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A Human Embryo of Streeter Age Group XXIII

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= Abstract =A human embryo considered to belong to stage XXIII of Streeter's developmental horizon is described. The embryo was obtained from a total hysterectomy specimen and prepared into serial sagittal sections in order to evaluate its gestational age.

The embryo was assigned to a total developmental score of 59.5 points based on numerical evaluation of the key organs, the points falling within the range of 48 to 60.5 points of Streeter stage XXIII. The crown-rump (CR) length of the embryo measures 30.5 mm and the ovulation age is estimated to be 47 ± 1 days by Streeter's criteria, and 56 days by Moore's criteria.

The distinguishing features of the embryo include the brain at the five-vesicle stage with well-demarcated flexures, dense matting of retinal pigment granules, the heart with distinct four chambers and a complete partitioning of the atrioventricular canal, prominent progressive dichotomous branchings of the secondary bronchi, the rotation of the duodenum to the right, fusion of the pancreatic buds fused, the superior and inferior parathyroid glands lying on the dorsal surface of the thyroid gland, and a discrete thymus overlapping the heart. All of the key organs are marked by characteristics registering stage XXIII except for the cochlea, cartilage and bone, both of which belong to stage XXII.

Key words: ***Embryo, Development, Streeter age group***

INTRODUCTION

The determination of the proper level of development of recovered human embryos, (e.g., abortion, hysterectomy) relative to other specimens, is done by setting up arbitrary stages or "horizons" established by detailed histogenetic data. Although general impressions determined from external characteristics and measurements of length had sufficed with simpler specimens, size alone may be an unreliable criterion. The assessment of the developmental status of the various organs in the case of embryos advanced into the more specialized levels may be necessary, because not all embryos undergo the same rate of growth.

In reference to this problem, Streeter (1951) established "developmental horizons" I to XXIII of which the horizons XIX to XXIII are of embryos aged 38 to 47 days, using a method of assigning point scores to the development of selected key organs. These key organs are the cornea, optic nerve, hypophysis, vomeronasal organ, submandibular gland, kidney, cartilage and bone.

This report describes a human embryo with a score of 59.5 total number of points based on rating the key organs, thus thought to belong to age group XXIII of Streeter's developmental horizon.

CASE REPORT

A human embryo (ESR #67) obtained from hysterectomy on a woman hospitalized at Seoul National University Hospital was fixed in 10% formalin, embedded in paraffin, serially sectioned in the sagittal plane in 7 μm thickness, prepared into slides and stained with hematoxylin-eosin. The total slides numbered 1 to 216 starting from the right to left side of the embryo (Fig. 1).

External view of embryo (Plate 1): In the terminal horizon XXIII demarcating the embryonic period, the head takes on a more distinct, rounded form, showing human characteristics, and the eyelids, which have been gradually thickening have

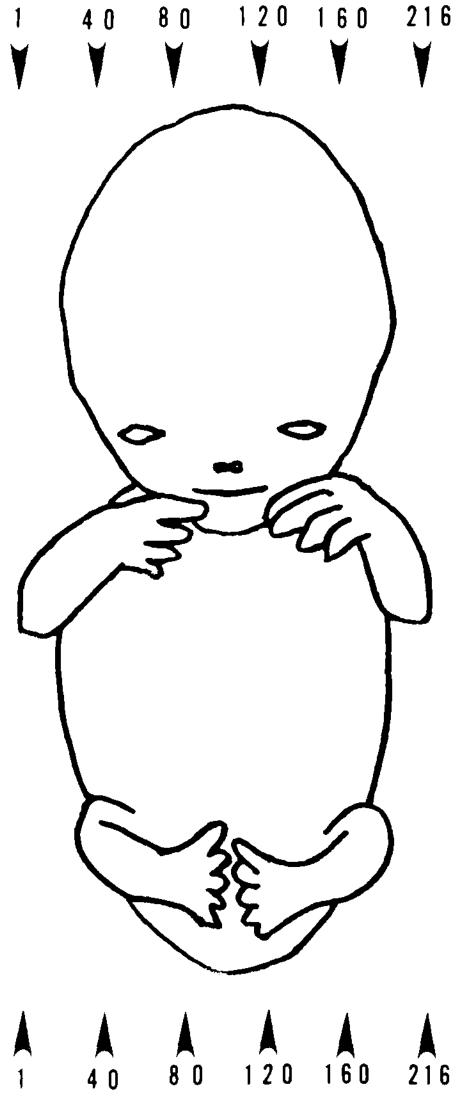

Fig. 1. Schematic reconstruction of the embryo in this report. Number represents the number of slide section.

