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RESEARCH ARTICLE

HISTOGENESIS AND DISTRIBUTION OF ISLETS IN HUMAN FETAL PANCREAS

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Abstract

The pancreas is a mixed exocrine and endocrine gland. Diabetes currently afflicts about 200 million people all over the world, and it is well known that the endocrine component of the pancreas has a direct correlation with the severity, morbidity, and treatment of the disease. Studies conducted in human fetal pancreas are very limited owing to ethical and technical issues. In cases of type I Diabetes mellitus, the knowledge of stepwise histogenesis of the endocrine component would be largely helpful to the surgeons for pancreatic transplant, and planning treatment protocols for pancreatic cancer.Study was performed on 30 aborted fetuses from 12 to 40 weeks, collected from Department of Obstetrics and Gynecology of the Institute, after due permission from institutional research and ethical committee. Pancreatic tissue was processed and stained with Hematoxylin and Eosin stains and examined for different components of pancreas correlating with development and distribution of islets.The parenchyma, exocrine and endocrine component of pancreases were observed. There was correlation between histogenesis of pancreas with regard to gestational age, gender, diabetic history of mother, congenital anomalies in fetus and distribution of islets throughout pancreas. Study would be helpful to know about the changes in histological development of fetal pancreas in relation to different gestational age and for planning treatment modalities for Diabetes and pancreatic diseases.

Introduction:-

The pancreas consists of exocrine and endocrine parts. Exocrine part consists of pyramidal shaped serous acini, filled with zymogen granules and interlobular ducts which are lined by low cuboidal epithelium without striation, which is a unique feature for identifying pancreatic histology slides. In the lumen of the acinus, there are cells of intercalated duct, known as Centro acinar cells (Standring, 2008). Endocrine part consists of Langerhans islets that are surrounded by acini and are covered by a reticular capsule. Islet of Langerhans comprises different types of cells like alpha, beta, delta, and parenchymatic polypeptide (PP) cells with capillaries (Bailey et al., 1984). In Haematoxylin and Eosin (HandE) stain, alpha cells stain slightly pinkish and beta cells stain slightly bluish in shades

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due to the nature of alpha cells being acidophilic and beta cells being basophilic. On the periphery of the cluster of islets, there are mostly alpha and delta cells, and in the core, there are beta cells (Eroschenko, 1996).
Diabetes currently afflicts about 200 million people all over the world, and it is well known that the endocrine component of the pancreas has a direct correlation with the severity, morbidity, and treatment of the disease (Centers for disease control anad prevention, 2007). Extensive research in the past has been carried out in animal models, which has helped us to know the different events occurring in the process of pancreatic development, but studies conducted in human fetal pancreas are very limited owing to the ethical and technical issues of obtaining human fetal pancreas.

The present research has obtained due ethical permission from the Institute and it would be helpful to know about the histological development of the fetal pancreas in relation to different gestation ages. In cases of type I Diabetes mellitus, where pancreatic transplant is needed, the knowledge of stepwise histogenesis of the endocrine component would be largely helpful to the surgeons. Along with this, the research would add substantial knowledge in the areas of pancreatic regeneration, surgical pancreatectomy, and treatment protocols for Diabetes mellitus and Pancreatic cancer. Through evaluation histogenesis in relation to different gestational ages, the research intends to draw attention to various associated developmental correlations that were not mentioned in previous studies.

Material And Methods:-

The research work was prospectively reviewed and approved by Institutional Ethics Committee of the Institute.Study was performed on 30 spontaneously and induced aborted fetuses collected from Department of Obstetrics and Gynaecology of the Institute,after a detailed history and proper consent. Age of fetuses ranged from 12 to 40 weeks and calculated by maternal history, ultrasonography and online gestational age calculator software. Fetuses were arranged in seven gestational group of four weeks' interval mentioned as: Group 1(12-16), Group 2 (17-20), Group 3 (21- 24), Group 4 (25-28), Group 5 (29-32), Group 6 (33-36) and Group 7 (37-40), as illustrated in **Table 1.**Foetuses were collected in 10% formalin immediately after abortion and medical termination of pregnancy. After stepwise dissection, fetal pancreas was removed as shown in **Fig.1.** Subsequently fetal pancreatic tissue within groups was processed and stained with Haematoxylin and Eosin stains (HandE) and examined for different components of pancreas correlating with development and distribution of Islets throughout pancreas in the specific age groups.The duration of our entire study was 18 months and the materials which were used during this whole process included 10% formalin, scalpel, weighing machine, Vernier calipers, measuring scale, Hand E stain, slides, cover slips, DPX.

Different parts of pancreas: head, body and tail were cut and kept in 10 % formalin (tissue fixation) for 24 hours. Tissue was then dehydrated by processing it with 70 to 100% alcohol for 2-hour interval for each step of dehydration.After dehydration clearing wasdone by different changes of xylene.After clearing, the tissue was embedded in paraffinwax.Once removed from heatsource, paraffin block was cooled and solidified.Paraffin block was then trimmedto size of 5 microns thickness with the help of microtome.

After section cutting, tissuewith paraffin (Ribbon) was dipped into floating water bath at 42 degreesCelsius for 1-2 minutes and then tissue was lifted on glass slide. Then it was placed on hot plate for 2-3 minutes to remove excess paraffinaround tissue.Clearing of tissue was done by two changes of Xylene. The tissue was then hydrated with descending concentration of alcohol (absolute alcohol, 95% alcohol, 80% alcohol, 70% alcoholand 50% alcohol) with 2-5 minutes interval and then washed by running water. Then it was dipped into Hematoxylin for 10 minutes, then in hydrochloric acid (HCl) and then into Eosin for 2 minutes for counterstaining. Slide was then washed in running tap water and was mounted with the help of Dibutylphthalate Polystyrene Xylene (DPX) and covered with a coverslip. Now the slide was ready to be viewed for histological analysis as illustrated in **Fig.2.**

Observation and Results:-

We have described the histological analysis of pancreas of each gestational age group under three headings: Parenchyma, Exocrine and Endocrine components.

Table 2 represents the histological findings in pancreas belonging to 12-16 weeks' gestational age group. The parenchyma was found to contain abundant mesenchyme and appeared as a collection of branched tubule lined with cuboidal epithelium. The exocrine component comprised groups of numerous developing acini lined by simple

cuboidal epithelium. The endocrine component consisting of Islets of Langerhans, were seen in budding stage, as illustrated in **Fig.3.**
In pancreas belonging to 17- 20 weeks' gestational age group, the parenchyma contained abundant mesoderm with developing acini cells along with interlobular and intercalated ducts, as shown in **Table 3.** The exocrine part showed proliferation of acini with their shield like arrangement around the Islets. The endocrine component comprised alpha, beta, delta and some undifferentiated cells in clustered form with alpha cells more in number than beta cells, as illustrated in **Fig. 4 and 5.**

As per **Table 4**, the pancreatic parenchyma in gestational age group of 21- 24 weeks, was organised into lobes and lobules. The exocrine part showed zymogen granules and acini with wide lumen, while the endocrine part showed higher concentration of Islets in head of pancreas, with beta cells now being more than alpha cells, as illustrated in **Fig. 6 and 7.**

The parenchyma of pancreas in gestational age group 25- 28 weeks was highly vascularised **(Table 5)**with exocrine part showing fully formed acini separated by connective tissues and endocrine part showing Islets encapsulated by reticulin fibres, as illustrated in **Fig. 8 and 9** (low and high magnification respectively).

