Diabetes affects rat visceral yolk sac cells viability and cell markers expression

Marlúcia B. Aires

Department of Morphology, Federal University of Sergipe, Sao Cristovao, Sergipe, Brazil

Correspondence: Marlúcia Bastos Aires
E-mail: marlucia_aires@yahoo.com.br
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Rodents have a well-developed visceral yolk sac (VYS) that acts as an active region for metabolic exchange and nutrition uptake until final fetal development in rats and may be affected by diabetes. Using Wistar rats, diabetes was induced by a single injection of alloxan at gestational day 8 (8 gd) and at 15 gd the collection of VYS was made. Flow cytometry was performed for VYS cell characterization, determination of mitochondrial activity, cell proliferation, DNA ploidy, cell cycle phases and caspase-3 activity. Fetal weight was reduced in the diabetic group. CD34, CCR2, and OCT3/4 expressions were significantly reduced, and CD90, CD117, and CD14 expressions were increased in the diabetic group. VYS cells with inactive mitochondria, activated caspase-3, and low proliferation were present in the diabetic condition. Severe hyperglycemia due to maternal diabetes was shown to have negative effects on pregnancy, VYS viability, and cell marker expression.

Keywords: visceral yolk sac; diabetes; embryo; pregnancy; cell markers

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Introduction

Diabetes is characterized by hyperglycemia as a result of problems in insulin production, insulin function, or both. Projections by the World Health Organization (WHO) suggest that the number of people with diabetes may reach 366 million people in 2030 [1]. The occurrence of diabetes during pregnancy may be classified into clinical diabetes, in cases previously diagnosed with type 1 or type 2 diabetes and gestational diabetes [2].

Maternal diabetes is a predisposing factor for embryonic lethality, congenital abnormalities and placental defects [3, 4, 5]. In rodents, the visceral yolk sac (VYS) acts as an active region for metabolic exchange and nutrition uptake [6, 7, 8], becoming an important tissue that persists active during the days following the placentation period from gestational day 12 (12 gd) onwards [9]. Recently, Aires et al. [10] expanded these studies by showing the effect of severe hyperglycemia induced by alloxan on pregnancy, VYS cell viability and cell marker expression at 15 gd in rats.

Diabetes induced by alloxan results in maternal severe hyperglycemia e reduced fetal weight

Diabetes in animals can be induced by partial pancreatectomy or by the administration of diabetogenic drugs such as alloxan that selectively destroy the pancreatic beta-cells. Experimentally-induced diabetes in pregnant animals, especially rodents, results in similar abnormalities to those observed in humans [11, 12]. In Aires et al. [10] study, diabetes was induced by one alloxan injection at gestational day 8 (8 gd) and at 15 gd the collection of fetuses and VYS was made. The pregnant rats showed marked hyperglycemia and reduced fetal weight, which is a common finding in severe diabetes [13]. Reduced fetal weight and size is
frequently observed in the severe diabetic condition [14] and could be related to yolk sac anomalies in rodents and humans [12, 15].

Diabetes affects the expression of cell markers by VYS cells

The yolk sac is considered the earliest site of hematopoiesis in mammals [16]. Moreover, the yolk sac is a source of mesenchymal stem cells (MSCs), which can differentiate into osteoblasts, chondrocytes and adipocytes in mice [17] and adipocytes and neurons in humans [18, 19]. For this reason, the VYS function seems to be critical during organogenesis until final fetal development in rats and could also be an important source of MSCs.

Aires et al. [10] showed the expression of stromal (CD90, CD44) and pluripotency (STRO-1, OCT3/4, NANOG) markers on VYS cells in both the control and diabetic groups confirming the existence of MSC in the rat VYS. However in diabetes, the functional significance of the increased CD90 expression and reduced OCT3/4 expression by VYS cells is not fully understood but could influence the mesenchymal niche and the cell differentiation. In fact, OCT3/4 and NANOG are considered crucial for stem cell pluripotency and are usually expressed by MSC [20]. Possibly, the hyperglycemic environment could reduce the pluripotency capacity of MSC in the yolk sac to some extent, but further studies are necessary to confirm this.

VYS cells from control and diabetic groups stained with hematopoietic (CD34, VEGFR1, CD117) and monocyte/macrophage (CD115, CD14, CCR2) markers. The immuno-positivity for CD117, VEGFR1 and CD34, markers for hematopoietic cell precursors, suggests that VYS cell at 15 gd retains the potential to generate hematopoietic lineages. In the yolk sac, primitive hematopoiesis is restricted to primitive erythroid, megakaryocyte and macrophage lineages [21] and, until 14 gd, yolk sac macrophages have features of active phagocytosis [22]. Besides the unaltered CD115 expression, the expression of CD14 and CCR2 was increased and decreased, respectively, suggesting that the hyperglycemic condition could influence cell differentiation of myeloid lineages.

Diabetes affects VYS cell viability, cell proliferation, and apoptosis

An important work showed that VYS mitochondrial function is maintained in normal pregnancy, even after the establishment of the chorioallantoic circulation (12 gd) [9]; therefore, the reduction of VYS mitochondrial activity in diabetes may be harmful for embryo nutrition. Aires et al. [10] showed the expression an increased number of VYS cells with inactive mitochondria (unviable cells) in the diabetic condition, indicating a reduction of mitochondrial transmembrane potential. An increase in sub-haploid VYS cells in the diabetic group corresponds to apoptotic cells with fragmented DNA or condensed chromatin [23], as evidenced by the significant amount of activated caspase-3 cells in that group. Moreover, VYS cells showed reduced proliferation index at 15 gd in diabetes. Therefore, the reduction of cellular viability, increased activated caspase-3 cells and low cell proliferation confirms the deleterious effects of diabetes for the VYS physiology and consequently for embryo/fetus development, even in late pregnancy.

Conclusion

In summary, in the model used by Aires et al., [10] the alloxan was administered at 8 gd and after seven days, at 15 gd, the VYS were collected; thus, the reduction of cellular viability, increased activated caspase-3 cells and low cell proliferation confirms the deleterious effects of diabetes for the VYS physiology and consequently for embryo/fetus development, even in late pregnancy. More detailed studies are necessary to elucidate the cellular and molecular events underlying yolk sac development and function during maternal diabetes.

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References