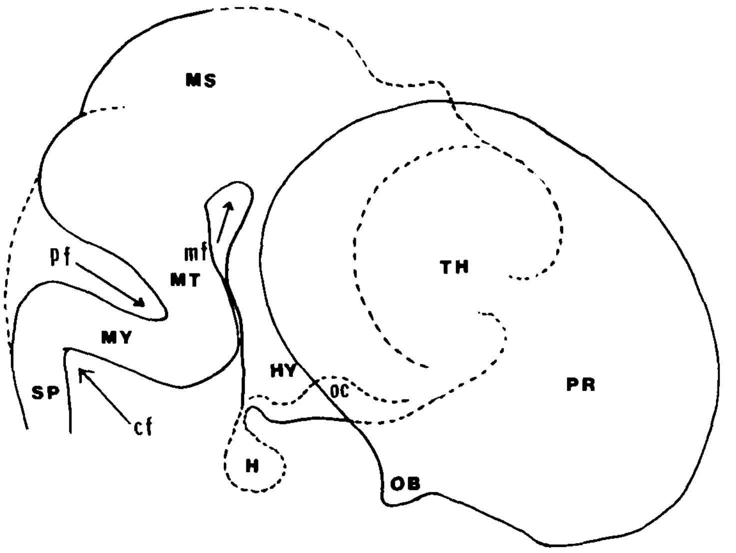

Fig. 2. Schematic reconstruction of the central nervous system.

CF: cervical flexure	OB: Olfactory bulb
H : Hypophysis	OC: Optic chiasm
HY: Hypothalamus	PF: Pontine flexure
MF: Midbrain flexure	PR: Prosencephalon
MT: Metencephalon	TH: Thalamus
MY: Myelencephalon	SP: Spinal cord

now encroached upon the eyeballs, but are united by epithelial fusion. The extremities have increased markedly in length compared to horizon XXII, and the digits are clearly defined. The forearm rises above the level of the shoulder, and prominent knee-bending is noted. The lengths of embryos of horizon XXIII, according to Streeter's collection, range from 26 to 32.2 mm; this embryo measures 30.5 mm, thus belonging to the more advanced relative position within the horizon. The estimated ovulation age is 47 $\pm$ 1 days.

Central Nervous System: The developing brain is at the five-vesicle stage showing a distinct telencephalon, diencephalon, mesencephalon, metencephalon and myelencephalon (Fig. 2). The parts of the prosencephalon have formed cavities contributing to the formation of the third ventricle, with its lateral walls representing the thalamus bulging into the ventricle and forming the massa intermedia. The cerebral aqueduct formed from the neural canal is seen in the midbrain, and the walls of the dilated cavity of the metencephalon have developed into the pons and cerebellum. The myelencephalon, which gives rise to the medulla oblongata, is presented with its lateral walls already rotated around an imaginary longitudinal axis in the floor plate, a movement caused by the pontine flexure; the flexure has caused divergence of the lateral walls of the pons and spreading of the gray matter in the floor of the fourth ventricle. The neuroepithelial cells from the ventricular zone are seen migrating into the mantle and marginal zones. The ventral area of the medulla contains a pair of fiber bundles, the pyramid, consisting of corticospinal fibers descending from the developing cerebral cortex.

The cervical flexure demarcates the rhombencephalon from the developing spinal cord. Areas of degenerating notochord are surrounded by the developing vertebral body. The notochord is expanding slightly to form nucleus pulposus of the intervertebral discs. Dorsal spinal ganglia are well-formed, with definite central and peripheral axonic processes (Plate 10).

Eye: The development of the eye is radically different from other general or special sense organs in that its receptive area, the retina, is in reality part of the wall of the brain carried out first as a vesicle, and then invaginated to form the double-layered eyecup with the lining of the cup containing the cells which are specialized to form the light-sensitive elements. According to Duke-Elder (1963) during the period which lasts from the 6th week to the 3rd month (10 to 68 mm) the neuro-epithelium comprising the sensory retina continues to proliferate and divides to give rise to two temporary layers of cells, the inner and outer neuroblastic layers, leaving between them a narrow zone devoid of nuclei (the transient fibre layer of Chievitz). The pars optica retinae of our embryo under study is marked by the commencement of cellular specialization in the inner neuroblastic layer, while the outer neuroblastic layer seems relatively undifferentiated. The most internal cells of the inner layer assumes the characteristics of ganglion cells, a transformation which according to Duke-Elder, is evident at the 17 mm stage. These consist of small cells with dark-staining nuclei and little cytoplasm, which are already sending long axonic processes over the inner surface of the retina. These fibers at the point of continuity with the optic stalk leave the eyeball and course together along the optic stalk to form the optic nerve.

There is also a dense matting of retinal pigment granules and well-developed underlying choriocapillaries. The lens is spherical, with the elongated primary lens fibers running in a direct antero-posterior course. The anterior chamber is evident, lined with flattened mesenchymal cells. In the posterior chamber is seen primitive vitreous body enmeshing the capillaries of the hyaloid plexus, formed by invasion of the optic cup by mesenchyme by way of the choroid fissure.

The front margins of the optic cup constituting the pars caeca retinae have grown forward to form the ectodermal part of the iris, but the appearance of radial folds to form a single ciliary process is not yet evident. Also, only the outer of these double-layered epithelium is pigmented (Plate 2).

Cardiovascular System: The four chambers of the heart, the right and left atria located posteriorly, and the right and left ventricle anteriorly, are distinctly apparent in our embryo, along with a complete partitioning of the atrioventricular canal. Although the primitive atrium is incompletely divided into right and left atria, since the subsequent fusion of the septum primum and the septum secundum is not yet seen, the ventricles are separated by the interventricular septum and the subendocardial tissue is fused, resulting in the closure of the interventricular foramen (Plate 11). Also evident are the well-formed semilunar valve swellings developed from localized proliferations of the subendocardial tissue at the orifices of both the aorta and the pulmonary trunk, as are the developing atrioventricular valves connected to the ventricular wall by chordae tendineae. The ventricular walls are cavitated and a spongework of muscle bundles that will remain as trabeculae carneae and papillary muscles are apparent, and are especially prominent in the left ventricle.

