Structural study on cultured isolated fetal rat pancreatic islets

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ABSTRACT

Objectives: To perform a morphometric and structural study of the cultured isolated fetal rat pancreatic islets.

Methods: Islets of the fetal rat pancreas, aged 22 days were isolated by the standard procedure of collagenase digestion and culture technique. The islets were cultured for 1, 2, 3, 4 and 5 days. The islets were counted under a dissecting microscope and islet diameter and purity were measured under a phase contrast microscope fitted with a calibrated grid. The islet specimens were fixed in buffered neutral formalin, dehydrated in alcohol and embedded in parablast. Sections were stained with hematoxylin and eosin. This study was conducted in King Fahd Medical Research Center, Faculty of Medicine, King Abdul-Aziz University, Kingdom of Saudi Arabia, during 2003 and 2004.

Results: The purity of the cultured islets gradually increased with time and was significantly different between the cultured groups. The number of the cultured islets gradually decreased with time. The islet diameter gradually increased with culture period. The islet purity and diameter were significantly different between the cultured groups. Parablast sections stained with hematoxylin and eosin showed that the shape and histological structure of the cultured islets were intact.

Conclusion: The results of the present work represent an extensive morphometric structural study of isolated cultured fetal islets. The culture islet diameter and purity showed gradual increase with culture period, while the islet number showed gradual decrease.

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The pancreatic islets of fetal rat can be isolated by the simple use of collagenase digestion and culture technique.[1] The isolation technique has become the standard procedure to harvest a large number of fetal pancreatic islets, which could be successfully cultured for further use in cryopreservation procedure and functional studies.[2-7] The fetal islets can be used for transplantation studies and were able to reverse diabetic state after transplantation.[2,6,8] Fetal pancreatic islets could provide more available islet source than the adult islets. Aborted fetuses could provide accessible source of fetal pancreatic islets for use in different studies or clinical trials. Nevertheless, the process of isolation inevitably leads to islet trauma and reduce the structural integrity of the isolated islets. It has been reported in the literature, that isolated islets showed damage to its central part which contains B cells and its peripheral part also.[9-13] The injury of the peripheral part of isolated islets presumably affects the hormonal secretory function of B-cells, which form the core of the islets.[12,14] Despite the recent surge in the use of the isolated fetal islets in research and clinical studies, there is no detailed study of the effect of the isolation and culture procedure on their morphology and structural integrity. The aim of the present work was to study the morphological criteria and structural integrity of isolated cultured pancreatic islets of the rat fetuses.

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Methods. ***Isolation and culture of the pancreatic islets.*** Six groups were studied in this research. Each group consisted of 10 fetuses. Fetal rat islets were isolated by the collagenase digestion and culture technique of Hellerstrom et al.[1] Pregnant rats were sacrificed by overdose and 22-day-old fetuses were extracted through a median abdominal incision. Each fetus was decapitated and the pancreas was removed aseptically and placed in a sterile ice-cold medium 199 (M199), pH 7.4. The medium was supplemented with penicillin (100 U/ml) and streptomycin (0.1 mg/ml) (from Gibco, Grand Island, New York, USA). Approximately 8-10 fetal pancreatic glands were obtained from each pregnant rat. After careful trimming of the pancreas from remnants of gut and spleen, it was chopped into fine (1 mm^3) pieces and washed twice with chilled M199. The pieces were transferred to a small sterile vial containing approximately 4 ml M199 supplemented with 6 mg collagenase (Sigma type V). Different concentrations of collagenase (1, 1.5 and 2 mg/ml) and different digestion times (8, 9, 10, 11, and 12 minutes) were used to obtain the optimal digestion conditions for this batch of collagenase. Collagenase at concentration of 1.5 mg/ml and incubation time of 11 minutes in a water bath at 37^{0}C were selected as the optimal digestion conditions. The digestion was subsequently terminated by adding 20 ml of ice-cold M199 followed by washing it 3 times. The digest was pelleted by centrifugation (500 rpm), resuspended in 10 ml culture medium, and transferred to a petri dish. The islets were then further purified by hand picking of the non-islet tissue with the help of a binocular dissecting microscope. The islets were then distributed in portions of 1 ml into tissue culture dishes, containing 4 ml of identical tissue culture medium. The isolated islets were cultured in 94 mm sterile polystyrene culture dishes. The culture medium consisted of RPMI-1640 (Sigma), pH 7.4, osmolality between 310 and 340 mOsm/kg. containing 11.1 mM glucose, 20 mM N-2-hydroxyethyl piperazine-N-2-ethane sulfonic acid buffer (HEPES buffer), penicillin 100 U/ml, streptomycin 0.1 mg/ml, gentamycin 10 µg/ml, nystatin 100 IU/ml. The medium was supplemented with 3% L-glutamine, and 5% heat inactivated fetal rat serum. The cultured islets were maintained at 37^{0}C in a humidified atmosphere of 5% (volume per volume [v/v]) carbon dioxide in air. The islets were cultured for 5 days and the culture medium was changed daily. After each culture period, the islets were detached from the bottom of the culture dish by aspirating gently with medium 199, and harvested with the aid of a 10 ml syringe attached to a 14-g cannula.

