Porcine Research

Mesenchymal erythropoiesis in the pig ontogenesis

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Abstract. The performed investigations on the mesenchymal erythropoiesis in the pig ontogenesis have confirmed a presence of two independent areas of the hemopoietic cells formation. That is a region of an extra-embryonic area represented by the yolk sack and the intra-embryonic zone of hemopoietic cells' localization, which includes the paraaortal mesenchyme and the AGM-region, the site of anlage of aorta, gonads and the primary renals - in the mesonephros area. The whole period of mesenchymal erythropoiesis in the pigs takes approximately three weeks: it arises on the 13^{th}-15^{th} day of the embryo's development and disappears by the 35^{th}-37^{th} day. A proliferative activity of the hemopoietic cells of mesenchymal erythropoiesis decreases by the 25^{th} day and practically fully disappears by the 35^{th} day of the pig embryo's development period. Content of hemoglobin in all the investigated cells did not differ significantly from that determined in analogical cells of the yolk sack.
Key Words: mesenchyme, stem cells, embryogenesis, haemopoiesis.

Introduction. It is well known, that in some period after fertilization an embryonic hemopoiesis is being arisen and the hemopoietic cells' formation source during that is represented by the embryonic mesenchyme. At the first stage of embryogenesis just one-sided directivity of differentiation of the stem cells towards the erythropoiesis is observed (Zambidis et al 2005; Tada et al 2006; Isern et al 2008). For a long time it was considered that the primary erythroblasts' formation is taking place inside the vessels of the yolk sack, the wall of which is proven to be as the primary hemopoietic organ in all the animals having the yolk sack (Ghatpande et al 2002), containing the polypotent stem cells, which give origin to all the propagules of hemopoiesis (Zambidis et al 2005; Tada et al 2006; Isern et al 2008; Sheng 2010). Later on it was shown, that the primary blood cells are being formed at the sites of mesodermal cells' small-scale accumulation. These cells have an important role in the process of early embryonic hemopoiesis, encountering everywhere in different organs and cavities of the body, especially in the paraaortal mesenchyme and the AGM-region, the site of anlage of aorta, gonads and the primary renals - in the mesonephros area (Emura et al 1983; Kritzenberger & Wrobel 2010; Lee et al 2011). They first arise at the beginning of gastrulating process, which occurs much earlier than the morphologically determined development of the blood islets. Their amount is not high, and the enlarged proliferation of the blood cells, unlike the hemopoietic islets of the yolk sack, is not encountered in the mesenchyme of the body cavity. It is correlated with a presumption, that at first the extra-embryonic mesoderm represented by hemangioblasts arranges the primitive erythroid lines very soon after exiting from the primary embryonic strip (Ferkowicz et al 2003; Ferkowicz & Yoder 2005; Huber et al 2004). It is the first, so-called angioblastic period of hemopoiesis (Jaffredo et al 2005). These cells are accepted to be named as hemocytoblasts. They differ by their big sizes and in case of the mammals also possess nuclei (Godin & Cumano 2005).

Considering the fact, that the data referring to the given stage of development of the pigs' primitive erythropoiesis are not available in the literature accessible to us, the goal of our research work became an investigation of the primary extra-yolk erythropoiesis in the pigs.

Material and Method. The investigation was encountered on 5 sows of the Large White breed of 11-12 months of ages when attaining 130-140 kg. After a process of covering the pregnant sows were slaughtered, correspondingly, on the 15th, 25th and the 35th day of pregnancy with aiming to study the growth and development of the mesenchymal erythropoiesis in pig embryos. Each time 5 embryos (fetuses) were taken for investigation. Euthanasia was provided according to the protocol Guide for the Care and Use of Laboratory animals, AVMA Guidelines (Institutional Review Board/Independent Ethics Committee of the Institute of Molecular Biology of NAS, IRB00004079).

In the capacity of fixing substances for the histological samples of embryos the Buen's liquid and the ethylic spirits of 96% concentration were used. The samples afterwards were embedded in paraffin with the following preparation of the serial histological slides of 5-8 mkm of width. The preparations were treated by the Hematoxylin solution B according to Weigert with an additional staining by eosin (Lillie 1965) and by azan according to Mallory (Gray 1954). For the cell analysis provision the slides were fixed in pure methanol and stained by Giemsa modified solution (azur B/azur II, eosin and methylene blue) according to the manufacturer's protocol (Sigma Aldrich). The cells were examined under the light microscope at the 1250 x magnification in a random sequence. At least 200 cells in each sample were classified. The morphological determination of stages of the primitive erythropoietic development was performed according to Baron et al (2013).