During 29-32 weeks' gestation period (**Table 6**), the pancreatic parenchyma seemed to contain lobes and lobules packed with serous acini. Although no branching was appreciated in the exocrine component, the Islets comprising the endocrine part were large and prominent, now distributed equally in head, body and tail of pancreas, as shown in **Fig. 10.**

However, the pancreatic parenchyma in 33-36 weeks of gestation (**Table 7**) contained less connective tissue but with enlarged exocrine as well as endocrine components, as shown in **Fig.11 and 12.**

Finally, the pancreas belonging to 37-40 weeks' gestational age group (**Table 8)** exhibited small amount of undifferentiated mesenchymal cells in parenchyma, exocrine part having mature acini and Islets now more concentrated in tail of pancreas, as seen in **Fig. 13 and 14.**

Discussion:-

Current Study was performed on 30 spontaneously and induced aborted fetuses in Department of Anatomy collected from Department of Obstetrics and Gynaecology of the Institute after a detailed history and proper consent.

Gupta et. al., 2002.studied on 40 aborted foetuses with gestational ages ranging from 12 to 40 weeks without congenital abnormalities. They were divided into five groups, each with a six-week gap. Haematoxylin and eosin, Aldehyde Fuschin, and Mallory's phosphotungstic acid haematoxylin were used in their study. They found mesenchyme tissue lodges with branching tubules and a large lumen in Group I (12-18 weeks). Sprouting was seen towards the tubule ends, resulting in the formation of primitive acini and islets. Lobes and lobules were more prominent between 13.2 to 18 weeks. The size of the islets of Langerhans was likewise enhanced, as was the capsule and vascularization. The number of islets of Langerhans grew with encapsulation and vascularization became denser in Group II (18.1–24 weeks). Mesenchymal tissue was reduced due to acinar proliferation. Interlobular tissue and ducts were found in Group III (24.1–30 weeks). The number of Langerhans islets increased and became more widely distributed. Acini and islets of Langerhans were increased in Groups IV (30.1–36 weeks) and V (36.1–40 weeks). Tails contained more islets than the rest of the head and body.

Ferner et al., 1951. studiedon 12fetal pancreases and observed that budding of islets from primitive tubules began at 10^{th} weeks of gestational age. They used Gomori stain to identify alpha and beta cells.

Banerjee et al., 2018. studied on 49 foetuses of different gestational age group from 10 weeks to 40 weeks, divided in 7 gestational groups with each group having 7 foetuses collected in normal saline and then proceeded for Haematoxylin and Eosin and Gomori Modified Aldehyde Fuschin stain. They observed that initially size and number of islets of Langerhans were small and gradually increased in 10 to 14 weeks. In 14.1 to 16 weeks'fetuses, number of connective tissue observed and alpha cells were more than beta cells. In 16.1 to 19 weeks'fetuses, delta cells were observed to be less in number and small in size than alpha and beta cells. In 19.1 to 23 weeks' fetuses,cluster of cells were seen in lobule forming acini and islets. In 23.1 to 26 weeks'fetuses, islets were encapsulated with alpha and beta cells being arranged in cords. In 26.1 to 33 weeks'fetuses, alpha, beta and delta

cells were well developed and arranged in encapsulated islets. In 33.1 to 40 weeks' fetuses, alpha cells were large but less in number than beta cells and delta cells were lesser compared to both alpha and beta cells.

The present study included 30 spontaneously and induced aborted fetuses with age ranging from 12 to 40 weeks, calculated by maternal history, ultrasonography and online gestational age calculator software. Fetuses were arranged in seven gestational group of four weeks' interval mentioned as: Group 1(12-16), Group 2 (17-20), Group 3 (21- 24), Group 4 (25-28), Group 5 (29-32), Group 6 (33-36) and Group 7 (37-40).

Weobserved that in Group 1**,** abundant mesenchyme was present with developing acini and budding islets. Histological picture of pancreas in Group 2 showed connective tissue arranged around ducts and Islets of Langerhans arranged in clusters. In Group 3, Islets were seen more concentrated in head of pancreas and in Group 4, highly vascularized parenchyma was observed with Islets encapsulated by thin reticulin fibres. In Group 5, Islets were equally distributed in head, body and tail of pancreas with absence of branching pattern. Group 6 showed lesser connective tissue arranged in parenchyma.

According to **Achaya et al., 1965.** the number of epithelial buds increases at several points along the duct system during the fetal stage of 8-11 weeks. Several cell buds along the tubule system's course, as well as terminal bud cells, develop basophilic cytoplasm early in the period.

In their separate studies onfetuses**, Conklin, 1962.**and**Jongmin et al.,2009**. found the islets to be related to the tubules. Islets were observed in 9–12 week fetuses as clusters of small eosinophilic cells attached to tubules, but they were separated from the tubules after vascularization due to connective tissue invasion**.**Our study results showed budding of islets and ductal system in 12-16 weeks of gestation. Pancreas belonging to 21-24 weeks of gestation showed gradually increasing size of islets of Langerhans, lined by cuboidal epithelium.

Robb, 1961. observed the "mantle islet stage" at around 12 weeks in his investigation on human fetuses, in which a zonal arrangement of cells was seen, with Beta cells in the centre and Alpha cells at the periphery. The embryonic islets sprout out of tubules, as seen at the fetus's earliest stage. However,**Jongminet.al., 2009.**reportedthe presence of almost same pattern with beta cells arranged in centre while delta and alpha cells arranged in periphery, around 20^{th} weeks onward.

Like et al., 1972.studied on 20 fetal pancreases, without any congenital abnormality, belonging to gestational agegroup of 8 to 23 weeks. They found branching of ducts and the budding of islets of Langerhans at 8 weeks of gestational age, which is quite earlier than what we observed. The size of the islets of Langerhans gradually increased between 12 to 16 weeks of gestation, with a larger number of alpha cells. However, by 20 weeks, beta cells had outnumbered all other cells. All Islet cells were organised into a cluster.**Dhendeet al., 2016.,Gupta et al., 2014.**and **Davis et al., 2019.** also reported the same findings.

In our study 30 fetuses were included from gestational age 12-38 weeks. Budding of Islets was seen in 12^{th} week of gestational age, unlike the above findings. In 17-20 weeks of gestation, Islets of Langerhans were formed in clusters and in 21-24 weeks of gestation, beta cells were more in number than alpha cells and arranged in centre and periphery respectively, which is similar to the findings reported by Jeon et al. as mentioned above.

Ramani et.al., 2015.collected 50 fetuses without any congenital anomalies between 12 to 40 weeks of gestational age. They showed that at 12 to18 weeks of gestation age, pancreatic parenchyma differentiated into lobes and lobules. At 18 to 24 weeks, Islets were organized into spherical circular clumps with alpha, beta and delta cells. Pancreas at 24 to 30 weeks of gestation showed well differentiated islets arranged with vasculature.

Manupati, 2012.examined17 fetuseswith gestational age of 6 to 40 weeks and concluded that as the gestational age increases, tissue contained numerous offshoots of epithelial tubules. Pancreatic tissuesin 16 to 18 weeks of gestation, got systematized into pancreatic lobes and lobules with budding of islets group of Langerhans. At 28 to 30 weeks of gestation, parechymatic tissue were more developed and exocrine part of pancreas was arranged with minimal mesenchymal tissue. Ducts became more prominent and abundant with offshoots. From 36 weeks onward, distinct pancreas tissue was arranged with minimal mesenchymal tissue and islets of Langerhans were large in number towards tail of pancreas.

In our study we found that during 12-16th week of gestation, mesenchyme was present in significant amount along with collection of branched tubules and developing acini. Pancreas at 17-20 weeks showed connective tissue arranged around ducts and at 21-24 weeks, showed parenchymal organization into lobes and lobules. Islets were encapsulated with thin reticulin fibre during 25-28th week of gestation.

Haldaret al., 2019.studied32 fetuses between 12 to 36 weeks of gestation and reported that in early weeks, small islets of Langerhans, primitive ducts, undifferentiated cells and acini and blood vessels were not demarcated. In later weeks of gestation, intralobular duct, separated by interlobular duct, acinar cells and islets of Langerhans were surrounded by a capsule. Islets of Langerhans began to develop during 2nd month of intrauterine life with alpha cells (arranged at periphery) appearing in middle of 3rd month,delta and beta cells (arranged at centre) appearing in 4th month of intrauterine life and becoming fully active in 5th month of intrauterine life.