Morphometric and histological study. Islet count, diameter and purity were measured in cultured islet specimens. The cultured islets were counted on 1, 2, 3, 4 and 5 days of culture under the dissecting microscope immediately after changing the culture medium and the counting was confirmed under phase contrast microscope. The total islet count could be estimated from the sample mean.[15] Islet diameter was measured under phase contrast microscope. The islets appeared under phase contrast microscope as dark brown rounded structures, which could be distinguished from the acinar and ductal tissue. A calibrated grid in the eyepiece was used to measure the diameter of the islets.

Purity of the islets was determined by estimating the relative volume of the islets to the non-islet tissue. This was carried out by inserting a grid in the eyepiece of the microscope and randomly counting the structures beneath the intersections of the crossbars, and determine the islet volume in relation to the non-islet structures,[16] as follows:

$$\text{Purity of islets} = \frac{\text{N of points which overly islets} \times 100}{\text{N of points which overly islet and non-islet tissues}}$$

wherein N=number. The isolated fetal islets were fixed in buffered neutral formalin, dehydrated in alcohol and embedded in paraplast (Sherwood Medical Co., St. Louis, MO, USA). Five µm serial sections were cut and stained with hematoxylin and eosin for histological study.

Statistical analysis. The results were presented as the arithmetic mean ± standard error of mean. One way analysis of the variance (ANOVA) was used for statistical evaluation of the data.

Results. Six groups of isolated fetal islets were examined in this study. Each group consisted of 10 fetuses. The islets were culture for 5 days and the number, diameter and purity of the islets were measured every day. Histological sections of the islets were stained with hematoxylin and eosin and examined under the light microscope. The freshly prepared isolated islets were recognized under the dissecting microscope by their characteristic rounded or oval shape, showing high opacity and milky white color, in contrast to the acinar tissue, which appeared irregular in shape and showed less opacity. Some of the cultured islets showed small blebs projecting from the well-defined surface. The boundary of the islet was ill-defined immediately after culture, but it became well-defined after culture. **Figure 1** shows cultured isolated fetal islets under the phase contrast microscope using green filter.

The mean number of the cultured islets gradually decreased with time. It was 259 ± 14.7 on the first day and then decreased to 126 ± 4.39 on the fifth day. The islet number was significantly different among the 5 days culture (p<0.001). The mean

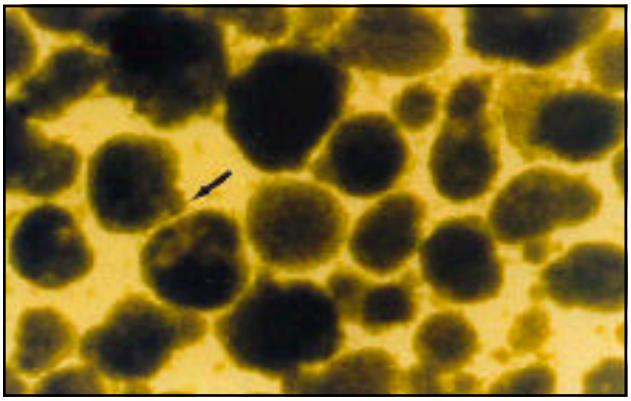

Figure 1 - Isolated fetal islets, cultured for 2 days. The islets appear homogenous with dark green tinge and well-defined rounded or oval shape. Phase contrast with green filter. Some of the islets show bleb projecting from their boundary (arrow) (x 100).

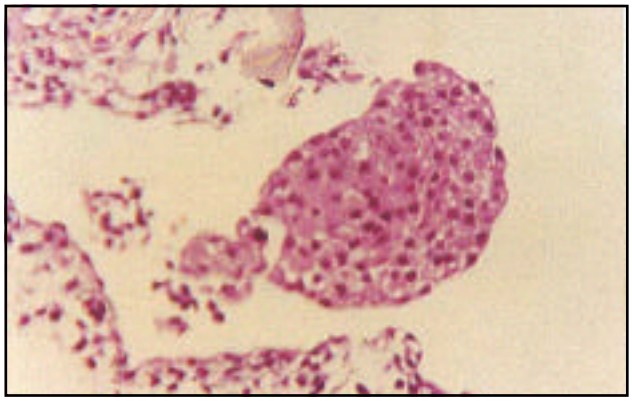

Figure 2 - An isolated fetal islet cultured for 3 days. The islet is stained with hematoxylin and eosin. It shows well defined border and well preserved structural integrity (x 200).

diameter of the cultured islets showed progressive increase during the first 3 days of culture. It increased from 92 ± 12 on the first day of culture to 175 ± 28.9 on the second day of culture and it became 256 ± 36.5 on the third day of culture and then it showed a slight increase during the forth (260 ± 35.5) and fifth (265 ± 34.8) days. The islet diameter was significantly different among the 5 days culture ($p<0.05$). The mean purity of the islets cultured for 5 days was 73.6 ± 2.39 one-day after cultured. Most of the impurities consisted of pancreatic acini, in addition to small ducts, blood vessels and lymph nodes. Handpicking of the non-islet tissues could raise the final mean purity to 94.8 ± 1.22, after 5 days of culture. The islet purity was significantly different among the 5 days culture ($p<0.001$). Islet sections of cultured isolated fetal islets stained with hematoxylin and eosin showed well preserved structural integrity of the islet cells during 5 days culture period. The islet boundaries were well-defined (**Figure 2**).

