analysis. The significance was evaluated by two-tailed Student t-test, and Mann-Witney u-test.

Results. Determination of the CSF serum levels in the sows blood during the whole gestation period are brought in the Figure 1.

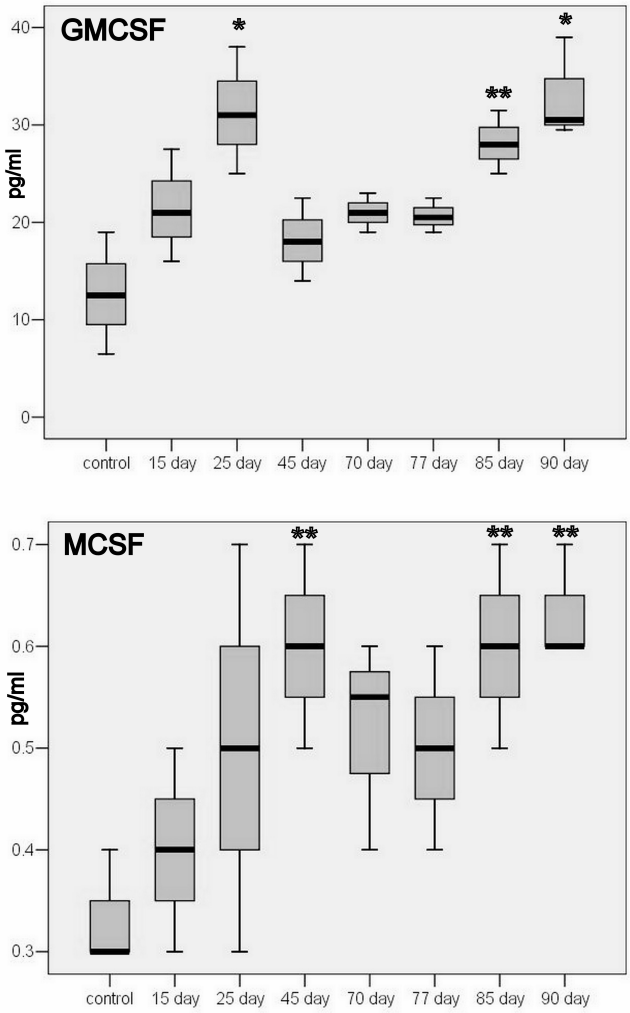

Figure 1. The CSF levels in the pregnant sows blood. On the abscissa axis – days of gestation; On the ordinate axis – the serum content of the GM-CSF and the M-CSF (pg/mL); * authentic comparably to control ($p<0.05$-$p<0.01$); ** tendency ($p<0.01$).

As it is apparent from the picture, the GM-CSF levels authentic increase on the 25th and the 90th days of gestation was revealed. On the 85th day there was a significant tendency to increase. The M-CSF level is not authentically changed in the sows sera, but we have revealed a tendency to growth of the M-CSF on the 45th, 85th and 90th days of gestation. Differently from the CSF, the Epo level in the sows blood remained practically unchanged till the end of gestation and just at the 90th day there was an increase detected. There are some data of Epo levels in the blood brought in the Figure 2 testifying to the Epo authentic increase just on the 90th days of gestation.

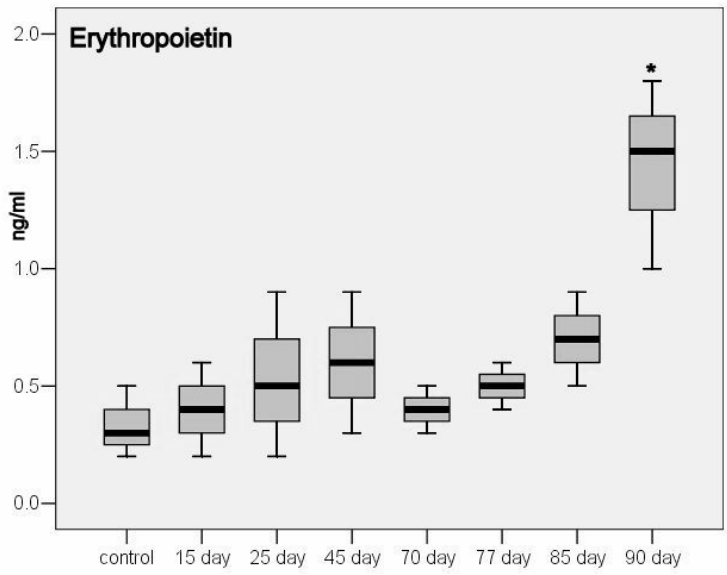

Figure 2. The Epo levels in the pregnant sows blood. On the abscissa axis – days of gestation; On the ordinate axis – the serum content of Epo (ng/mL); * authentic comparably to control ($p<0.01$).