Hemoglobin quantification. Hemoglobin amount in cells was determined spectrophotometrically on unstained preparations. Wavelength scans of the diluted rat blood consistently showed the greatest absorbance at 414 nm, which is the Soret peak from hemoglobin (Tanaka et al 1989).

Statistical analysis. The significance was evaluated by two-tailed Student's t-test. P values <0.05 were considered significant. SPSS version 17.0 software package (SPSS Inc., Chicago, IL, USA) was used for statistical analyses.

Results and Discussion. By the provided investigation it was shown, that in the 15th day pig embryo having a length from 3.0 to 6.0 mm and a weigh from 2.5 to 5.5 mg, and in those which already have in average 6 pair of somites an onset of differentiation of mesoderma onto the intra - and the extra-embryonic mesodermas was observed. The latter participates then in the process of defense and trophic membranes formation; whereas the intra-embryonic mesoderma gives raise the primary strip origination resulting in the brain and eyes primordiums (anlages) generation on its caudal terminal. The so-called primary heart, being an unpaired organ in adult forms, at that time of development is represented by the paired tubular formation. In the pericardial region of mesenchyme the disseminations of erythroid cells are coming apparent (Figure 1).

These loci of erythropoiesis are well distinguished in the 15th and the 25th days pig embryos, but practically fully disappeared in the 35th day pig embryos in the latters a single and sometimes the unique loci are observed in the AGM-region (Figure 2). At the same time (in the 15th-25th days embryo) at the primary embryonic strip stage, beyond the embryo's body the extra-embryonic mesodermal cells accumulations are coming raise with the following formation of the so-called bloody islets (Figure 3).

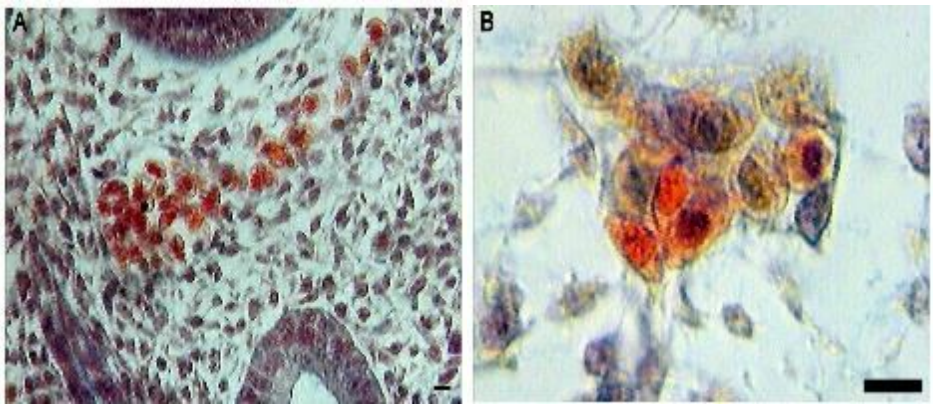

Figure 1. The primary primitive erythropoiesis in the pericardial region of the 15 day pig embryo. Staining is by hematoxilin-eosin according to Karachi (A) and by azan according to Heidenhain (B). The scale used is of 10 μm.

