Development of fetal rat pancreatic islet A cells

A quantitative and immunocytochemical study

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ABSTRACT

Objective: To perform a detailed quantitative immunocytochemical study of the development of fetal rat pancreatic islet A cells.

Methods: Pancreases were obtained from 19 and 21-day-old fetal rats. Ten rats were used per each group. Non-fasting blood glucose levels were measured to confirm that the animals were normoglycemic. The pregnant rats were anesthetized by ether inhalation, and the fetuses were removed from their uteruses. They were fixed in buffered neutral formalin, dehydrated and embedded in paraplast and serially sectioned (5 μm). We examined 32-48 islets (8-12 per section) for each fetus. Sections were stained by avidin biotin complex technique. A quantitative study was performed on the pancreatic islet A cells. Carl Zeiss software from Zeiss was used in this study. This study was carried out at the Department of Anatomy, King Abdul-Aziz University, Jeddah, Kingdom of Saudi Arabia during the period January to December 2005.

Results: The volume density and the number of A cells showed a significant increase during the last days of gestation. All other parameters showed a significant increase during the last days of gestation. The A cell nuclear diameter and volume did not increase significantly during the last days of pregnancy. The A cells were well stained and occupied the peripheral part of the islets.

Conclusion: The present study represented a detailed quantitative immunohistochemical study and demonstrated that the size of the endocrine tissue and the islet A cells increased significantly during the last days of gestation.

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The glucagon producing A cells start to appear in the endocrine pancreas on the 12 day of pregnancy in rats, the B cells appear on the 14 day and the PP cells appear on the 19 day.[1,2] The endocrine and exocrine parts of the pancreas develop from the foregut ectodermal progenitors as initial epithelial buds[3-6] and then the islets are formed from differentiating epithelial stem cells.[7] The A and B cells develop in the pancreatic primordium from day 12 of gestation.[2]

The endocrine fetal pancreas of the rat has been used as an animal model to investigate the effect of various factors and different maternal diseases on the pancreatic development. Fetuses of obese mothers were reported to have blood insulin level higher than that of fetuses of non-obese mothers.[8] Maternal diabetes can lead to fetal islet hypertrophy and predispose to diabetes.[9]

However, a detailed quantitative immunocytochemical study of the development of the glucagons producing A cells is not present. The immunocytochemical staining would permit the specific localization of the A cells and the performance

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of accurate quantitative study. The aim of this study was to investigate fetal A cell pattern, namely the number, volume density and distribution at 19 and 21 days of gestation. The islet volume density, diameter and volume were measured. The A cell nuclear diameter and volume were also assessed. This study will provide a quantitative model for the development of fetal endocrine tissue and A cells, which can help further investigation in this field.

Methods. ***Animal and tissue preparation.*** Included in this study were 19 and 21-day-old fetal rats. The fetuses were obtained from normoglycemic lewis albino pregnant rats. Non-fasting blood glucose levels were measured to confirm that the animals were normoglycemic. Each group, 10 rats were examined. The pregnant rats were anesthetized by ether inhalation and the fetuses were removed from their uteruses. The pancreases were removed from the fetuses and fixed in buffered neutral formalin, dehydrated, embedded in paraplast (Sherwood Medical Co., St. Louis, Mo., USA) and serially sectioned (5 μm). Ten pancreases of each fetal age were examined. This study was carried out at the Department of Anatomy, King Abdul-Aziz University, Jeddah, Kingdom of Saudi Arabia during the period January to December 2005. The ethical consent was taken from the ethic committee at the Faculty of Medicine, King Abdul-Aziz University.

Immunocytochemical staining. Four sections, 20 sections apart were obtained from each pancreatic specimen. The sections were stained by avidin biotin complex (ABC) technique.[10] This method was used to localize the glucagon producing A cells. The primary antibody used was rabbit antiporcine glucagon serum (optimal dilution 1:5000). The secondary antibody used was biotinylated swine antirabbit immunoglobulin (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.