Respiratory System: The larynx is bounded cranially by the epiglottis developed from the hypobranchial eminence. Laryngeal cartilages are evident in the branchial mesoderm and clearly delimited rings of cartilage are discernible along the trachea, but have not reached the lobar and segmental bronchi. The right primary bronchus is subdivided into three secondary bronchi and the left into two bronchi. Progressive dichotomous branching of the secondary bronchi forming the teriary bronchi with surrounding mass of mesenchyme developing into bronchopulmonary segments are prominent throughout the lung mesenchyme (Plate 12).

Digestive System: A narrow-lumened esophagus is seen dorsal to and roughly parallel the trachea and ventral to the bodies of the lower cervical and upper thoracic vertebrae, embedded in what resembles visceral mesoderm. The endodermal epithelium of the esophagus is still composed of pseudostratified columnar epithelial cells, many of which are ciliated prior to its gradual replacement by stratified squamous epithelium, probably by metaplasia (Hamilton, 1972).

The digestive tube as a whole shows the four characteristic fundamental layers throughout the entire tract. Although the histological differences in the mucosal layer which are important distinguishing characteristics of the various adult regions of the digestive tube are not quite apparent, the primordial gastric pits are recognizable. The small intestine is yet devoid of any villi projecting from the mucosa. The duodenum has rotated to the right, becoming C-shaped, and the ventral pancreatic bud carried dorsally with the bile duct has fused with the dorsal pancreatic bud. Upon fusion of the pancreatic buds, the ducts have anastomosed, and the main pancreatic duct is clearly observable along with the cystic and hepatic ducts connected to the common bile duct, the entrance of which is carried around to the dorsal aspect of the duodenum as the duodenum grows and rotates. The liver fills most of the abdominal cavity and shows diffuse hemopoietic foci. The hepatic cords of which have acquired lumens are arranged in mazes of interlacing cords, bestowing the hepatic parenchyma an anastomosing tubular pattern resembling the basic architectural pattern of the adult liver (Plate 13).

Other Organs: The thyroid diverticulum has grown and divided into the right and left lobes connected by an isthmus and lies anterior to the second and third tracheal rings. The thyroglossal duct has disappeared, but a persistent portion of the inferior end of the thyroglossal duct comprising the pyramidal lobe (Plate 15) is seen extending superiorly from the isthmus. Microscopically, the thyroid primordium, which previously consisted of a solid mass of endodermal cells has been broken up into a network of epithelial cords by invasion of the surrounding vascular mesenchyme. These cords are divided into small cellular groups with the formation of a few lumens in the cellular clusters. The cells have not yet become arranged in a single layer around the lumen. The fourth pharyngeal pouch has lost its connection with the pharynx and its dorsal portion has developed into the superior parathyroid glands, which are seen on the dorsal surface of the thyroid gland. The dorsal bulbar portion of the third pharyngeal pouch has also differentiated into inferior parathyroid glands which, after caudal migration, have separated from the thymus and are seen to lie on the dorsal surface of the thyroid gland as well.

The epithelium of the elongate ventral portions of the two pouches of the third pharyngeal pouch has proliferated, migrated medially, and fused to form the thymus. The descended thymus is noted at a more inferior position than the inferior parathyroid glands and is seen resting superior to the upper limit of the pericardial sac below and extending into the root of the neck above. The discrete thymus appears to be somewhat divided into two developmentally separate parts by a longitudinal fissure (Plate 14).

DISCUSSION

In compiling developmental horizons of human embryo age groups XIX to XXIII Streeter chose key organs which undergo marked histologic transformation during the period of observation. For each of the key organs, Streeter arbitraily selected progressing morphological characteristics. Each of these characteristics was given a numerical point score, depending on the degree of progression. The assignment of total developmental points per embryo is based on "schedules of scoring points". Each organ was graded; developmental characteristics were assigned numerical point scores. In this manner, the total developmental point score per embryo, as summated from the scores of the individual organs, was established and utilized in determining not only the horizon to which the embryo belongs, but also its relative position within that horizon (Table 1).

Table 1. Number of developmental points per embryo

Horizon	Number of points for youngest embryo within a given horizon	Number of points for oldest embryo with a given horizon
XIX	10	16.5
XX	19	29.5
XXI	30	39
XXII	40.5	46
XXIII	48	60.5

Table 2 shows a list of the key organs and the corresponding advancing characteristics as observed under the microscope of our embryo. The total developmental points were 59.5; since the range of points of Streeter horizon XXIII is from 48 to 60.5 (Table 1) our embryo is thought to belong to the older age group within horizon XXIII.

