Immunohistochemical and morphometric study of the development of fetal and newborn rat pancreatic islets

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ABSTRACT

Objectives: The aim of this study is to perform a detailed morphometric immunohistochemical study of the development of fetal and newborn rat pancreatic islets.

Methods: Twenty-four pancreases were obtained from 19 and 21-day-old fetal rats, one and 4-day-old newborn rats. They were fixed in buffered neutral formalin, dehydrated and embedded in paraplast. Sections were stained with anti-insulin antibodies. A morphometric study was performed on the pancreatic islets at the Department of Anatomy, King Abdul-Aziz University, Jeddah, Kingdom of Saudi Arabia, between 2001 and 2002.

Results: The volume density of B cells showed a gradual increase during the last days of gestation and a slight increase during the first 4 days after birth. All the other morphometric parameters showed a gradual increase during the last days of gestation and during the first days after birth. The B cell nuclear diameter and volume showed a slight increase after birth. The B cells were well stained and present in the central part of fetal and newborn islets, while, the other islet cells were present in the periphery of the islets.

Conclusion: The size of the endocrine tissue, which was represented by the islet diameter, islet volume, islet volume density, total number of islet cells, number of B cells and volume density of B cells showed a progressive increase during the perinatal period.

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The pancreas develops from the primitive epithelium of the foregut as dorsal and ventral primordia. Initial epithelial buds are formed on the 12 day of gestation. The epithelium starts to elongate and branch on 13-14 days. The tubular structure appears on the 15-16 days, while the acinar structure appears on 17 day or more.[1,2] The beta cells (B cells) are found between 12 and 14 days of gestation.[3,4] The fetal rat endocrine pancreas has been used as an animal model to study the effect of maternal diseases and different factors on its development. Despite the extensive studies on the development of fetal rat endocrine pancreas, a detailed quantitative immunohistochemical study is not available. The use of immunohistochemical stain has permitted specific and clear localization of islet B cells, which contain insulin granules. The application of immunohistochemical technique in this study would allow the performance of accurate measurement of the different morphometric parameters. This investigation was therefore design to study the fetal and newborn islet volume density, diameter, cell number and B cell pattern, namely the number, volume density and distribution at 19 and 21 days of gestation and at one

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and 4 days after birth. Fetal and newborn nuclear diameter and volume were also measured.

Methods. ***Animal and tissue preparation.*** Rat fetuses of 19 and 21 days fetal age obtained from normoglycemic pregnant rats were used. Newborn rats at one and 4 days after birth were also studied. Non-fasting blood glucose concentrations were measured to confirm that the animals were normoglycemic. Pregnant rats were anesthetized by ether inhalation and the fetuses were removed from their uteruses. The pancreases were fixed in buffered neutral formalin, dehydrated, embedded in paraplast (Sherwood Medical Co, St. Louis, MO, United States of America [USA]) and serially sectioned (5 µm). Six pancreases of each fetal and newborn age were examined, with a total of 24 pancreases. This study was carried out at the Department of Anatomy, King Abdul-Aziz University, Jeddah, Kingdom of Saudi Arabia, between 2001 and 2002.

Immunohistochemical staining. Four sections, 20 sections apart were obtained from each pancreatic specimen. The sections were stained by indirect immunoperoxidase method[5] to localize the insulin-producing B cells. The primary antibody used was guinea pig anti-swine insulin serum (optimal dilution 1:500). The secondary antibody used was rabbit anti-guinea pig immunoglobulin conjugated with peroxidase (dilution 1:200). All sera and antisera were obtained from Dako Corporation, Carpinteria, CA, USA. The chromogen substrate used was 3, 3-Diaminobenzidine tetrahydrochloride (Sigma, St. Louis, Mo, USA). The sections were counterstained with Harris' hematoxylin to facilitate nuclear identification.