Quantitative analysis. In each fetus, we examined 32-48 islets (eight to twelve per section). The point counting method of Weibel[11] was used to calculate the volume density of A cells per islet (V_{vA}) and the volume density of the islets per pancreatic tissue (V_{vi}). The sections were examined at a magnification of × 1000 to estimate V_{vA} and at a magnification of × 400 to estimate V_{vi}. The number of stained A cell nuclei per islet profile was divided by the mean islet area to estimate the numerical density of stained A cells per islet profile (N_{Aa}, no./μm^2). The nuclei were counted by direct counting method at a magnification of × 1000. The numerical density of stained A cells per unit volume of islet (N_{va}, no./μm^3) was calculated by a variant of DeHoff and Rhines formula.[12]

$$N_{va} = \frac{N_{Aa}}{D_n + t}$$

Where N_{Aa} represents the number of nuclear profile per unit area estimated in sections of thickness t and D_n represents the mean corrected nuclear diameter.

The numerical density of stained A cells per unit volume of islet (N_{va}, no./μm^3) was multiplied by islet volume to estimate the absolute number of A 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 $d_i = 2\sqrt{ab}$[12] 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[12] was used to calculate the mean islet diameter (D_i).

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

Where N represents the total profiles measured.

Similar steps were used to estimate the A cell nuclear profile diameter (d_n). Fifty nuclei were measured for each slide at a magnification of × 1000. The corrected mean nuclear diameter (D_n) was estimated by the Abercrombic method.[12]

$$D_n = d_n \times 4/\pi$$

The equation $V = 4\ \pi/3 \times (D/2)^3$ was used to calculate the mean islet volume and the mean A cell nuclear volume from the mean corrected islet diameter (D_i) and mean corrected nuclear diameter (D_n).[12]

Carl Zeiss software from Zeiss was used in this study.

Statistical analysis. The means were compared by Mann Whitney U test. All the statistical analysis was made using the Statistical Package for Social Sciences software and Excel. The difference was considered as significant when $p<0.05$.

Results. The mean islet axial ratio of the 19-day-old fetuses was 1.25 ± 0.0748, while for the 21-day-old fetuses 1.21 ± 0.06, indicating that the islets could be

Table 1 - Volume density of A cells per islet, number of stained A cells per islet, volume density of the islets per pancreatic tissue, islet diameter, islet volume and number of total cells per islet of 19 and 21-day-old fetal rats (n=10).

Parameters	19-day-old fetuses	21-day-old fetuses
Volume density of A cells/ islet (V_{vA})	0.137 ± 0.011	0.196 ± 0.00474*
Number of stained A cells/islet	18.2 ± 2.7	51.7 ± 2.21*
Volume density of the islet/ pancreatic tissue (V_{vi})	0.029 ± 0.004	0.051 ± 0.00415*
Islet diameter (μm)	45 ± 1.33	62.4 ± 1.31*
Islet volume (μm^3)	48026 ± 4362	127822 ± 8328*
Number of total cells/islet	117 ± 11.1	246 ± 19.2*

Values are presented as mean ± SEM
*p<0.05, 19-day-old fetuses versus 21-day-old fetuses

Table 2 - A cell nuclear diameter and volume of 19 and 21-day-old fetal rats (n=10).

Parameters	19-day-old fetuses	21-day-old fetuses
Nuclear diameter (μm)	6.82 ± 0.124	7.14 ± 0.147^{ns}
Nuclear volume (μm^3)	166 ± 8.78	163 ± 17.1^{ns}

Values are presented as mean ± SEM
^{ns}p>0.05, 19-day-old fetuses versus 21-day-old fetuses

considered as spheroid structures. The mean nuclear axial ratio of the 19-day-old fetuses was 1.22 ± 0.045 while for the 21-day-old fetuses 1.13 ± 0.0356.

The volume density of A cells per islet, the absolute number of A cells, the volume density of the islets per pancreatic tissue, the islet diameter, the islet volume and the absolute number of total cells per islet of 19 and 21 day old fetuses are shown in **Table 1**.