Patil et al., 2013 studied30 aborted fetuses between 12 to 40 weeks of gestational age with the help of Mallory's phosphotungstic acid, Gordon and Sweet stains and Haematoxylin and eosin stain. Fetuses were classified into five groups according to their gestational age.They observed branched tubules with lumen, primitive acini and spherical small islets during 12 to 13 weeks of gestation. At 13.1 to 18 weeks, they reported increased size of islets with appearance of lobes, lobules and undifferentiated mesenchymal tissue. Pancreas at 18.1 to 24 weeks showed enlarged Islets, covered by capsule and better differentiated intralobular and interlobular ducts. During 24.1 to 30 weeks and 30.1 to 40 weeks of gestation, number of islets kept increasing with more prominent ductal system. They found that at 22 to 24 weeks'gestational age, fetal pancreas was most suitable for transplantation.

Patilet al., 2013. in their studyon 30 fetal pancreas of gestational age 12-40 weeks, reported that in 12 to 13-week fetal pancreas, splitted tubules were well organized in pancreatic tissue and endocrine part starts to appear as an offshoot. Pancreas in 13 to 18 weeks, had well defined parenchymatic lobes and lobules with increased number of islets of Langerhans. Ducts were separated from tubules and endocrine component was seen to be more prominent and covered by reticulin capsule in pancreas belonging to 18-24 weeks of gestation.

According to **Proschina et.al, 2019.** fetus of8th to 12th week gestational age showed primitive ducts and branched budding islets of Langerhans. All these structures were surrounded by mesenchymal tissue. During 10th to 11th weeks, alpha cells were found to be more in head of pancreas, as compared to other cells. Differentiation of alpha, beta and delta cells was seen during 7th to 14th week of gestational age. At around 16 weeks, alpha Cells get arranged at one pole and beta cells at aopposite pole. Later alpha cells are arranged at periphery and beta cells come towards the center of islets.

In our study, sprouting of islets was seen between 12- 16th weeks of gestation with abundant mesenchyme and acini having small lumen.The lumen became larger during 33-36 weeks. Differentiation of alpha, beta and delta cells was seen in 16 weeks of gestation.

Sandler et al., 1982. in their fetal study, reported dense granulations in alpha and beta cells during 22-24 weeks, which is in correspondence with our study where granulations appeared between 21-24 weeks of gestation.

According to **Ramani et al., 2018**.and others, the first islet cell groups appeared in the central region of pancreatic tissue at around 18–24 weeks of gestation.Around the 30th or 40th week, the cell buds that would become islets were spotted encased in clusters of capillaries.

Present study shows smallest islets as solid knots of cells budding out from the ducts at 12 to 16 weeks of gestation.At 8-12 weeks of gestation, the parenchyma begins to arrange into lobes and lobules with substantial mesenchymal tissue, according to **Manupati, 2012.** Acini are better developed at 16-18 weeks, and connective tissue separates them around 28-30 weeks. The present studyshowsappearance of zymogen granules in ducts of acini with wide lumen at around 21- 24 weeks of gestation while **Hamilton et al., 1972.** reported presence of secretory granules in third month of intrauterine life in alpha and beta cells.

At 10thweek of gestation, **Clark et al., 1983.**reported the development of duct of acini covered with cuboidal epithelium with better identifiable lobes and lobules by 12thweeks, which is almost similar to our findings showing developing acini and islets of Langerhans in 12-16 weeks of gestation.

Ferneret al., 1951.recognised the alpha and beta cells in the human pancreatic islets as early as 10–13 weeks by Gomori'smethod, in contrast to our study where alpha and beta cells were appreciated in 17-20 weeks of gestation by haematoxylin and eosin stain.

Grube et al., 1983.in his study on microanatomy of Islets,concluded that human fetal pancreas during gestational age of 20-24 weeks, might be a suitable donor transplant material as alpha and beta cells start secreting from this time onwards and capillaries present around the Islets Cells can grow well if transplanted.Our present study also supports this view as we also observed that during this gestational age group, size of islets of Langerhans is large with maximum number of beta cells in centre and alpha cells in periphery.

Laitio et.al., 1974. observed that abundant mesenchyme was present in fetal pancreas having gestational age of 9 weeks and pancreatic tubules were lined by simple columnar epithelium.While the present study showed large amount of mesenchyme at 12-16th weeks of gestation.At gestational ages of 24 to 32 weeks, **Adda et al., 1984.** observed the emergence of definitive ductal structures such as intercalated, intralobular, and interlobular ducts while the same was observed at 21-24 weeks of gestation in our present study. In 25-28 week of gestation, interlobular connective tissue with minimum mesenchymal tissue was seen.

Conclusion:-

The study would add substantial knowledge in the areas of pancreatic regeneration, surgical pancreatectomy, and treatment protocols for diabetes mellitus and pancreatic cancer. Through evaluation of variations in morphometry and histology in relation to different gestational ages, the research intends to draw attention to various associated developmental correlations.

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Conflict of interest:
Nil.

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Figure Legends:-

Fig.1: Stepwise dissection (a) opening up of abdominal cavity and (b) dissecting out pancreas.

Fig.2: Steps for tissue processing and staining of slide.

Fig.3: Fetal pancreas no.1 (Transverse section) **A** – developing acinus with small lumen. **B –** Abundant mesenchyme with some undifferentiated cells. **C-** Budding of islets of Langerhans.

Stain: Haematoxylin and eosin. Magnification 10x

Fig. 4: Fetal pancreas no. 8 (Transverse section) **A** –acinus in pancreatic parenchyma. **B-** Cluster of islets of Langerhans. **C-** Blood vessels in mesenchyme**. D-** abundant mesenchyme with some undifferentiated cells.

Stain: Haematoxylin and Eosin. **Magnification** 10x.

Fig.5: Fetal pancreas no. 8 (Transverse section) **A** – Interlobular duct lined by simple cuboidal epithelium. **B-** Cluster of islets of Langerhans. **C**- Acini.

Stain: haematoxylin and eosin. **Magnification** 40x.

Fig. 6: Fetal pancreas no.13 (Transverse section) **A** – Acini. **B**- Cluster of islets of Langerhans lined by cuboidal epithelium. **C**- Interlobular duct. **D**- Mesenchyme, parenchyma showed organisation into lobes and lobules.

Stain: haematoxylin and eosin**. Magnification** 10x

Fig. 7: Fetal pancreas no.13 (Transverse section) Acini ducts lined by simple columnar epithelium with some compound epithelium, wide lumen with some zymogen granules.

Stain: Haematoxylin and eosin. **Magnification** 40x

Fig. 8: Fetal pancreas no.19 (Transverse section) **A-** fully formed acini. **B-** Islets of Langerhans**. C –** Intercalated duct.

Stain: Haematoxylin and eosin. **Magnification** 10x

Fig. 9. Fetal pancreas no.19 (Transverse section) **Arrow-** Islets of Langerhans lined by thin reticulin fibre.

Stain: Haematoxylin and eosin. **Magnification** 40x

Fig. 10: Fetal pancreas no.22 (Transverse section) **A-** acini (no branching pattern was seen). **B**- Islets of Langerhans.

Stain: Haematoxylin and eosin. **Magnification** 10x

Fig. 11: Fetal pancreas no.25 (Transverse section) **A-** Acini with large lumen. **B**- Interlobular duct lined by simple cuboidal epithelium. **C**- Islets of Langerhans.

Stain: Haematoxylin and eosin. **Magnification** 10x

Fig. 12: Fetal pancreas no.25 (Transverse section) **A-** Interlobular duct lined by simple cuboidal epithelium. **B-** Acini with large lumen. **C**- Large cluster of Islets of Langerhans**. D-**Reticuin fibre around islets.