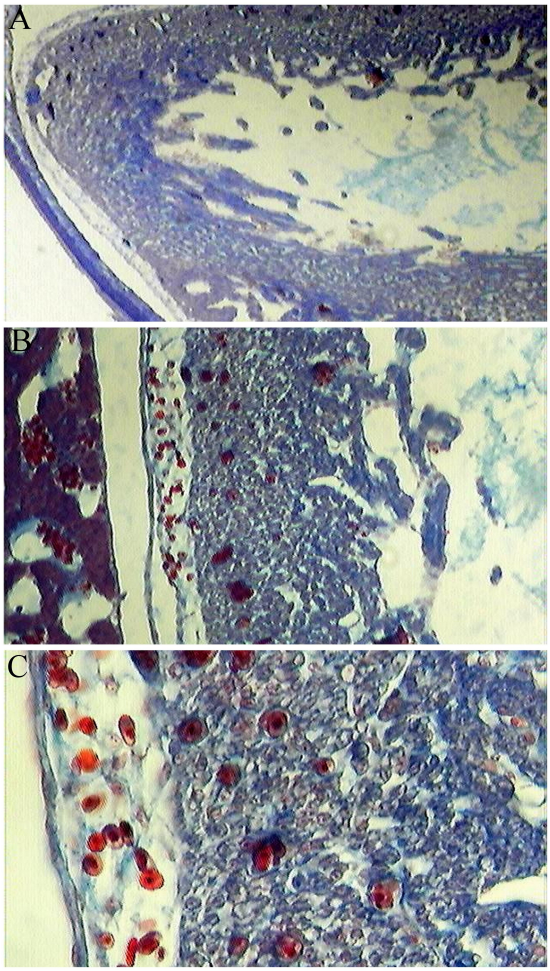

Figure 2. General appearance of multiple loci of the primary primitive erythropoiesis in the pericardial region of the 25 days pig embryo. Staining is by azan according to Mallory. Magnification used – A-40x; B-100x; C-250x.

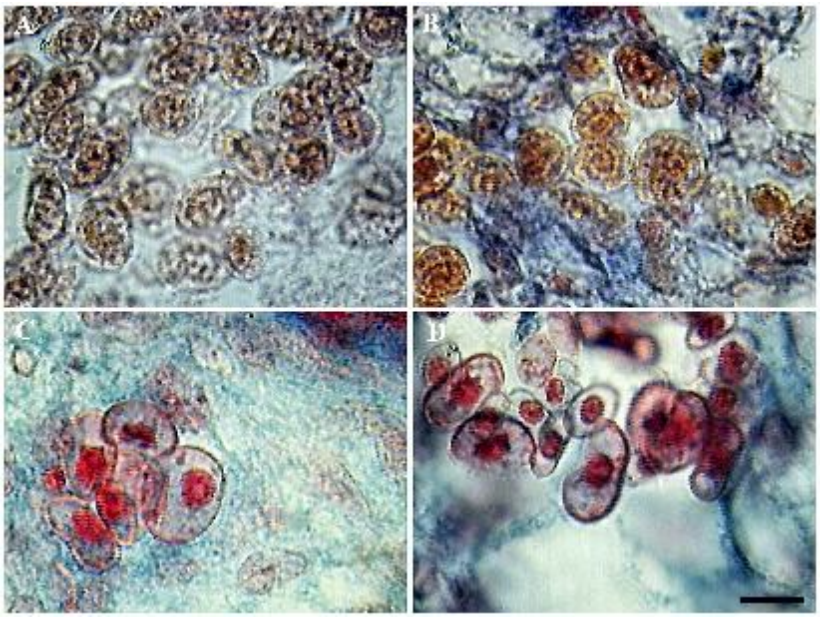

Figure 3. The cells of the primary primitive erythropoiesis in the pericardial region of the 25 days pig embryo. A, B - staining is by hematoxilin-eosin according to Karachi, C, D - staining is by azan.

At the Figure 1 the mesodermal bloody islet of the 15th day pig embryo is represented. In its structure the erythroid cells of blood are well distinguished, which are accepted to be named as hemangioblasts. Almost they all contain nuclei and are at different stages of differentiation. Amongst them both the low-differentiated and the more advanced in differentiation blasted forms, as well as the nucleated forms of primitive erythrocytes are available. Their population content is represented in the following manner (Table 1): on the 15th day of embryo's development an overwhelming majority (approximately 63%) is made up by hemangioblasts and the rest part (approximately 37%) is represented by the primary erythrocytes. Already by the 25th and the 35th days the hemangioblasts' number is gradually decreasing and their moiety is not attaining 50% of the population by the 35th day of the embryo's development. Herein, the number of megaloblasts decreases more than two times and the number of small erythroblasts increases many times. At the same time amount of erythrocytes gradually enhances and by the 35th day makes up approximately 50% of erythroid cells whole number in the mesenchymal islets. Herein, it is important to note, that along the embryo's age increase the same tendency of increasing of the small erythrocytes number is observed, what is probably connected with attenuation of the angioblastic period of hemopoiesis along the embryo's development process.