Key Organs: In order to assess the proper age of the embryo under study, a list of eight key organs and their advancing characteristics have been compiled as shown in Table 2. Further explanations and numerical evaluations are as follows:

The body of the cornea, which is the first of the key organs, is a thick mass delaminating into looser and more compact layers, sharply layered with a well-defined cuboidal mesothelial layer (Descemet's endothelium). It then becomes thick and fibrous. The body is 3 or more times as thick as the epithelium, and finally formed of thin, elongated endothelioid cells in horizons XXI, XXII, XXIII, and latter part of XXIII, respectively. The corneal body (mesoderm) of our embryo is a stratum more than 3 times as thick as the epithelium, with the nuclei of endothelioid cells thin and elongated. Thus assigned the score of 8 points which equates to the latter developmental position within the Streeter horizon XXIII (Plate 3).

Table 2. A list of key organs and their observed advancing characteristics as selected by Streeter for embryo ESR #67. Points at the level of the horizon are numerical values assigned to the characteristics.

Key Organ	Streeter Characteristics	Points	Horizon
Cornea	Corneal body: 3 or more times as thick as epithelium formed of thin, elongated cells	8	XXIII
Optic Nerve	Definite nerve sheath, bundles of fibers	9	XXIII
Cochlea	Tip turns up second time	6	XXII
Hypophysis	No trace of stalk. Oriented epithelial follicles, abundant angioblasts and capillaries present	6	XXIII
Vomeronasal Organ	Retrogression; shrinking sac	6	XXIII
Submandibular Gland	Lumens in terminal branches, angiogenesis beginning around epithelium	10	XXIII
Kidney	Long secretory tubules, high epithelium in some tubules	8	XXIII
Cartilage and Bone	Early osseous band, borders of shell sharp	6.5	XXII
Total Points		59.5	XXIII

	Streeter XXIII	ESR #67
Points	48 - 60.5	59.5
CR	26 - 32.3 mm	30.5 mm

The second of the key organs, the optic nerve, takes on the characteristics of the latter portion of horizon XXIII; in the transformation of the optic stalk into a nerve tract there is a distinct condensation or alignment of the cells in the bordering mesenchyme, forming a definite nerve sheath layer. The neuroglial supporting tissue is well advanced with striate arrangement of nuclei and bundles of nerve fibres. Score of 9 points are thus assigned (Plate 4).

Thirdly, the cochlea, especially the cochlear duct, is evaluated. The cochlear duct practically completes its characteristic spiral growth during the period covered by age groups XIX to XXIII. According to Streeter horizon XXI the duct points definitely downward, grows horizontally, and then in XXII begins to point upward for the second time. Fig. 3 shows that the tip of the reconstructed cochlear duct is coiled upwards for the second time. It thus belongs to horizon XXII and is assigned 6 points.

The hypophysis, which is the fourth key organ, is characterized by findings belonging to horizon XXIII (Plate 5). A small portion of pars neuralis is evident, and the epithelial stem has regressed with

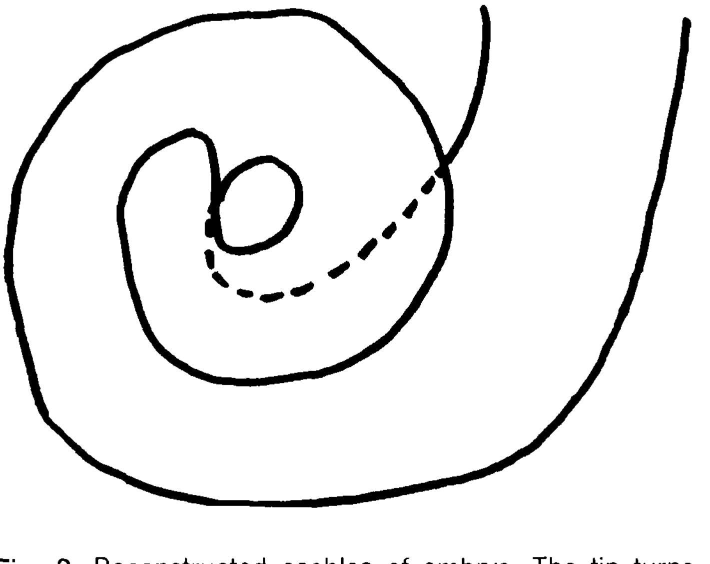

Fig. 3. Reconstructed cochlea of embryo. The tip turns up second time.

practically no trace of stalk remaining. The epithelium of the ventral part of the anterior lobe is markedly proliferated, partly subdivided into lobuli which project into the mesodermal component of the gland. In the vascular component of the anterior lobe, epithelial follicles are oriented with abundant angioblasts and capillaries. Small lateral processes of the pars intermedia are also observed, which, as shown by Atwell (1927), will fuse across the midline and develop into the pars tuberalis. According to Streeter, the pars tuberalis has not yet begun to grow forward in the terminal horizon XXIII. This is true of our embryo. A score of 6 points are given, in level with horizon XXIII.

The vomeronasal organ, the fifth key organ, arises from thickenings in the nasal epithelium as shallow grooves in group XIX, the grooves resulting in the formation of long, tapering ducts in subsequent groups. In group XXIII the duct begins to narrow, expanding caudally into blind tubular sac. Since our embryo shows an intermediately long duct, the retrogressing narrow caudal neck of which expands into a blind shrinking sac, a score of 6 points of horizon XXIII are given (Plate 6).