Discussion. Isolation of the fetal pancreatic islets has become a commonly used procedure to obtain a relatively pure endocrine tissue for transplantation experiments or for functional metabolic studies.[2,6,8] In the present work, fetal rat islets were isolated from the pancreas by the collagenase digestion and culture technique of Hellerstrom et al.[1] This was selected since it is the most commonly used technique for the isolation of fetal pancreatic islets.

In the present study, the number of the cultured islets gradually decreased with time and the difference was statistically significant. This could be explained by the inevitable loss of some islets during changing of the culture medium, which was carried out daily. Some of the non-islet tissue was difficult to be distinguished from the islets on the early days of culture but it could be distinguished from the islets on the subsequent culture days and was removed. The decrease in islet number may be also due to the death of some of the islets. The purity increased gradually during the 5 culture days. It increased from 73.6 ± 2.39 on the first day to 94.8 ± 1.22 on the fifth day and the difference was statistically significant. This could be explained by the removal of the non-islet tissue. Histological sections stained with hematoxylin and eosin showed that the shape and histological structure of the cultured islets were intact and well-preserved (**Figure 2**). Previous reports also reported that the structural and histological integrity of the isolated fetal islets were well-preserved.[16-20] The islet boundaries were ill-defined immediately after isolation but the boundaries became well-defined after culture. The irregular peripheral region may indicate that the isolation procedure had injured the peripheral parts of the islets. This injury could be due to enzymatic digestion of the peripheral parts of the islets by the collagenase enzyme and mechanical trauma during the process of isolation. Injury to the peripheral cells of the isolated islets was reported in the isolated islets by Slavin et al,[9] Trimble et al,[10] El-Naggar et al[11] and Bertuzzi et al.[12] Slavin et al[9] attributed the injury of the peripheral part of the islets to the metabolic changes occurring in the islet cells during the isolation procedure. Trimble et al[10] reported that the intraportal transplanted isolated islets contained less A cells that the control pancreatic islets. El-Naggar et al[11] also reported injury to the peripheral cells of the isolated islets. They showed that the peripherally arranged A, D and PP cellular populations of the cultured rat islets showed significantly lower percent volumes and numbers than those of the intact islets. Bertuzzi et al[12] demonstrated that peripheral glucagon secreting cells (A cells) are lost during the process of

isolation. However, the process of isolation was also reported as injury to the central part of the islets.[13]

The islets cultured for the 5 days period showed a progressive increase in diameter. It increased from 92 ± 12 on the first day to 265 ± 34.8 on the fifth day. The increase in the diameter of the cultured islets could be attributed to either increase in the size or the number of the constituting islet cells. The presence of small blebs projecting from the well-defined surface of the cultured islets (**Figure 1**) may suggest proliferation of some of the peripheral cellular population of the islets such as A and D cells. A similar finding was reported by Yderstraede and Flindt-Egebak.[20] The peripheral region of the cultured islets was irregular immediately after isolation and then became well-defined and rounded after culture. This was noticed in the histological and phase contrast examination. This finding may also suggest the proliferation of the peripheral islet cell population. Our finding was supported in the literature. Dudek et al[17] demonstrated immunocytochemically as an increase in the mass of insulin and glucagon positive cells in cultured islets of 21.5-day-old fetal rat. Georgiou and Mandel[18] found that proliferation of beta cells of cultured fetal pancreas was highest during the first 3-weeks of culture but these cells rapidly declined in number thereafter as the delta-cell mass increased. Leduque et al[19] found, with immunocytochemical staining of cultured fetal rat islets, that the number and percentage of A and D cells increased with time, at the expense of the percentage of B cells. Whether the changes in the peripheral parts of the cultured islets can lead to changes in the hormonal secretory function of the B cells, is not yet known. However, there are various reports indicating the presence of communications between the cells of the pancreatic islets. Pipeleers et al[14] found that B cells in the isolated islets of the adult rat released 30 times more insulin in response to glucose as compared with that released from purified single B cells. Bertuzzi et al[12] reported that glucagon increase insulin secretion of cultured pancreatic islets. This study presented a detailed morphometric study of the cultured fetal pancreatic islets, however, morphometric study of the cultured pancreatic islets was reported before by Trimble et al,[10] El-Naggar et al,[11] Bertuzzi et al[12] and Yderstraede et al.[20]

In conclusion, the results of the present work represent an extensive morphometric structural study of isolated cultured fetal islets. The culture islet diameter and purity showed gradual increase with culture period, while, the islet number showed gradual decrease.

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