The performed investigation of the pig embryos erythroid cells' dimensional indices on the 15th, 25th and the 35th days of development have revealed, that as the mean values of the erythroblasts' square areas, as well as the dimensions of the small and the big erythroblasts on the 15th and 25th days authentically differed from each other, whereas the differences between their dimensions on the 25th and the 35th days were registered to be as not authentic. The analogical data were obtained by us regarding erythrocytes (Table 1). It is also noteworthy, that the nuclear-plasma volume relations in all the types of erythroid cell are decreasing with age. Anyway, in this case in small erythroblasts and erythrocytes on the 15th and the 25th days of embryo's development this relation attains 0.5, whereas in their big analogues it does not prevail 0.4. But on the 35th days the nuclear-plasma volume relationship drops, making up 0.3.

Table 1

Changes in the population content and the square area of nucleus, cytoplasm and cells of erythroblasts and erythrocytes of the 15th, 25th and the 35th day of the pig embryos

Type of cells	*%*	*Square area in μm²*		
		Cell	*Nucleus*	*Cytoplasm*
		15th day		
Erythroblast				
Small	5.0	54.6±0.9	18.0±1.6	36.6±1.2
Large	58.0	83.3±1.7	22.9±0.5	60.4±1.5
Total	63.0	80.8±1.7	22.9±0.4	58.3±1.5
Erythrocyte				
Small	9.0	54.1±1.2	15.2±0.7	38.9±0.8
Large	28.0	86.1±2.3	23.2±0.6	62.9±2.1
Total	37.0	82.6±2.3	22.4±0.6	60.2±2.0
		25th day		
Erythroblast				
Small	32.0	36.5±2.0	12.3±0.6	24.2±1.8
Large	26.0	59.8±1.4	16.8±0.9	43.1±1.5
Total	58.0	48.4±2.2	14.6±0.7	32.8±1.9
Erythrocyte				
Small	32.0	35.8±1.7	11.3±0.6	24.5±1.5
Large	10.0	60.9±2.5	17.8±1.9	41.1±4.1
Total	42.0	41.8±2.2	13.3±0.9	28.5±1.8
		35th day		
Erythroblast				
Small	22.0	36.1±2.0	10.9±0.5	25.2±2.0
Large	26.0	62.1±7.5	14.3±0.6	47.8±7.3
Total	48.0	49.1±4.6	12.6±0.6	36.5±4.0
Erythrocyte				
Small	36.0	30.0±4.6	8.2±0.8	21.8±3.8
Large	16.0	61.7±5.3	11.7±0.7	50.0±5.8
Total	52.0	45.8±4.6	10.0±0.6	35.9±4.2

The data obtained by us regarding to the dynamics of changes of the hemoglobin content in mesenchymal erythroid cells at the different stages of the pig embryos' development (Table 2) have revealed, that the maximal content of hemoglobin was registered on the 15th day of embryogenesis, when the mesenchymal erythropoiesis is being the only way of hemopoiesis, whereas already on the 25th and the 35th days of pregnancy the hemoglobin content was authentically decreased, probably, because of its attenuation in connection with a dramatical minimization of its significance during the process of embryogenesis. Along with the embryo's age further increase the dynamics of hemoglobin content changes in the cells of blood does not display such an abrupt tendency to deceleration.

Table 2

Content of hemoglobin in the mesenchymal erythroblasts and erythrocytes of the pig embryos on the 15th, 25th and the 35th days of pregnancy (pk/g)

Type of cells	*N*	*Cell*
	15th day	
Erythroblast		
Small	5.0	34.8±0.9
Large	58.0	53.1±1.2
Total	63.0	51.5±1.7
Erythrocyte		
Small	9.0	34.5±1.2
Large	28.0	54.8±2.3
Total	37.0	52.6±2.3
	25th day	
Erythroblast		
Small	32.0	23.2±2.0
Large	26.0	30.1±1.4
Total	58.0	30.8±2.2
Erythrocyte		
Small	32.0	22.8±1.7
Large	10.0	38.8±2.5
Total	42.0	26.6±2.2
	35th day	
Erythroblast		
Small	22.0	23.0±2.0
Large	26.0	39.6±7.5
Total	48.0	31.3±4.6
Erythrocyte		
Small	26.0	19.1±4.6
Large	26.0	39.3±5.3
Total	52.0	29.2±4.6

Figure 4 highlights the last loci of the primary primitive erythropoiesis in the pericardial region of the 35 days pig embryo.

The performed investigation of the mesenchymal erythroid cells nuclei distribution according to the ploidity classes (Figure 5) have revealed a presence of the hyperdiploid and even tetraploid cells on the 15th day of embryo's development and the hyperdiploid ones on the 25th day of embryo's development. At the later stages of embryogenesis the main mass of cells is represented by the diploid population.