Next, the submandibular gland is evaluated. In horizon XXIII lumina as well as early angioblastic tissue appear in many terminal branches of the submandibular ducts. In our embryo, the gland consists of long ducts and many lumen-containing branches with angiogenesis beginning around the epithelium and mesoblasts forming layers around the glands. These findings equate to horizon XXIII, and 10 points are credited (Plate 7).

In embryos of horizon XX, Bowman's capsules of the kidneys, the seventh key organ, are spoon--shaped, in horizon XXII short secretory tubules and numerous large glomeruli present, and in XXIII long secretory tubules seen. In our embryo numerous large glomeruli as well as high epithelium in some of long secretory tubules are observed, thus suggesting that 8 points be given in accordance with the younger groups of horizon XXIII (Plate 8).

Finally, the cartilage of bones in horizon XIX gradually shows a clearing center. This is beginning of calcification as osteoblasts are fomed in horizon XXI. The developing osseous band then forms the early shaft shell in XXII, but the borders of shell are not yet sharp in XXII. The cartilage of the femur of our embryo is characterized by calcified cartilage matrix and periosteal ossification; since borders of the shaft shell are distinct and hence more advanced than horizon XXII, a score of 6.5 is assigned (Plate 9).

All of the above observations lead to the conclusion that our embryo belongs to Streeter age group XXIII. The estimated ovulation age is 47 $\pm$ 1 days. The assignment of ages of the embryos by Streeter in 1948, however, was based on comparative study done with macaque monkey embryos, and is now known to be slightly different especially for stages XIV to XXIII. Therefore, the general acception that the embryo at stage XXIII is at least 56 days old instead of 47 $\pm$ 1 days old, should be taken into consideration, provided that the embryo meets the criteria as given by Moore in 1982. These criteria are 1) the head is rounded and shows human characteristics, 2) the external genitalia still have a sexless appearance, 3) a distinct bulge is still present in the umbilical cord, and 4) the tail has disappeared.

The external appearance, as well as the process of organogenesis were observed to be normal in the embryo under our study.