Morphometric analysis. Thirty-two to 48 islets (8-12 per section) were examined for each fetus and newborn. The point counting method of Weibel[6] was used to calculate the volume density of the islets per pancreatic tissue (Vvi) and the volume density of stained B cells per islet (Vvb). The sections were examined at a magnification of x 400 to estimate Vvi and at a magnification of x 1000 to estimate Vvb. The number of stained B cell nuclei per islet profile was divided by the mean islet area to estimate the numerical density of stained B cells per islet profile (NAb, no./µm^2). The nuclei were counted by direct counting method at a magnification of x 1000. The numerical density of stained B cells per unit volume of islet (Nvb, no./µm^3) was calculated by a variant of DeHoff and Rhines formula:[7]

$$N_{vb} = \frac{N_{Ab}}{Dn + t}$$

Where NAb represents the number of nuclear profile per unit area estimated in sections of thickness (t) and Dn represents the mean corrected nuclear diameter. The Nvb, no./µm^3 was multiplied by islet volume to estimate the absolute number of B cells per islet. Similar steps were used to estimate the absolute number of total cells per islet. The major (a) and minor (b), at right angle to (a), axes of the islet were measured by a graticule of a calibrated linear scale. The equation di = 2 ÷ ab 7 was used to estimate the islet profile diameter. The mean axial ratio of the islets was calculated. Assuming that the islets are spheroid structures, the formula of Fullman 7 was used to calculate the mean islet diameter (Di).

$$Di = \frac{\pi}{2} \times \frac{N}{1/d1 + 1/d2 - 1/dN}$$

Where N represents the total profiles measured. Similar steps were used to estimate the B cell nuclear profile diameter (dn). Fifty nuclei were measured for each slide at a magnification of x 1000. The corrected mean Dn was estimated by the Abercrombic method[7] (Dn = dn x 4/ π). The equation (V = 4 π/3 x (D/2)^3 was used to calculate the mean islet volume and the mean B cell nuclear volume from the mean corrected Di and mean corrected Dn.[7]

Statistical analysis. The data was analyzed statistically by one way ANOVA. All the statistical computations were made using the Statistical Packages for Social Sciences and Excel. The difference was considered as significance when $p < 0.05$.

Results. The mean islet axial ratio of 19 was 1.23 ± 0.0439 and the 21 day old fetuses was 1.21 ± 0.0266, while, the mean islet axial ratio of one was 1.18 ± 0.0378 and the 4-day-old newborns was 1.20 ± 0.0442, indicating that the islets could be considered as spheroid structures. The mean Vvi, the mean Vvb, the mean Di, the mean islet volume, the mean absolute number of stained B cells per islet and the mean absolute number of total cells per islet of 19 and 21 day old fetuses are demonstrated in **Table 1**. **Table 2** shows the mean values for one and 4-day-old newborns. The mean volume density of the B cells increased gradually during the last days of gestation and then showed a slight increase over the first 4 days after birth (**Table 1 & 2**). All the other parameters showed a gradual increase during the last days of gestation and during the first 4 days after birth. Analysis of variance test showed that all these parameters were significantly different between the 4 groups ($p<0.05$). The mean B cell Dn and volume of 19 and 21-day-old fetuses are shown in **Table 3**. **Table 4** shows the mean B cell Dn and volume of one and 4-day-old newborns. At 19 and 21 days of gestation (**Figure 1 & 2**) and at one and 4 days after birth (**Figure 3 & 4**), the islets were nearly rounded, well defined, and the B cells were present in the central part of the islet.

Discussion. The results found in this study showed that the volume density of B cells increased gradually during the last days of gestations. This increase in B cell volume density may be due to the rapid increase in B

Table 1 - Volume density of the islets per pancreatic tissue, volume density of B cells per islet, islet diameter, islet volume, absolute number of stained B cells per islet and absolute number of total cells per islet of 19 and 21 day old fetal rats (N = 6)*.

Parameter	19-day-old fetuses	21-day-old fetuses
Vvi	0.0396 ± 0.00379	0.0502 ± 0.00534
Vvb	0.382 ± 0.0309	0.534 ± 0.0363
Islet diameter (µm)	41 ± 2.31	69.7 ± 4.8
Islet volume (µm³)	39041 ± 5524	191057 ± 36598
Stained B cells/islet	36 ± 2.67	156 ± 7.68
Total cells/islet	111 ± 11.5	316 ± 27.7

*values are presented as mean ± SEM,
Vvi - volume density of the islets per pancreatic tissue
Vvb - volume density of B cells per islet, B cells - beta cells

Table 2 - Volume density of the islets per pancreatic tissue, volume density of B cells per islet, islet diameter, islet volume, absolute number of stained B cells per islet and absolute number of total cells per islet of one and 4 day old newborn rats (N = 6)*.