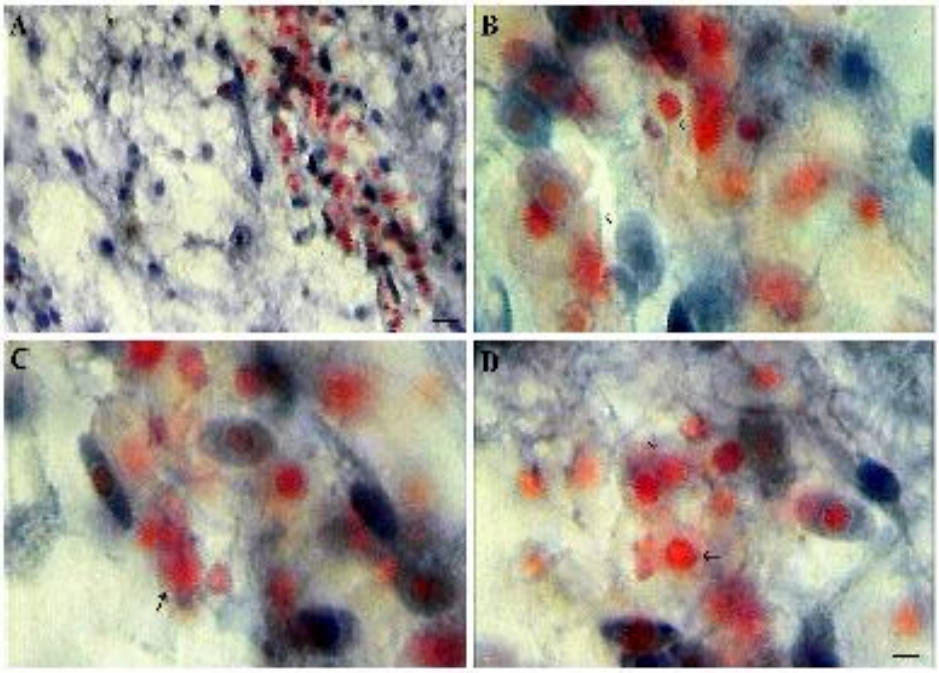

Figure 4. The last loci of the primary primitive erythropoiesis in the pericardial region of the 35 days pig embryo. Staining is by azan according to Mallory. The scale used – A - 40 μm; B, C, D - 10 μm.

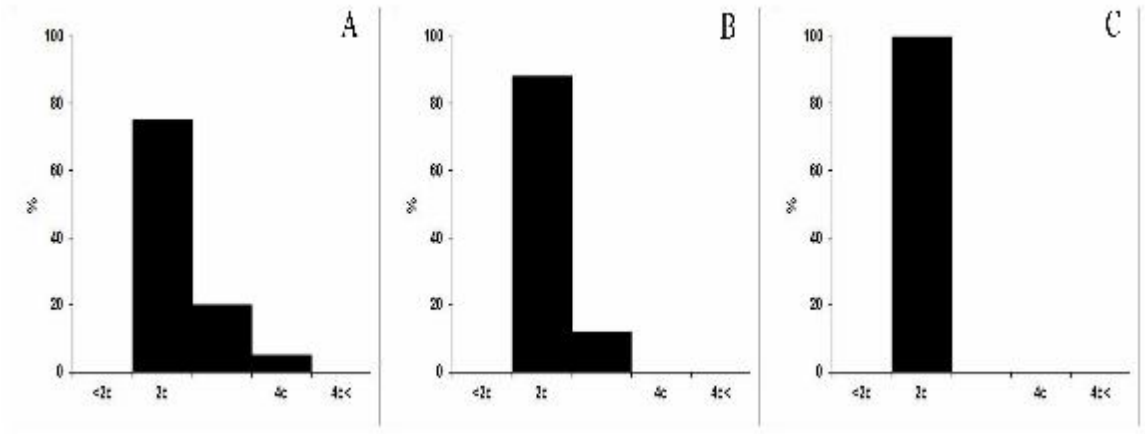

Figure 5. The mesenchymal erythroid cells nuclei distribution according to the ploidity classes. A – the 15th day of embryo's development; B - the 25th day of embryo's development; C - the 35th day of embryo's development.