Stain: Haematoxylin and eosin. **Magnification** 40x

Fig. 13: Fetal pancreas no.38 (Transverse section) **A-** Interlobular duct lined by simple cuboidal epithelium in thin connective tissue septa. **B-** Large cluster of Islets of Langerhans. **C**- Acini with large lumen.

Stain: Haematoxylin and eosin. **Magnification** 10x

Fig. 14: Fetal pancreas no.38 (Transverse section) **A-** Acini with Centro acinar cell. **B-** Large cluster of Islets of Langerhans.

Stain: Haematoxylin and eosin. **Magnification** 40x

Table1:- Group of fetuses with gestational week.

Groups	Gestational week	Number of foetuses
1	**12-16 Weeks**	**05**
2	**17-20 Weeks**	**06**
3	**21-24 Weeks**	**06**
4	**25-28 Weeks**	**02**
5	**29-32 Weeks**	**06**
6	**33-36 Weeks**	**04**
7	**37-40 Weeks**	**01**
	Total	**30**

Table 2:- Histological analysis of pancreas between 12-16 weeks of gestation.

Serial no.	Gestational age group(Weeks)	Pancreatic parenchyma	Exocrine	Endocrine

1.	**12-16 weeks**	Abundant mesenchyme present. Parenchyma appeared as collection of branched tubules lined by **cuboidal epithelium.** Pancreatic lobule consist **central duct** surrounded by several tubules. All are embedded in **mesenchyme.** **Some undifferentiated cells were seen in mesenchymal tissue.** 15 week onward general pattern of pancreas is more mature .	Blood vessels and numerous primitive ducts with few acini appeared. Groups of numerous developing acini lined by simple cuboidal epithelium were seen. At the branched tubules budding some developing acini were seen. Acini was arranged in small clumps with small lumen in pancreatic parenchyma seen 15 weeks onwards.	Smallest islets as solid knots of cells budding out from the ducts without capillaries were seen. Some smallest group of islets consist of a cord of cells grown out from duct. This stage is known as **"budding stage."** Some granulated cells seen in islets group after 16 weeks onward.

Table 3:- Histological analysis of pancreas between 17-20 weeks of gestation.

Serial no.	Gestational age group (weeks)	Pancreatic parenchyma	Exocrine	Endocrine
2	**17-20**	Abundant mesoderm with some developing acini cells. Interlobular ducts were seen, lined by simple cuboidal epithelium. Connective tissue is arranged around ducts. Interlobular, intercalated ducts were seen.	Proliferation of acini were seen. Acini arranged around islets like a shield for islets of Langerhans .	Islets of Langerhans arranged in clusters. Mesenchymal tissue arranged around islets clumps and made an indistinct lining. Blood vessels arranged near the clusters. Alpha, beta, delta and some undifferentiated cells arranged in clusters. Alpha cells were more in number than beta cells.

				Alpha cells reorganised by acidophilic in nature with large nuclei.

Table 4:- Histological analysis of pancreas between 21-24 weeks of gestation.

Serial no.	Gestational age group (weeks)	Pancreatic parenchyma	Exocrine	Endocrine
3	**21-24**	Parenchyma shows organisation into lobes and lobules. Some undifferentiated mesenchymal tissue was seen.	Acini ducts were lined by single columnar epithelium and some with compound epithelium. Wide lumen of acini. Zymogen granules were seen.	Islets of Langerhans cluster size gradually increased lined with cuboidal epithelium. **Islets concentration more in head region of pancreas**. From 22 weeks onwards beta cells were more in number than alpha cells. This gestational age is group suitable for transplantation of pancreas.

Table 5:- Histological analysis of pancreas between 25-28 weeks of gestation.

Serial no.	Gestational age group (weeks)	Pancreatic parenchyma	Exocrine	Endocrine
4	**25-28**	Highly vascularised Intralobularconnective tissue with minimum mesenchymal tissue.	Fully formed acini separated by connective tissue	Islets encapsulated by thin reticulin fibre.

Table 6:- Histological analysis of pancreas between 29-30 weeks of gestation.

Serial no.	Gestational age group	Pancreatic parenchyma	Exocrine	Endocrine
5	**29-32**	Lobes and Lobules packed with serous acini	No branching pattern was seen	Islets group of Langerhans is more mature. Islets were large and prominent. Equal distribution of islets of Langerhans in head,body and tail part

				of pancreas.

Table 7:- Histological analysis of pancreas between 33-36 weeks of gestation.

Serial no.	Gestational age group	Pancreatic parenchyma	Exocrine	Endocrine
6	**33-36**	Less connective tissue was arranged in parenchyma.	Acini with large lumen with centroacinar cells clearly appeared.	Size of islets were large with well-defined reticular lining capsule.

Table 8:- Histological analysis of pancreas between 37-40 weeks of gestation.

Serial no.	Gestational age group	Pancreatic parenchyma	Exocrine	Endocrine
7	**37-40**	Small amount of undifferentiated mesenchymal tissue was seen.	Thin septa among mature acini were seen. Centroacinar cells were arranged Mature acini were seen	**Islets concentration more in tail portion**. Large, vascular islets with well-defined reticulin fibre capsule. Alpha, beta and delta cells arranged.

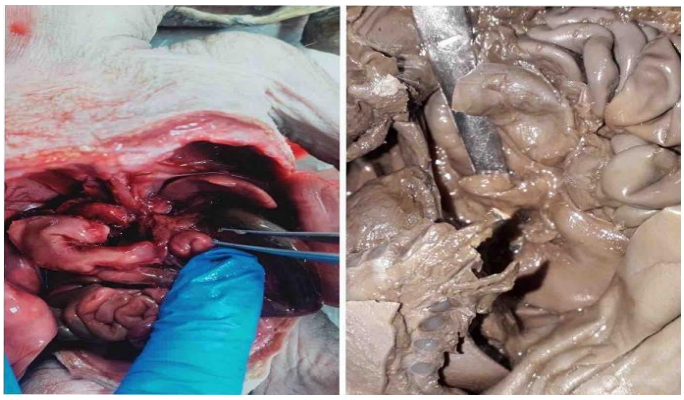

Fig. 1:- Stepwise dissection (a) opening up of abdominal cavity and (b) dissecting out pancreas.

Fig. 2:- Steps for tissue processing and staining of slide.