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= 국문초록 =

배아 (Streeter 연령군 XXIII)의 1예

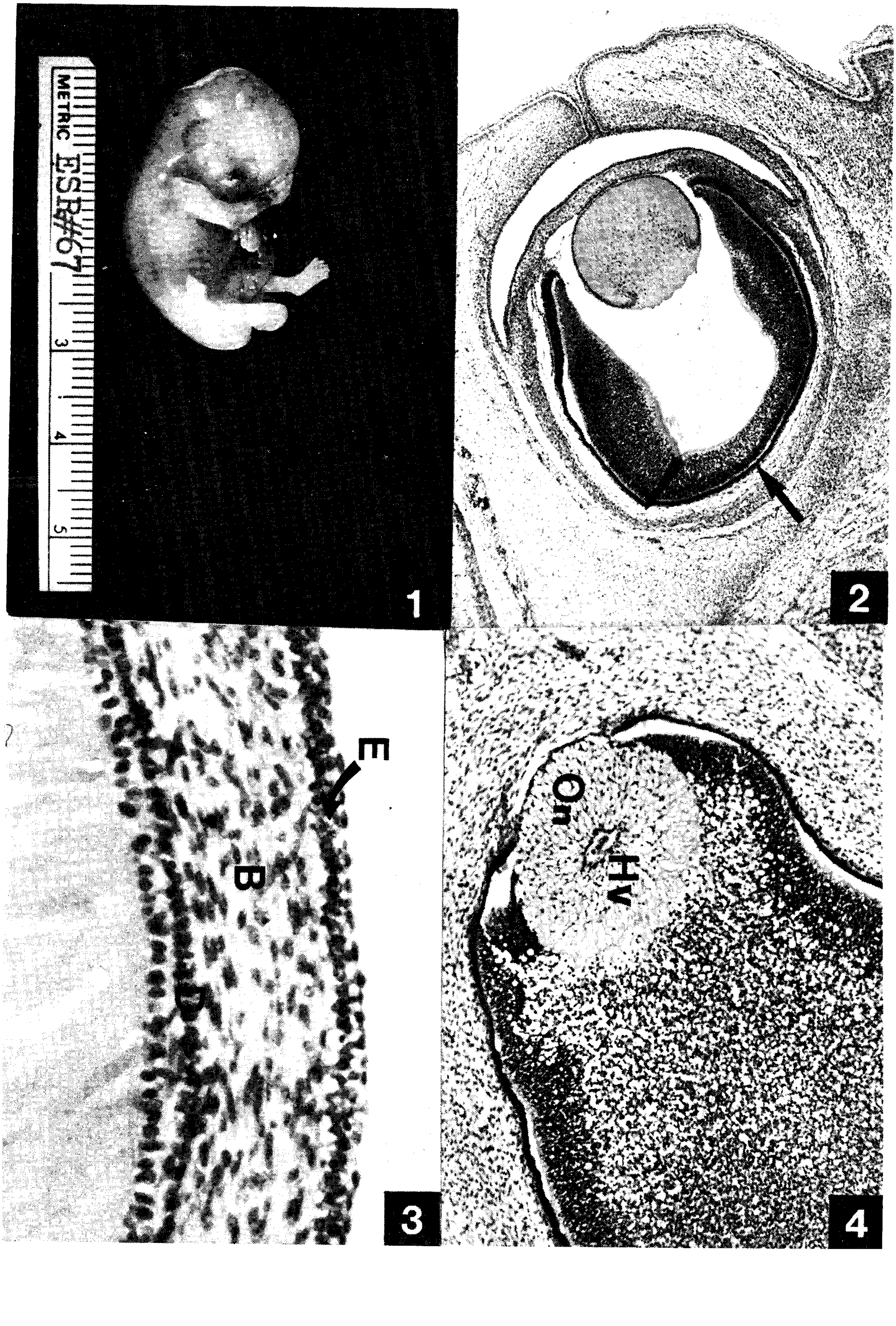
서울대학교 의과대학 병리학교실

지제근 · 하은신

저자들은 자궁적출술을 받은 환자에서 우연히 발견된 배아를 연속절편하여 얻은 216장의 시상 절편표본을 관찰하고 재구축한 결과 이 배아는 Streeter의 제 23연령군에 속하는 정상배아라고 판단되어 이를 발생학적으로 기술하였다. 본 배아의 형태학적 특징은 뇌가 잘 구분되는 flexures와 함께 5개와 vesicle로 구성되었고, 망막색소 과립이 강하게 뭉쳐 있었고, 심은 네개의 방실과 심방실의 완전한 구분이 되었고, 이차 기관지가 현저한 이분 분지 되었고, 십이지장이 우측으로 회전하여 췌아와 합했고, 상하 부갑상선이 갑상선의 배부에 있었고, 흉선이 심장위에 있었다.