Parameter	one-day-old newborns	4-day-old newborns
Vvi	0.0710 ± 0.00574	0. 0.117 ± 0.0116
Vvb	0.536 ± 0.026	0.583 ± 0.0272
Islet diameter (µm)	90.8 ± 3.9	108 ± 1.09
Islet volume (µm³)	437075 ± 31763	660773 ± 21084
Stained B cells/islet	236 ± 39.8	392 ± 10.5
Total cells/islet	467 ± 63.8	769 ± 28.6

*values are presented as mean ± SEM,
Vvi - volume density of the islets per pancreatic tissue,
Vvb - volume density of B cells per islet, B cells - beta cells

Table 3 - B cell nuclear diameter and volume of 19 and 21 day old fetal rats (N = 6)*.

Parameter	19-day-old fetuses	21-day-old fetuses
Nuclear diameter (µm)	6.87 ± 0.162	6.78 ± 0.250
Nuclear volume (µm³)	168 ± 10.8	165 ± 17.4
Nuclear axial ratio	1.16 ± 0.0321	1.09 ± 0.035

*values are presented as mean ± SEM

cell mass and insulin content of the pancreatic islets. From the first to the 4th postnatal day the volume density of B cells showed a slight increase. These findings are in agreement with those of Freie et al[8] Aerts and Van Assche,[9] McEvoy and Madson,[10] and Noda.[11] The Vvi, which reflects the amount of endocrine tissue inside the pancreas showed a gradual increase during the last days of pregnancy and during the first 4 days after birth. This indicates that the endocrine tissue expands rapidly during the perinatal period. A similar finding was reported by Freie et al,[8] Aerts and Van Assche,[9] and Wang et al.[12] The rat fetal endocrine pancreas starts to appear early during the gestational period. Gomez Dumm et al[3] reported that alpha cells (A cells) appear on the 12 day of gestation, B cells appear on the 14 day and PP cells appear on the 19 day. McEvoy and Madson[10] demonstrated that A and B cells increased rapidly during the last days of gestation. Noda[11] reported that alpha and B cells were detected on the 16 day of pregnancy. The A cells were more numerous than B cells and the volume density of B cells increased rapidly during the following fetal period. He also reported that the insulin level increased rapidly during the same period. Glucagon secreting cells (D cells) and B cells were detected in the pancreatic primordium on day 12 of gestation.[4] In human at early stage of development, the pancreas is formed of an epithelial mass contains central ducts surrounded by dense mesenchyme. The B, D and A cells start to appear within the central ducts of the epithelial mass at 8 week of development.[13] The pancreatic islets develop as buds from the ducts. The islet buds were detached from the duct on weeks 17-20 to form separate islets containing B and other endocrine cells.[14] In the present study, the islet diameter and volume showed a gradual increase during the last days of gestation and the first 4 days after birth. In consistent with this finding, the absolute number of B cells per islet and the absolute number of total cells per islet showed a similar increase. These findings indicate a rapid increase of the endocrine tissue during the perinatal period. This increase in the size of the endocrine tissue may explain the rapid increase in pancreatic insulin content during the last days of gestation and the first days after birth.[8,11,12] The result reported in this study showed that the nuclear diameter and volume of B cells did not increase from 19-21 days of gestation. This may indicate a similarity of the B cells size at 19 and 21 days of gestation. The nuclear diameter and volume increased slightly during the following days of gestation and the first 4 days after birth. This may be due to the increase in the size of B cells. The result demonstrated that the B cells were well stained and occupied the central part of the islets, while, the other islet cells were present at the periphery of the islet. This islet cellular pattern is similar to that of the adult pancreatic islets.[15,16]

In conclusion, the data reported in this study showed that B cell volume density increased gradually during the last period of gestation and showed a slight increase during the first 4 days after birth. The amount of

Table 4 - B cell nuclear diameter and volume of one and 4 day old newborn rats (N=6)*.