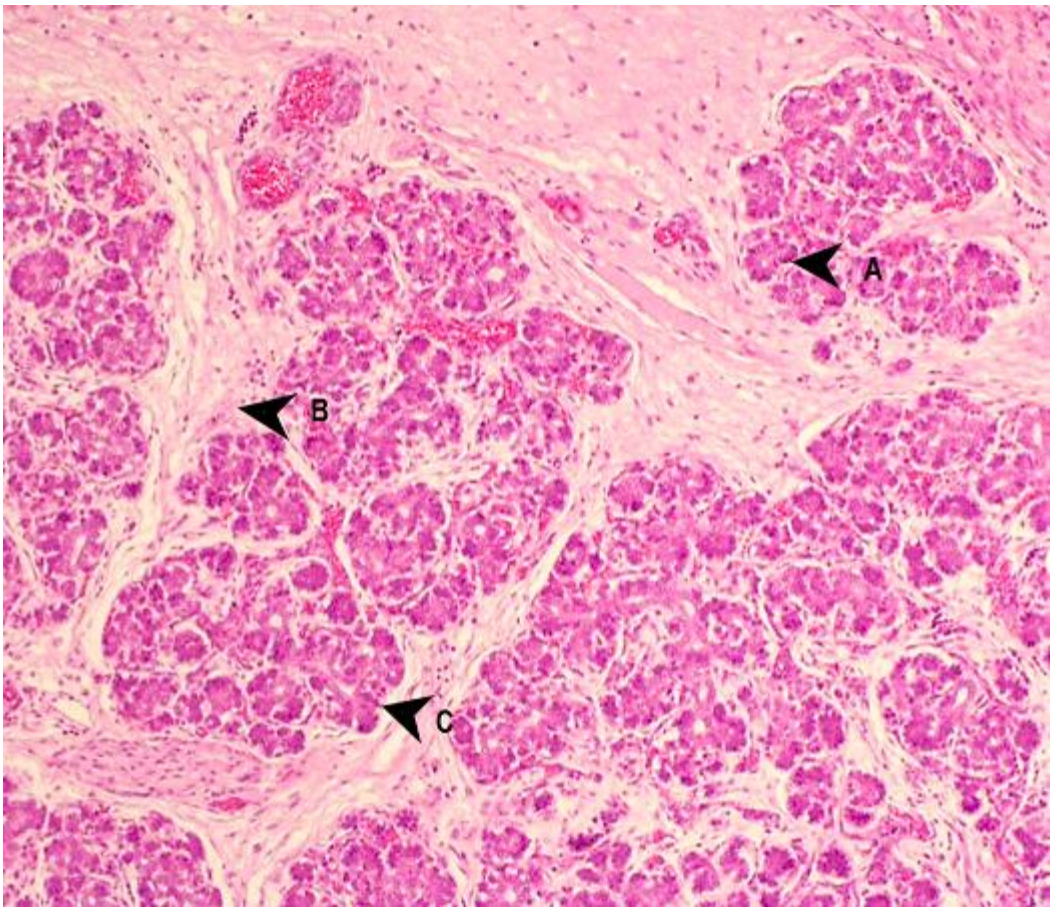

Fig. 3:- Fetal pancreas no.1 (Transverse section) **A** – developing acinus with small lumen. **B –** Abundant mesenchyme with some undifferentiated cells. **C-** Budding of islets of Langerhans.
Stain: Haematoxylin and eosin. Magnification 10x

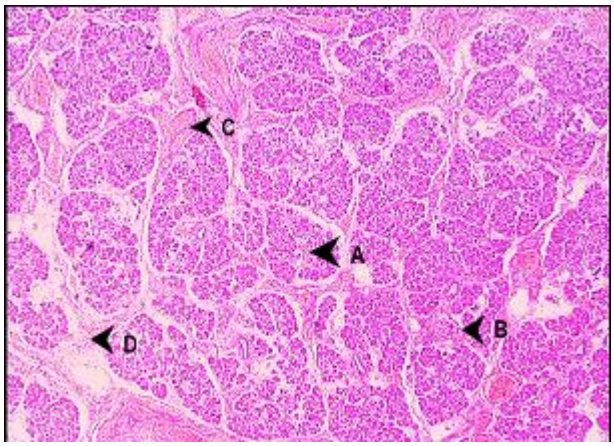

Fig. 4:- Fetal pancreas no. 8 (Transverse section) **A** –acinus in pancreatic parenchyma. **B-** Cluster of islets of Langerhans. **C-** Blood vessels in mesenchyme**. D-** abundant mesenchyme with some undifferentiated cells.
Stain: Haematoxylin and Eosin. **Magnification** 10x.

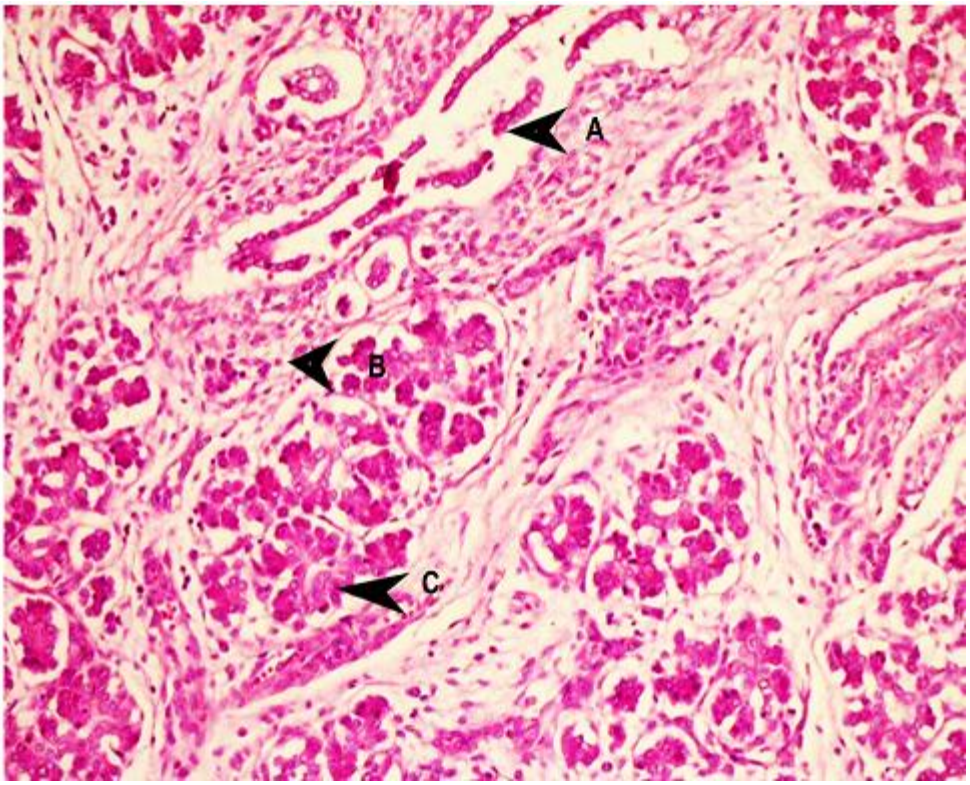

Fig. 5:- Fetal pancreas no. 8 (Transverse section) **A** – Interlobular duct lined by simple cuboidal epithelium. **B-** Cluster of islets of Langerhans. **C-** Acini.
Stain: haematoxylin and eosin. **Magnification** 40x.

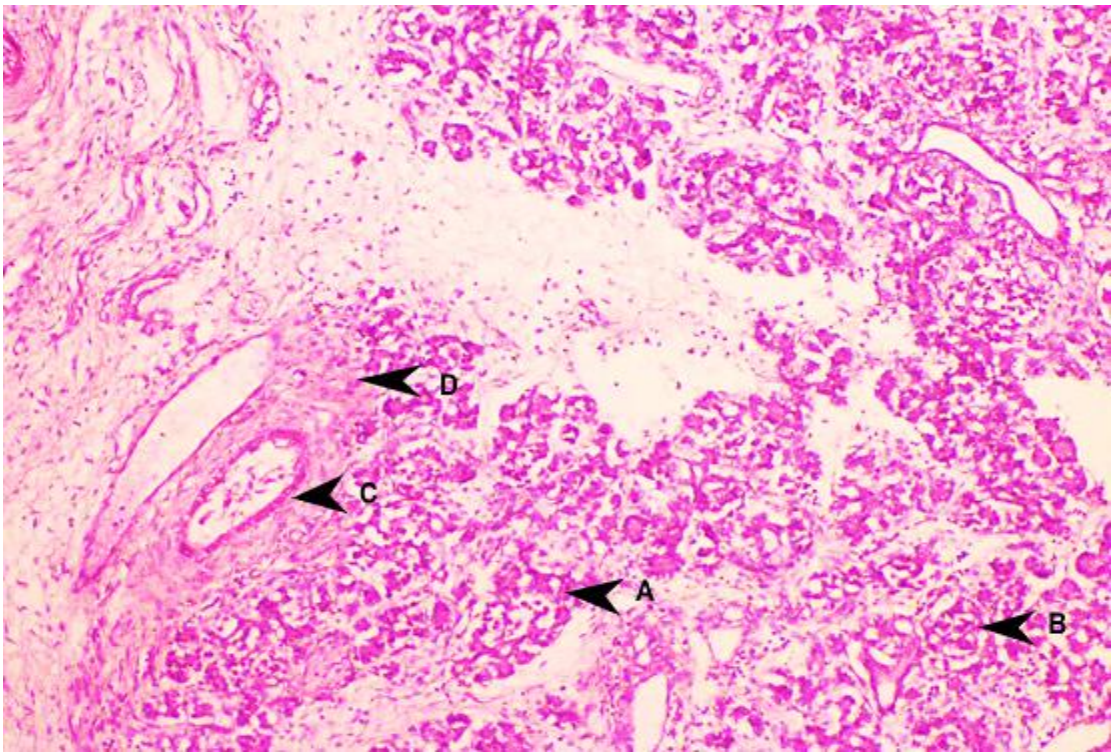

Fig. 6:- Fetal pancreas no.13 (Transverse section) **A** – Acini. **B-** Cluster of islets of Langerhans lined by cuboidal epithelium. **C-** Interlobular duct. **D-** Mesenchyme, parenchyma showed organisation into lobes and lobules.
Stain: haematoxylin and eosin**. Magnification** 10x