LEGENDS FOR PLATES

Plate 1. The external form of the embryo.
Plate 2. (No. 179) The eye. The arrow points to the pigmented outer layer. H&E, X40
Plate 3. (No. 180) The corna. The body formed of thin, elongated endothelioid cells is a stratum more than 3 times in thickness as the epithelium. The epithelium (E), body (B), and Descemet's endothelium (D). H&E, X200
Plate 4. (No. 150) The optic nerve. Axonic processes of ganglion cells of the inner layer of retina form the optic nerve seen leaving the eyeball. The optic nerve (On), hyaloid vessels (Hv). H&E, X100
Plate 5. (No. 123) The hypophysis. The pars neuralis (PN), lumen of Rathke's pouch (R), and pars distalis (PD). H&E, X100
Plate 6. (No. 123) The vomeronasal organ. The arrow points to the blind shrinking sac of the expanded caudal end. H&E, X40
Plate 7. (No. 151) The submandibular gland. Many lumen-containing terminal branches as indicated by the arrow are seen as well as angiogenesis begining around the epithelium. H&E, X40
Plate 8. (No. 126) The kidney. Long secretory tubules are noted with high epithelium in some tubules. The glomerulus (G) and tubule (T). H&E, X100
Plate 9. (No. 173) Cartilage and bone. The femur shows calcified matrix and a distinct early osseous border indicated by the arrow. The femur (F). H&E, X40