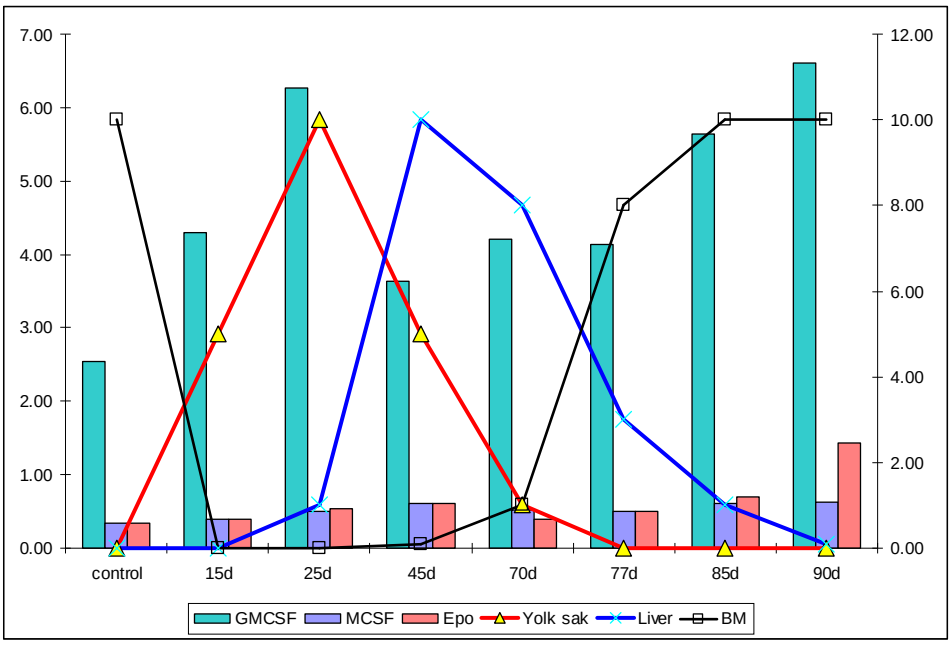

Figure 3. Comparison of the embryonal erythropoiesis periodization with the CSF and Epo levels in the pregnant sows (histogram). The erythropoiesis linear sketches in the yolk sac, liver and the bone marrow (conventional units).

In the Figure 3 the embryonal erythropoiesis periodizations are represented schematically. That was given in details in the previous research works (Tatoyan et al 2016). From the sketch it is becoming obvious, that the GM-CSF levels increase is followed by the yolk sac erythropoiesis transfer to the erythropoiesis in the liver and coincides with the erythropoiesis transfer into the bone marrow. The M-CSF levels changes had the similar dynamics, but were of a lesser expression. Increase of the maternal Epo occurred at the final stage of pregnancy and did not have any connection with the macrophagal CSF.

Discussion. Although the macrophages role in the erythropoiesis process is hard to overestimate, the mammals embryo macrophages remain comparably less investigated, and the embryo development of pigs macrophages practically is not investigated at all. Herein, the mammals embryonal macrophages differentiation predetermining conditions and mechanisms are not pretty clear. The main factors, contributing to the macrophages differentiation are the GM-CSF and the M-CSF, which being produced in the mothers organism play an important role in the processes of implantation, placentation and development of embryos in some mammals, including the humans (Rahmati et al 2015). Anyway, the maternal GM-CSF and M-CSF influence onto the macrophages maturation and differentiation of the hepatic EI and then on their transfer into the normoblastic erythropoiesis in the pigs embryos are not studied yet.

Sooner it was previously shown, that approximately by the pigs embryogenesis 20-25th days in the embryos vessels two types of cells are observed: the megaloblasts, deriving from the yolk sac and the normoblasts, deriving from the liver (Tatoyan et al 2016). In the modern literature an appearance of non-nucleated embryonal erythrocytes is often linked to the hepatic EI formation and functioning onset, and so, to the macrophage EI formation. Namely, an extrusion of erythroblasts nucleus occurs there (Sonada et al 1998). But at the earlier stages (the 15th days of embryos development) the primitive non-nucleated erythrocytes appearance does not seem to be connected with their nuclei extrusion by macrophages (Tatoyan et al 2016), so this issue still remains open.

So, the macrophages input into the non-nucleated erythrocytes formation becomes understandable just after the 25th day of the pig embryos development, when the first peak of increase of the GM-CSF level and of the M-CSF at a lesser extent comes rise, which levels increase remains not authentic just because of their tangible distortions.

According to Gregor et al (1999), GM-CSF is capable to penetrate through the placental barrier in humans. A penetration of the maternal GM-CSF occurred in small amounts (about 2.5% of the factor penetrated the placenta from the added to the initial level of the GM-CSF in the maternal circulation). Thus, we have shown a possibility of the maternal macrophage CSF involvement into the process of embryonal erythropoiesis in pigs, particularly of the GM-CSF.

Involvement of the macrophage CSF, particularly the GM-CSF, in the processes of embryonal erythropoiesis in mature mice have been shown previously (Jegalian et al 2002), and the earlier research works revealed a stimulating action of the xenogenic GM-CSF not just onto myelopoiesis, but also on erythropoiesis in mice (Nishijima et al 1997). Herein, it was noted by a number of authors, that GM-CSF in conditions in vitro and in vivo has had an inhibitory effect on the erythroid cells differentiation (Hermine et al 1996; Udupa & Sharma 1996). So, we can conclude that the GM-CSF stimulatory action is not connected with the erythroid cells differentiation, but is linked to the processes of activation and differentiation of macrophages, as well as to the central macrophages EI. For the benefit of our suggestion the data testify also to the GM-CSF as a powerful stimulator of the erythroid burst-forming unit in conditions in vitro (Aglietta et al 1993). The GM-CSF stimulatory effect onto the erythropoiesis in the patients suffering from the bone marrow pathology was also shown (Vadhan-Raj et al 1988).

Conclusions. Thus, it was shown by us, that the maternal macrophage CSFs are probably involved into the process of embryonal erythropoiesis. Increase of the maternal macrophage CSF levels precedes the yolk sac erythropoiesis transfer into the erythropoiesis in liver, and then coincides with the erythropoiesis in the embryos bone marrow. The changes in the CSF levels are not correlated with the changes in erythropoietin levels.

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Received: 25 May 2016. Accepted: 28 June 2016. Published online: 30 June 2016.
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How to cite this article:
Tatoyan M., Semergyan Z., Karalyan Z., 2016 Association of the maternal colony-stimulating factors with the pig embryos normoblastic erythropoiesis. Porc Res 6(1):24-30.