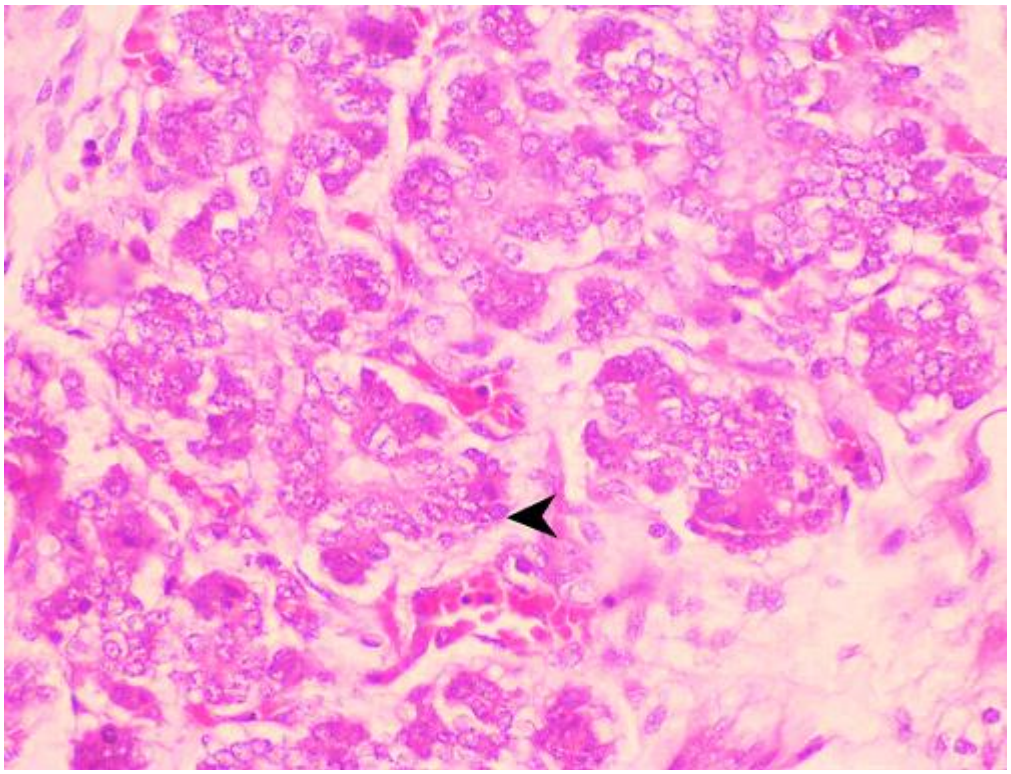

Fig. 7:- Fetal pancreas no.13 (Transverse section) Acini ducts lined by simple columnar epithelium with some compound epithelium, wide lumen with some zymogen granules.
Stain: Haematoxylin and eosin. **Magnification** 40x

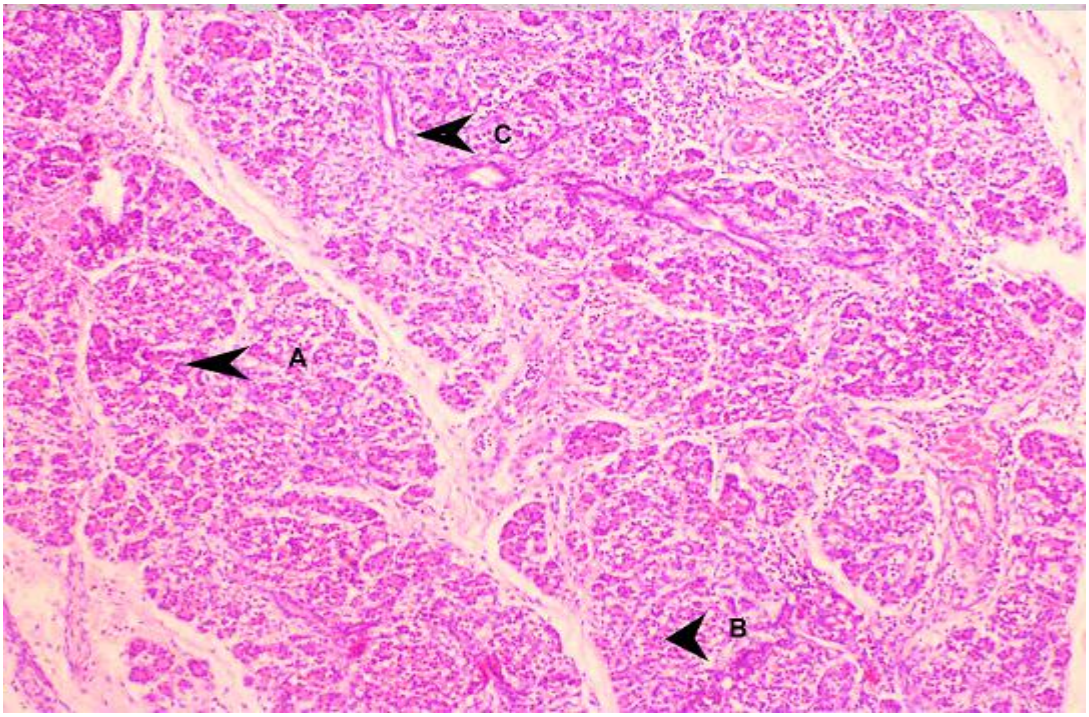

Fig. 8:- Fetal pancreas no.19 (Transverse section) **A-** fully formed acini. **B-** Islets of Langerhans**. C –** Intercalated duct.
Stain: Haematoxylin and eosin. **Magnification** 10x