Plate 10. (No. 97) The'spinal cord (SC). Short arrows point to the notochord (NC) and the curved arrow, to the abdominal aorta. The spinal ganglia (SG). H&E, X40
Plate 11. (No. 131) The heart. The ventricles are separated by the interventricular septum (IVS), right ventricle (RV) in communication with the pulmonary artery (PA). The left ventricle (LV), left atrium (LA), and bicuspid valves (BV). H&E, X40
Plate 12. (No. 127) The lung. The left primary bronchus (P). Secondary bronchi (S), and terminal bronchioles (T). H&E, X40
Plate 13. (No. 97) The bile duct system. The ventral pancreatic bud (VP) has fused with the dorsal bud (DP), forming the main pancreatic duct (PD). The duodenum (D), gallbladder (GB), cystic duct (CD), and hepatic duct (HD). H&E, X40
Plate 14. (No. 111) The thymus (T) lies superior to the pericardial sac below and extends to the neck above. H&E, X40
Plate 15. (No. 133) The thyroid gland (TG). The arrow points to the pyramidal lobe. H&E X40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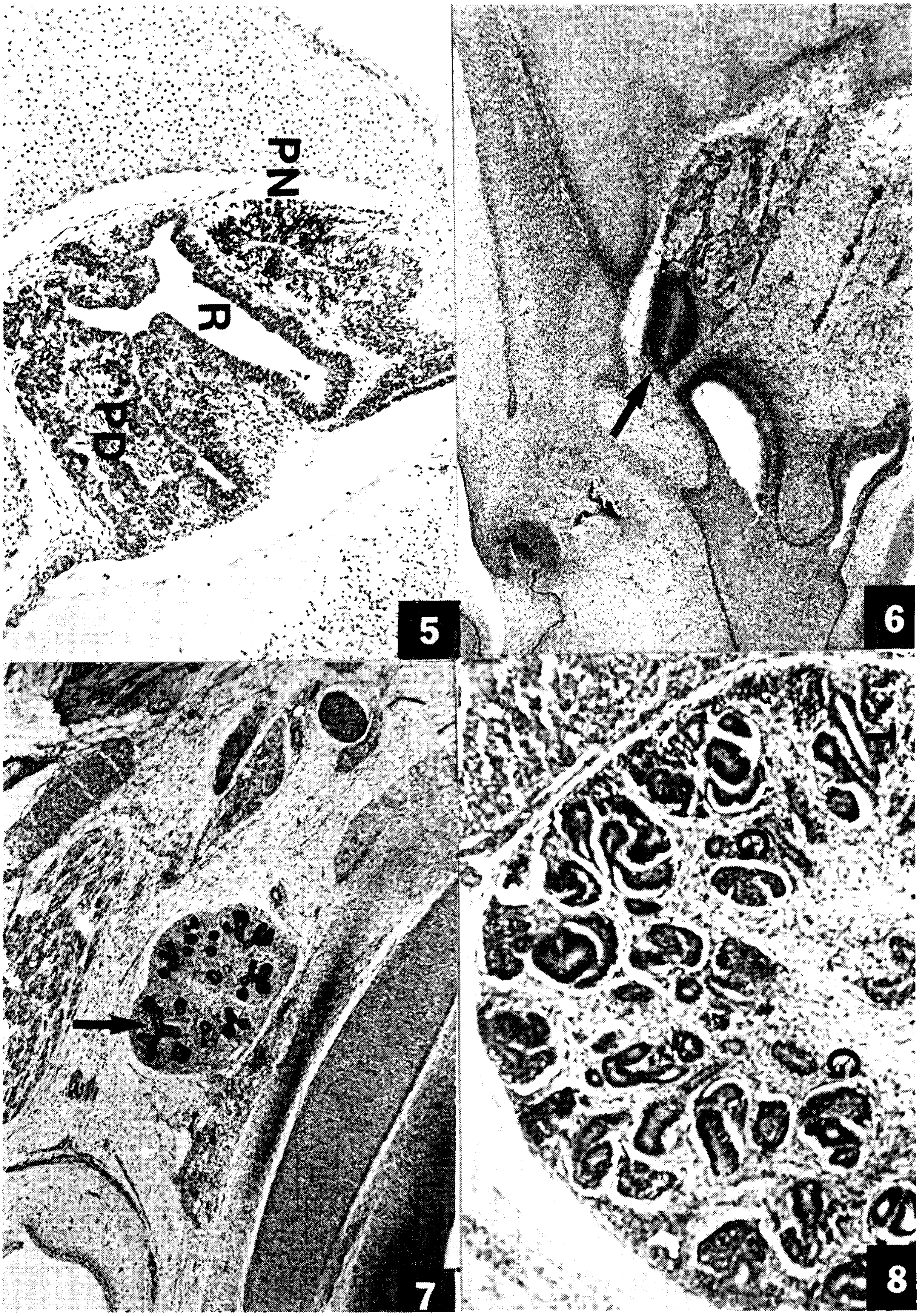
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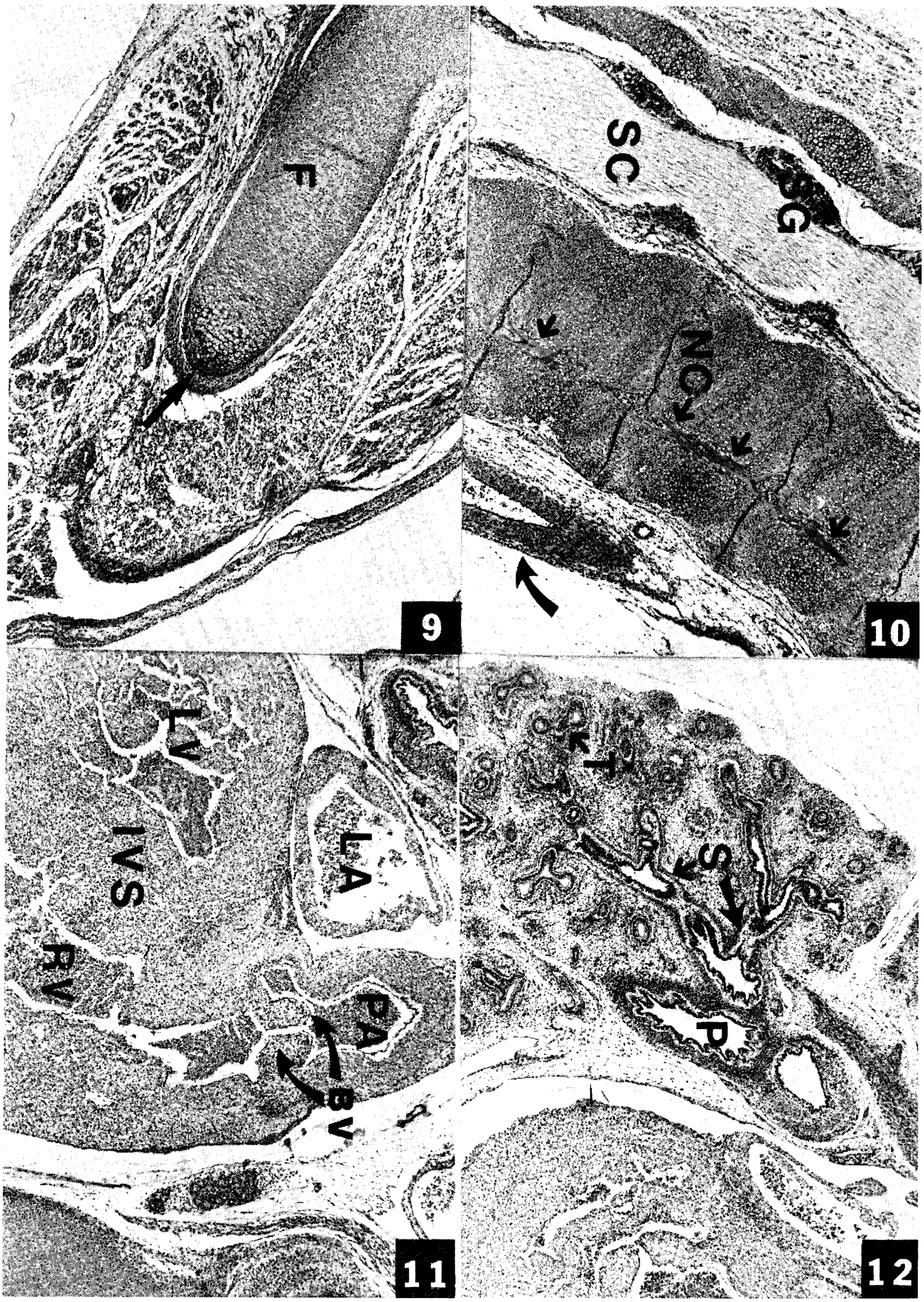
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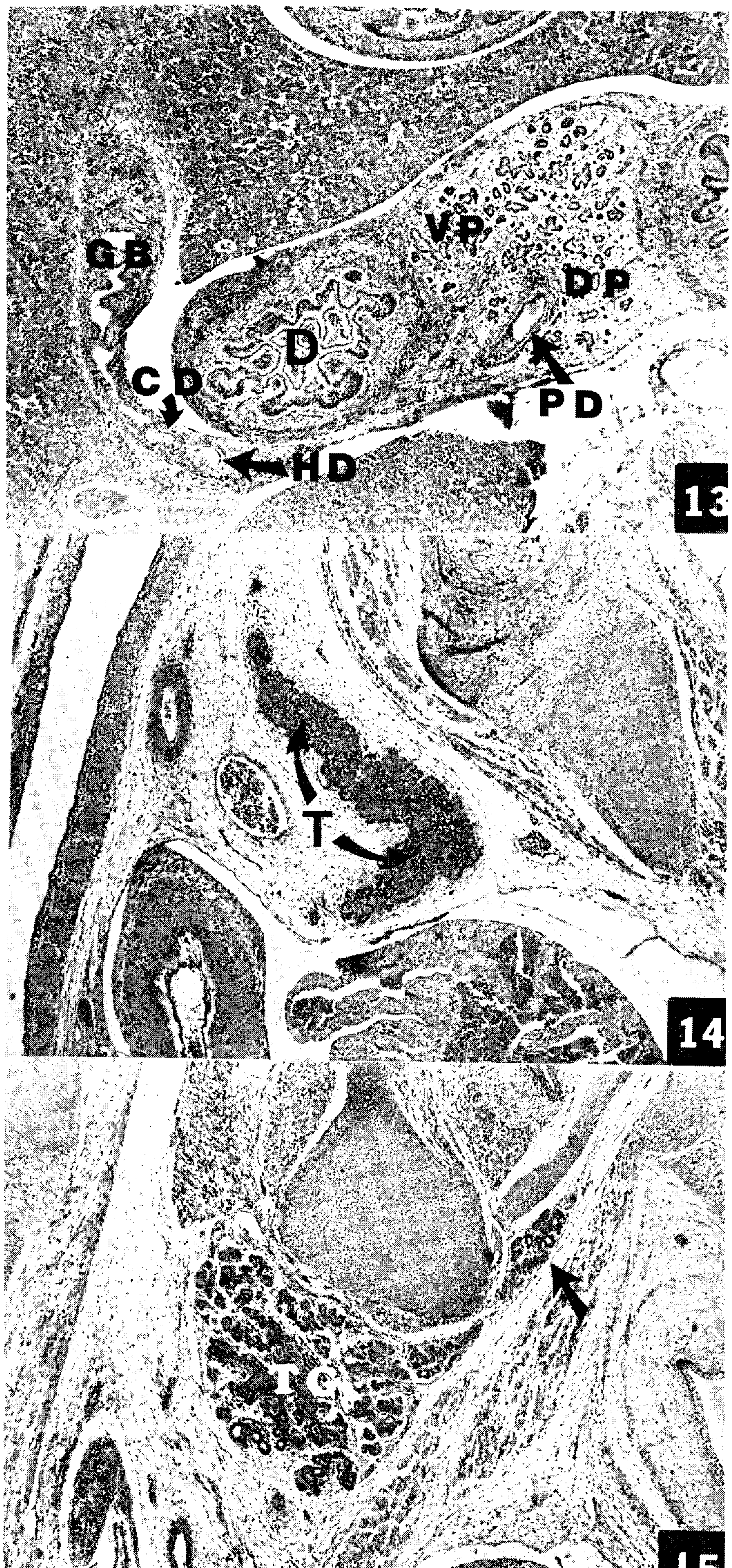
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