The volume density of A cells showed a significant increase during the last days of gestation. All other parameters also showed a significant increase during the last days of gestation. Mann Whitney U test showed that all these parameters were significantly different between the 2 groups (p<0.05).

The A cell nuclear diameter and the volume of 19 and 21 day old fetuses are shown in **Table 2**. The A cell nuclear diameter and volume were not significantly different between the 2 groups.

At 19 and 21 days of gestation (**Figures 1 & 2**) the islets were nearly rounded, well defined and the A cells occupied the peripheral part of the islet.

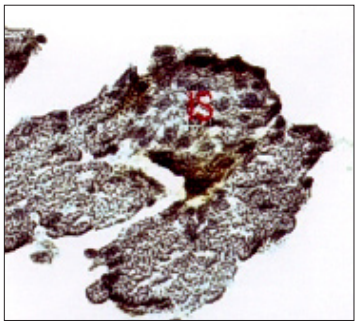

Figure 1 - Light micrograph of pancreatic islet (IS) of 19-day-old fetus. The islet is stained with avidin biotin complex technique to show A cells. ×200.

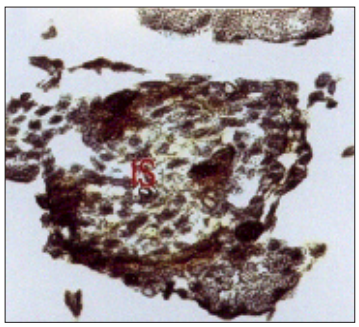

Figure 2 - Light micrograph of pancreatic islet (IS) of 21-day-old fetus. The islet is stained with avidin biotin complex technique to show A cells. ×200.

Discussion. The results obtained in this study revealed that the volume density of glucagon producing A cells, which represents the amount of islet tissue containing A cells increased significantly during the last days of gestation. This result may suggest that the amount of A cell mass and glucagon content of the pancreatic islets show a significant increase during the last days of gestation. This finding is in agreement with that of McEvoy and Madson[13] and Noda.[14] The volume density of pancreatic islet also increased significantly during the last days of pregnancy. The islet volume density reflects the amount of endocrine tissue. Subsequently, this result suggests that the size of endocrine tissue increased during the last period of pregnancy and confirms our result regarding the increase in A cell volume density. A similar finding was reported by Freie et al[15] and Aerts and Van Assche.[16] In this study the A cell number and total islet cell number increased significantly during the last days of gestation. In consistent with these findings the islet diameter and volume also showed a significant increase. These results may indicate that the endocrine tissue expands rapidly and the islet glucagon content increases significantly during the

last days of gestation. Similar findings were reported in the literature. McEvoy and Madson[13] found that the number of A and B cells showed a rapid increase during the last days of pregnancy. Noda[14] reported that the A and B cells were present on the 16 day of pregnancy and they showed a rapid increase during the following gestational period. It is clear that the different pancreatic islet cells of fetal rats appear early during development. Gomez Dumm et al[1] and Park and Bendayan[2] reported that the A cells start to appear in the islet on the 12 day of gestation, the B cells appear on the 14 day and PP cells were detected on the 19 day. In comparing with islet development in human, the pancreas starts to develop from an epithelial mass that contains central ducts surrounded by dense mesenchyme. The A, B and D cells begin to develop within the central ducts at 8 week of development.[17] The pancreatic islets appear as buds from the ducts and are separated from the ducts on weeks 17-20.[3]

The A cell nuclear diameter and volume did not increase from 19-21 days of gestation. This may indicate a similarity of the A cell size at 19 and 21 days of gestation. The A cells were well stained and occupied the peripheral part of the islet. This cellular pattern of A cells is similar to that of adult pancreatic A cells.[18,19]

In conclusion, the present study represented a detailed quantitative immunohistochemical study and demonstrated that the size of the endocrine tissue and the islet A cells increased significantly during the last days of gestation.

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