Conclusions. In distinct from the widely accepted depiction of the blood islets formation process, during which the primitive erythropoiesis is coming raise in intravenous way in the yolk sack, our data obtained showed that in the pigs it arises in extra-vascular way. Consequently, we could confirm the hypothesis of that, during the process of the mammals' embryogenesis the hemopoietic cells' intra-embryonic localization zone formation is taking place first. The mesenchymal erythroid cells in the pig embryo come appear on the 13-15 days of the embryo's development and disappear fully by the 35-37 days of it. The data obtained by us regarding to the nuclear-plasma volume relations declination till 0.3 by the 35th day of embryogenesis are connected with a decrease of the cells' nuclei dimension, what indirectly testifies about the proliferative activity's attenuation in erythroid cells of the given region of hemopoesis. These data got are in

high correlation with the results of investigation of DNA content in the nuclei of erythroid population of the mesenchymal islets, which in turn testify about the fact, that the whole population of erythroid cells is diploid by the 35 days of embryogenesis. The mentioned fact, most likely, could be explained by the almost complete disappearance of this locus of embryonic hemopoiesis by the 35th day of embryogenesis.

References

Baron M. H., Vacaru A., Nieves J., 2013 Erythroid development in the mammalian embryo. Blood Cells Mol Dis 51(4):213-219.

Emura I., Sekiya M., Ohnishi Y., 1983 Two types of immature erythrocytic series in the human fetal liver. Arch Histol Jpn 46(5):631-43.

Ferkowicz M. J., Yoder M. C., 2005 Blood island formation: longstanding blood island formation: observations and modern interpretations. Experimental Haemotology 33(9):1041-1047.

Ferkowicz M. J., Starr M., Xie X., Li W., Johnson S. A., Shelley W. C., Morrison P. R., Yoder M. C., 2003 CD41 expression defines the onset of primitive and definitive hematopoiesis in the murine embryo. Development 130(18):4393–4403.

Ghatpande S., Ghatpande A., Sher J., Zile M. H., Evans T., 2002 Retinoid signaling regulates primitive (yolk sac) hematopoiesis. Blood 99(7):2379-2386.

Godin I., Cumano A., 2005 Of birds and mice: hematopoietic stem cell development. Int J Dev Biol 49(2-3):251-257.

Gray P., 1954 The microtomist's formulary and guide. Blakiston, New York.

Huber T. L., Kouskoff V., Fehling H. J., Palis J., Keller G., 2004 Haemangioblast commitment is initiated in the primitive streak of the mouse embryo. Nature 432(7017):625–630.

Isern J., Fraser S. T., He Z., Baron M. H., 2008 The fetal liver is a niche for maturation of primitive erythroid cells. Proc Natl Acad Sci USA 105(18):6662-6667.

Jaffredo T., Bollerot K., Sugiyama D., Gautier R., Drevon C., 2005 Tracing the hemangioblast during embryogenesis: developmental relationships between endothelial and hematopoietic cells. Int J Dev Biol 49(2-3):269-277.

Kritzenberger M., Wrobel K. H., 2010 Role of the mesonephros as a transient haematopoietic organ in the bovine embryo. Anat Histol Embryol 39(6):534-545.

Lee K. Y., Fong B. S., Tsang K. S., Lau T. K., Ng P. C., Lam A. C., Chan K. Y., Wang C. C., Kung H. F., Li C. K., Li K., 2011 Fetal stromal niches enhance human embryonic stem cell-derived hematopoietic differentiation and globin switch. Stem Cells Dev 20(1):31-38.

Lillie R. D., 1965 Histopathologic technical and practical histochemistry. 3rd edition, McGraw-Hill, NY.

Sheng G., 2010 Primitive and definitive erythropoiesis in the yolk sac: a bird's eye view. Int J Dev Biol 54(6-7):1033-1043.

Tada T., Widayati D. T., Fukuta K., 2006 Morphological study of the transition of haematopoietic sites in the developing mouse during the peri-natal period. Anat Histol Embryol 35(4):235-240.

Tanaka S., Fujimoto J., Ishii E., Hata J., 1989 Human yolk-sac tumor antigen 2G10: biochemical characterization and significance as a serum antigen. Int J Cancer 44(5):788-794.

Zambidis E. T., Peault B., Park T. S., Bunz F., Civin C. I., 2005 Hematopoietic differentiation of human embryonic stem cells progresses through sequential hematoendothelial, primitive, and definitive stages resembling human yolk sac development. Blood 6(3):860-870.

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