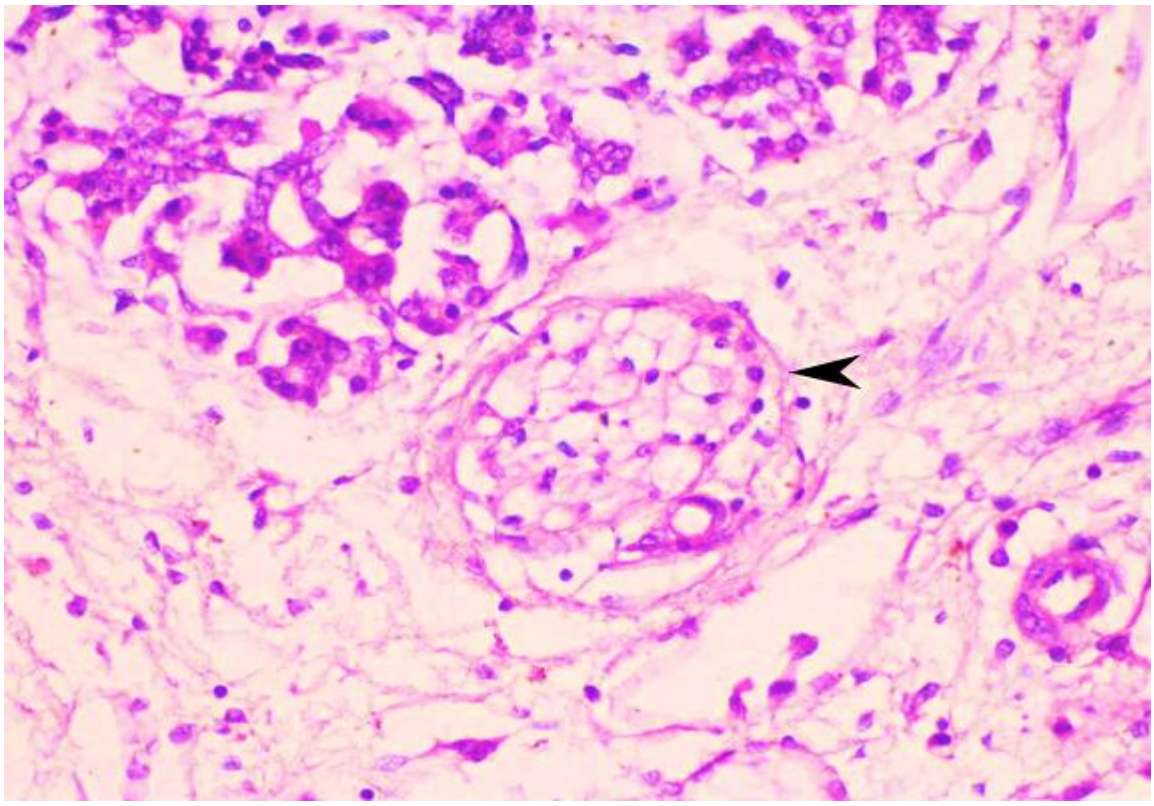

Fig. 9:- Fetal pancreas no.19 (Transverse section) **Arrow-** Islets of Langerhans lined by thin reticulin fibre. **Stain:** Haematoxylin and eosin. **Magnification** 40x

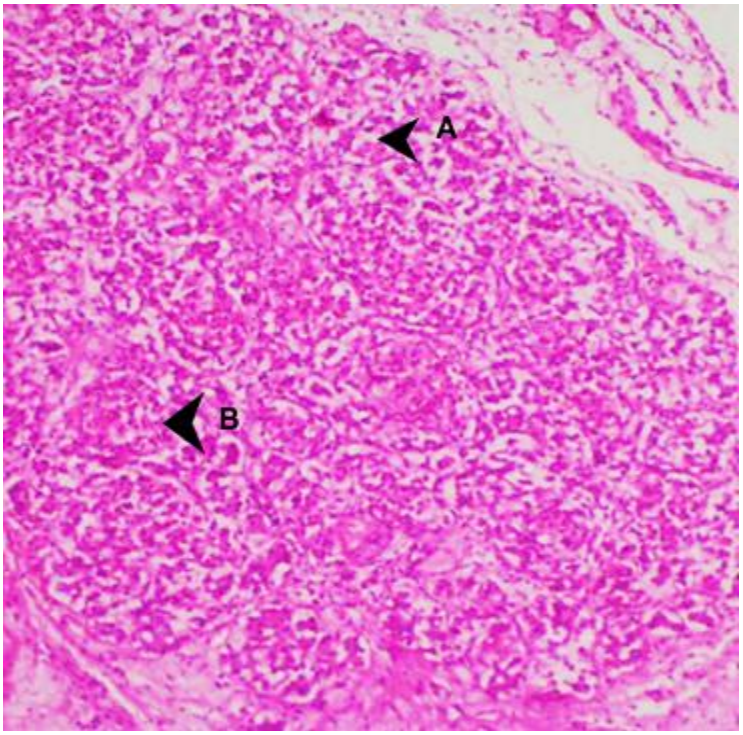

Fig. 10:- Fetal pancreas no.22 (Transverse section) **A-** acini (no branching pattern was seen). **B**- Islets of Langerhans.

Stain: Haematoxylin and eosin. **Magnification** 10x

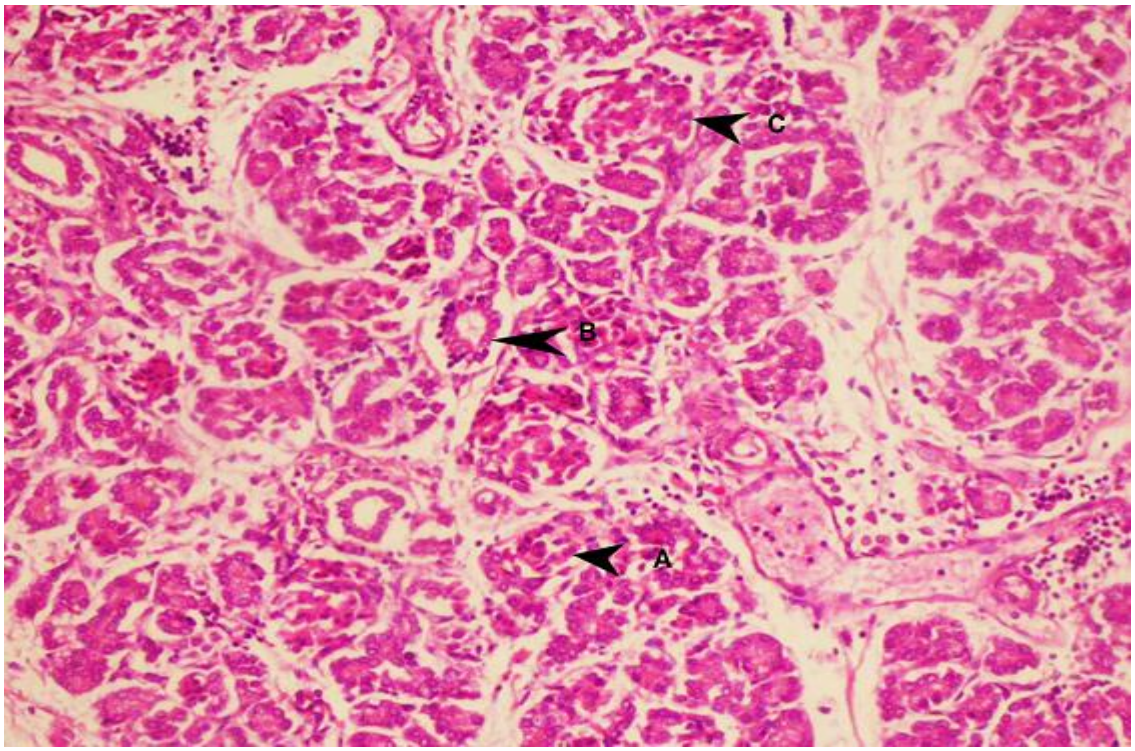

Fig. 11:- Fetal pancreas no.25 (Transverse section) **A-** Acini with large lumen. **B**- Interlobular duct lined by simple cuboidal epithelium. **C-** Islets of Langerhans.
Stain: Haematoxylin and eosin. **Magnification** 10x