Parameter	one-day-old newborns	4-day-old newborns
Nuclear diameter (μm)	7.00 ± 0.105	7.51 ± 0.146
Nuclear volume (μm^3)	192 ± 15.7	222 ± 12.7
Nuclear axial ratio	1.24 ± 0.0759	1.15 ± 0.0558

*values are presented as mean ± SEM

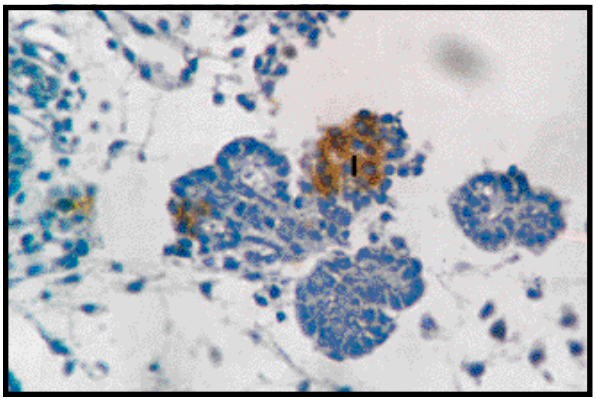

Figure 1 - Light micrograph of a 19-day-old fetal rat pancreatic islet. The islet is stained with indirect immunoperoxidase method to demonstrate islet B cells x 200. I - pancreatic islet.

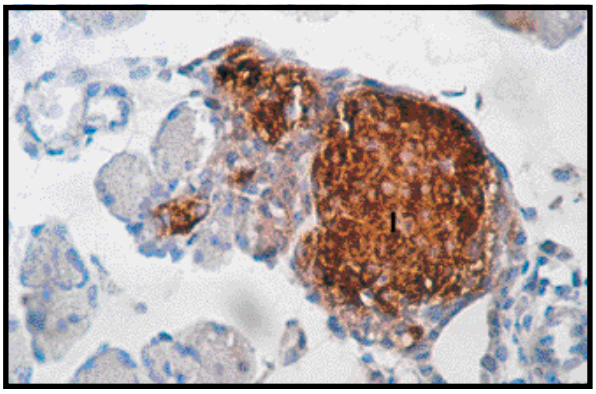

Figure 3 - Light micrograph of one-day-old newborn rat pancreatic islet. The islet is stained with indirect immunoperoxidase method to demonstrate islet B cells x 200. I - pancreatic islet.

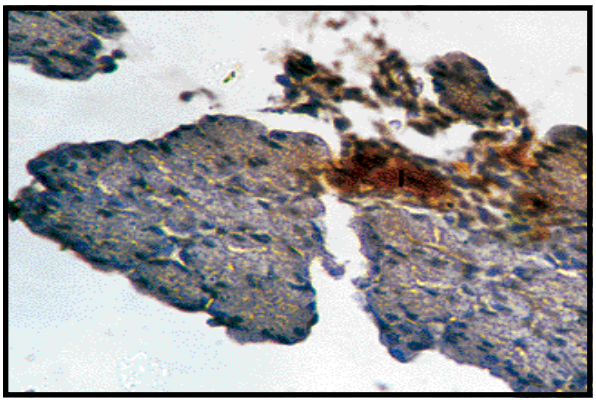

Figure 2 - Light micrograph of 21-day-old fetal rat pancreatic islet. The islet is stained with indirect immunoperoxidase method to demonstrate islet B cells x 200. I - pancreatic islet.