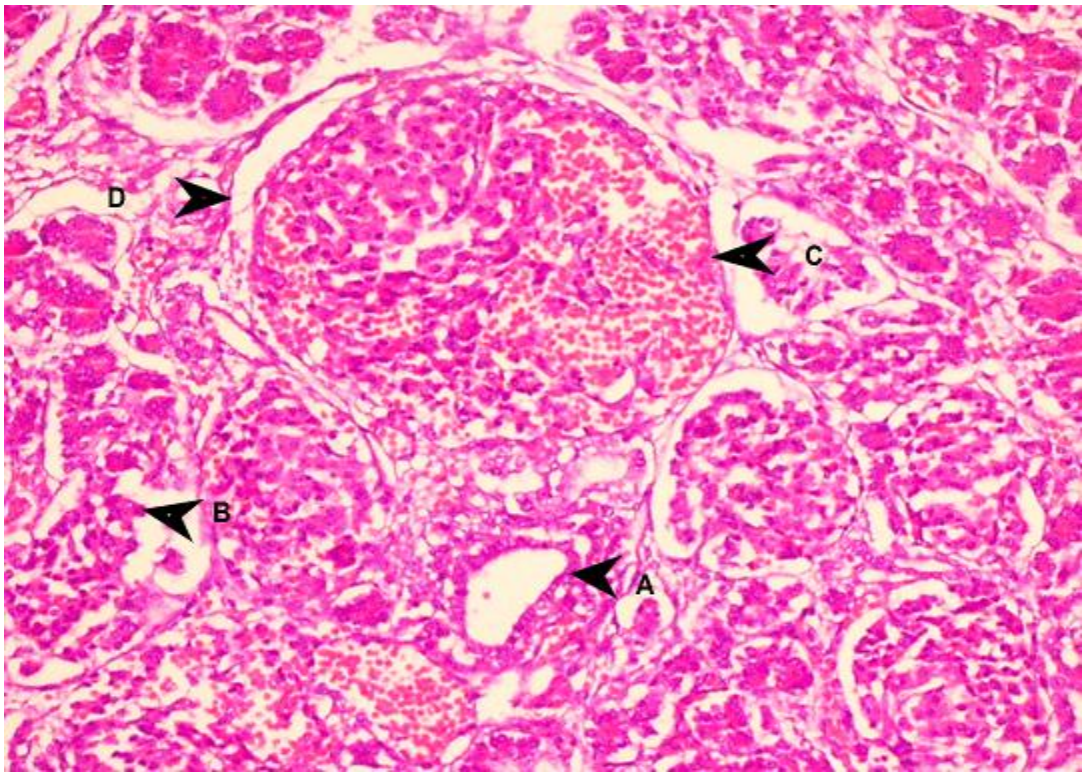

Fig. 12: Fetal pancreas no.25 (Transverse section) **A-** Interlobular duct lined by simple cuboidal epithelium. **B-** Acini with large lumen. **C-** Large cluster of Islets of Langerhans**. D-** Reticuin fibre around islets.
Stain: Haematoxylin and eosin. **Magnification** 40x

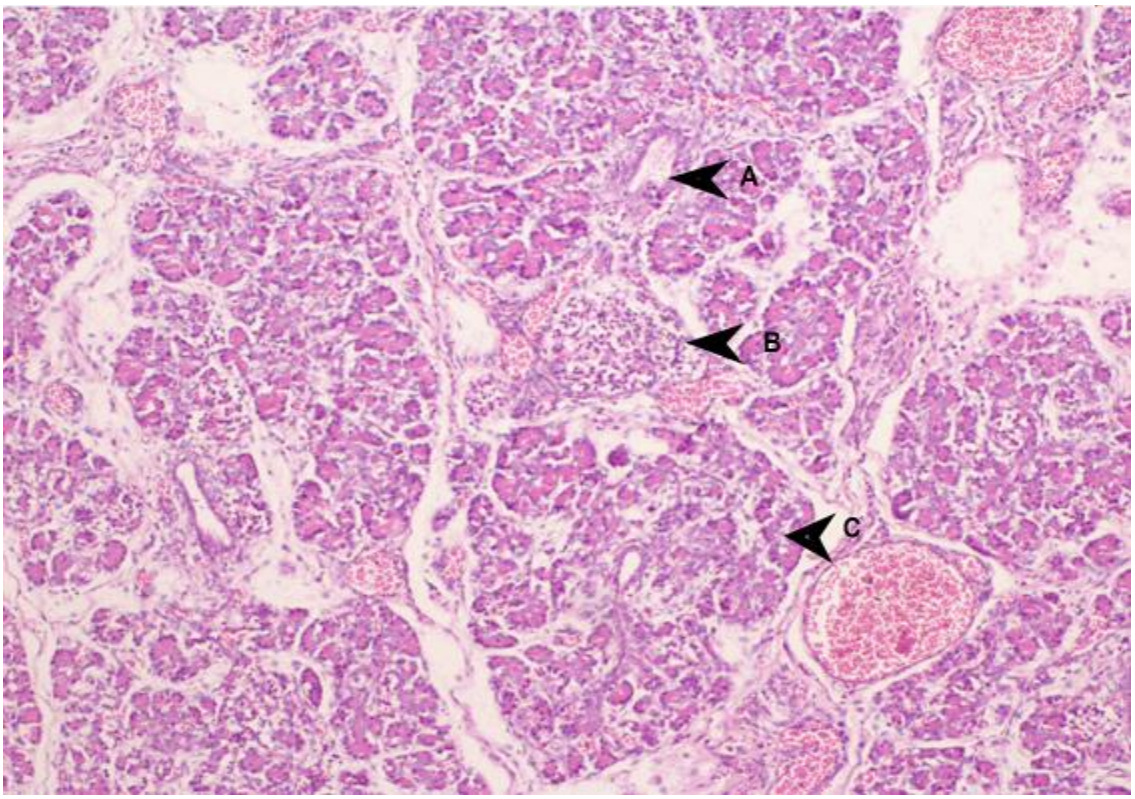

Fig. 13:- Fetal pancreas no.38 (Transverse section) **A-** Interlobular duct lined by simple cuboidal epithelium in thin connective tissue septa. **B-** Large cluster of Islets of Langerhans. **C-** Acini with large lumen.
Stain: Haematoxylin and eosin. **Magnification** 10x

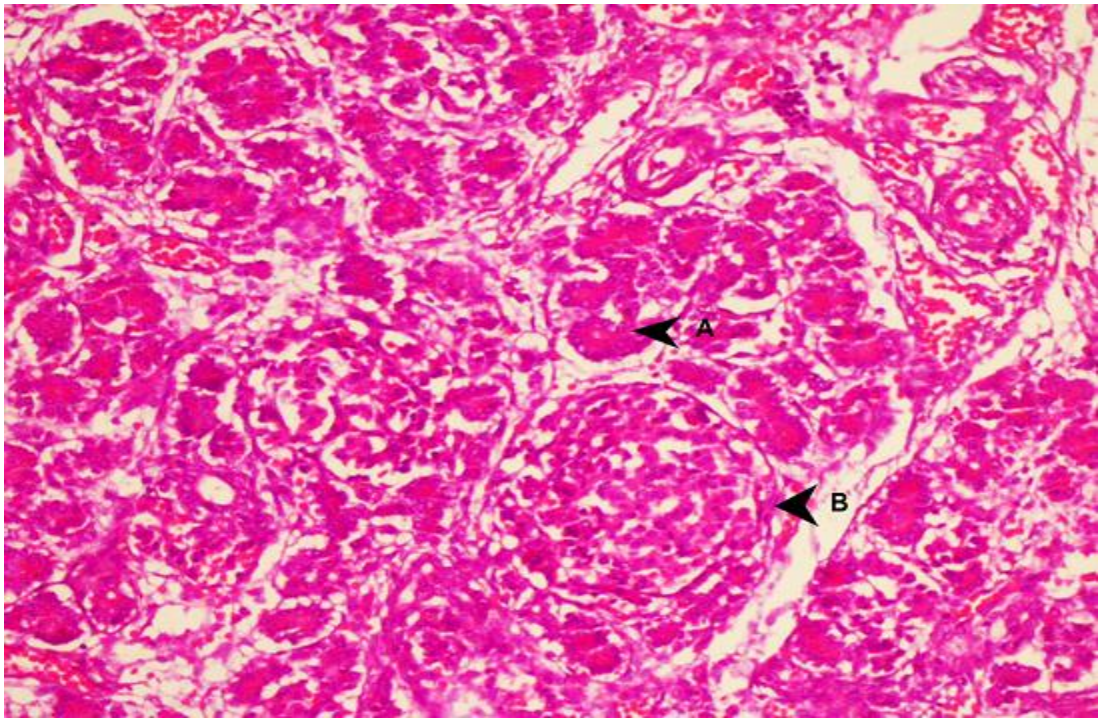

Fig. 14:- Fetal pancreas no.38 (Transverse section) **A-** Acini with Centro acinar cell. **B-** Large cluster of Islets of Langerhans.
Stain: Haematoxylin and eosin. **Magnification** 40x