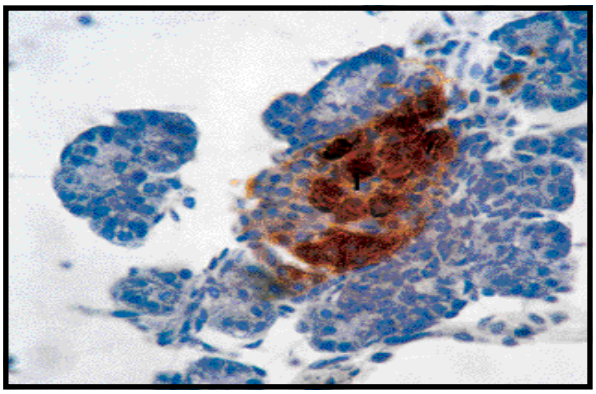

Figure 4 - Light micrograph of 4-day-old newborn rat pancreatic islet. The islet is stained with indirect immunoperoxidase method to demonstrate islet B cells x 200. I - pancreatic islet.

endocrine tissue represented by the islet diameter, the islet volume, the islet volume density, the number of B cells per islet, and the total number of cells per islet showed a gradual increase during the last days of gestation and continued to increase during the first days after birth. The B cell nuclear diameter and volume showed a slight increase after the 21 day of gestation and during the first days after birth.

References

1. Hisaoka M, Haratake J, Yamamoto O. Morphological development of the rat fetal pancreas. ***Journal of University of Occupational and Environmental Health*** 1992; 14: 1-12.
2. Hisaoka M, Haratake J, Hashimoto H. Pancreatic morphogenesis and extracellular matrix organization during rat development. ***Differentiation*** 1993; 53: 163-172.
3. Gomez Dumm C, Galeano A, Genoro S, Gagliardino J. Appearance of immunoreactive endocrine cells during the development of the rat pancreas, with special reference to polypeptide-secreting cells. ***Acta Anat (Basel)*** 1987; 130: 158-162.
4. Park I, Bendayan M. Development of the endocrine cells in the rat pancreatic and bile duct system. ***Histochem J*** 1993; 25: 807-820.
5. Sternberger L. The unlabelled antibody peroxidase-antiperoxidase (PAP) method. In: Cohen S, McClusky RT, editors. Immunocytochemistry. 2nd ed. New York (NY): John Wiley and Sons, 1979; 104-169.
6. Weibel E. Principles and methods for the morphometric study of the lung and other organs. ***Lab Invest*** 1963; 12: 131-155.
7. Williams M. Quantitative methods in Biology. In: Glauert AM, editor. Practical methods in electron microscopy. 1st ed. Amsterdam: North-Holland Publishing Company; 1977. p. 48-62.
8. Freie H, Pasma A, Bouman P. Quantitave analysis of pancreatic islet development and insulin storage in the fetal and newborn rat. ***Acta Endocrinol*** 1975; 80: 657-666.
9. Aerts L, Van Assche F. Rat foetal endocrine pancreas in experimental diabetes. ***J Endocrinol*** 1977; 73: 339-346.
10. McEvoy R, Madson K. Pancreatic insulin, glucagon, and somatostatin positive islet cell populations during the perinatal development of the rat. II. Changes in hormone content and concentration. ***Biol Neonate*** 1980; 38: 255-259.
11. Noda K. Studies on developmental changes in rat pancreatic endocrine system during perinatal period. ***Nippon Sanka Fujinka Gakkai Zasshi*** 1989; 41: 441-448.
12. Wang R, Bouwens L, Kloppel G. Beta-cell proliferation in normal and streptozotocin-treated newborn rats: site, dynamics and capacity. ***Diabetologia*** 1994; 37: 1088-1096.
13. Polak M, Bouchareb-Banaei L, Scharfmann R, Czernichow P. Early pattern of differentiation in the human pancreas. ***Diabetes*** 2000; 49:225-232.
14. Hahn von Dorsche H, Reiher H, Hahn H. Phases in the early development of the human islet organ. ***Anat Anz*** 1988; 166: 69-76.
15. Baetens D, Malaisse-Lagae F, Perrelet A, Orci L. Endocrine pancrea: three-dimensional reconstruction shows two types of islet of Langerhans. ***Science*** 1979; 206: 1323-1325.
16. Bani Sacchi T, Bani T. New views on the identification of the various cell types in the pancreatic islet of the rat. An ultrastructural and morphometrical study. ***Acta Anatomica*** 1985; 122: 1-17.