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VARIATION OF LEPTOMENINGEAL HEMORRHAGE LOCALIZATIONS IN FETI. IMPLICATIONS IN FORENSIC EVALUATION

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Abstract: The relatively high frequency of leptomeningeal hemorrhage instances in premature feti initiated a research project for the anatomic evaluation of dissemination pathways and of the collection of blood in this hemorrhage episode. The variation study of leptomeningeal hemorrhage localizations, also called subarachnoidian hemorrhages, requires a dissection protocol in order to visualize the lamellar structures of the pachymeninx, of the trabecular structures of the leptomeninx and of the relief forms generated by the neuronal structures. The analysis and evaluation of leptomeningeal hemorrhage study was carried out on 20 feti aged between 8 and 32 weeks, and 4 healthy adult brains aged between 39 and 50 years for the analysis and evaluation of relief forms at the level of the diencephalon and the brain stem. Our methods aim at fixation of tissues, dissection of the scalp and opening of the brain box by cutting the calvaria opercules or by topographically cutting on planes: midsagittal, parasagittal and horizontal. We believe that anatomic localizations heterogeneity of leptomeningeal hemorrhages is dictated by the morphogenesis of the dissemination pathways and the collection of bleedings as consequences of the evolution derivatives of the primitive neuronal tube, on the one hand, and the differentiation of the neuronal structures that participate in the making of relief forms, on the other. In the forensic evaluation of deceased feti of variable prematurity degrees suffering from leptomeningeal hemorrhages the following steps are taken: evaluation of the factors generating the hemorrhage episode, the prematurity degree, whether the fetus was alive or died antepartum, intrapartum or postpartum.

Keywords: dissection protocol, intracranial hemorrhage, pachimeninx, leptomeninx, prematurity.

INTRODUCTION

The study of leptomeningeal hemorrhages in feti – also called subarachnoidian hemorrhages – is of particular interest in identifying their emergence and evolution in time and space, antepartum, intrapartum and postpartum. In the first stage of our study we worked out a dissection protocol in order to visualize the lamellar conjunctive structures of the cranial dura mater, on the one hand, and the identification of the relief forms of the neuronal structures in the diencephalon and the brain stem, on the other. In the second stage we analyzed and evaluated the extraventricular localizations of the migration pathways and of the collection spaces of leptomeningeal hemorrhages, in morphogenetic dynamics of neuronal structures. The heterogeneicity of information in anatomic litarature regarding the names of leptomeningeal spaces and choroidian structures generated debatable issues regarding the interpretation and integration of our observations [1-4].

The aim of our work is the analysis and anatomic evaluation of the dissemination pathways and of the collection spaces for leptomeningeal hemorrhages in feti. The objective of the present paper is to work out a dissection protocol in order to visualize the compartmental conjunctive structures in the brain box and the analysis of the relief forms in the diencephalon and the brain stem with the view of evaluating leptomeningeal hemorrhages in feti of varied prematurity degrees.

MATERIALS AND METHODS

A) Materials

The study was carried out on 20 feti aged between 8 and 32 weeks and 4 adult brains, free from chronic or acute lesions taken from 2 men and 2 women

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aged between 39 and 50 years. The gestational age of feti was calculated in millimetres, on the basis of Crown – Rump value (Table 1).

B) Methods

By using the forthcoming methods, including our dissection protocol, we aimed at fixing/preparing the tissues, dissecting the scalp and opening the brain box.

Methods for tissue fixing

The fetal tissues were fixed in Baker 5% formaldehide-Calcium solution, buffered to Ph=7.4 in the unpaired umbilical vein visualized through the dissection of the umbilical cord. The samples taken from adults were pre-fixed in Baker 10% formaldehide-Calcium solution for 10 days and post-fixed in Baker 5% formaldehide-Calcium solution for 6 months [5].

Method for scalp dissection

In the first stage the fetus was suspended vertically, keeping it by the cervical region between the thumb and the forefinger. Then, the scalp was cut following the bi-mastoidian line that intersects the anterior fontanelle, and made two loops, an anterior one and a posterior one. The anterior loop, partly detached, was turned over the eyebrow arcades, while the posterior one was turned up, close to the external occipital protuberance. Thus we visualized the operculum of the calvaria in three ways – frontally, parietally and occipitally.

The frontal operculum corresponds to the frontal bone and is anteriorly delimited, towards the medial part, by the frontal part of the sagittal suture and posteriorly by the coronal suture (Fig. 1A-Index number:3;4 and Fig. 1D-Index:op fr).

The parietal operculum corresponds to the parietal bone and is delimited medially by the parietal part of the sagittal suture, and posteriorly by the lambdoid suture (Fig. 1B-D - Index number:5;6 ; Fig. 1F-Index number:5).

The occipital operculum corresponds to the squamous part of occipital bone and appears delimited anteriorly by the lambdoid suture (Fig. 1B-Index number:8; Fig. 1C-Index: op occ; 1F-Index number:8; 9).

Methods to open the brain box

The opening of the brain box was carried out by using the following methods: cutting the opercula of the calvaria and by topographically cutting up the sagittal and horizontal planes.

a) Cutting up the opercula of the calvaria

The opening of the brain box was carried out straight on the hemineurocranium by severing the three operculae of the calvaria, following the three routes of the future sections: the coronal suture, the sagittal suture, the lambdoid suture, the occipitomastoid suture, the parietomastoid suture and the squamous suture (Fig 1A-Index number:1; Fig.1B-Index numbers:5;9; Fig.1C-Index:op.occ; Fig.1D-Index :op.par).

After the removal of the opercula, we easily visualized the superior sagittal sinus and the falx cerebri and we cut into the longitudinal cerebral fissure in order to severe the corpus callosum and the cerebral peduncle in midsagittal plane. Then we extracted the right hand side cerebral hemisphere from the brain box. In the space thus formed the relationships between the lamellar structures of the cranial dura mater become visible: the falx cerebri, the falx cerebelli and the tentorium cerebelli (Fig.1E-Index numbers:15;14;12).

b)Topographic cutting methods of the brain box in sagittal and horizontal planes:

We used 3 methods of topographic cutting which we termed according to the cutting plane: midsagittal, parasagittal and horizontal. They enable the study of the spatial distribution of lamellar structures of the dura mater and the analysis of the compartments marked by the respective structures.

b1) Cutting in midsagittal plane:

The cutting of the brain box in midsagittal plane was carried out on the trace marked by the localization of the future the sagittal suture, on the line uniting the anterior fontanelle (Syn. Bregma) and the posterior fontanelle (Syn.Lambda). We employed this method: 1. to visualize the structures of the cranial dura mater that separates the supra- and sub-tentorial

Table 1. Antropological structure of the feti under study

Age (in weeks)	*Number of cases*	*Fetal Crown – Rump Length (mm)*	*Fetal Weight (grams)*	*Prematurity degree*
8	2	30 - 50	10 - 14	
16	1	165	300	Premature 4th degree
20	1	250	600	
24	4	275 - 300	1100 - 1200	Premature 3rd degree
28	8	325 - 350	1550 – 1750	Premature 2nd degree
32	4	380 - 400	2400 - 2600	Premature 1st degree

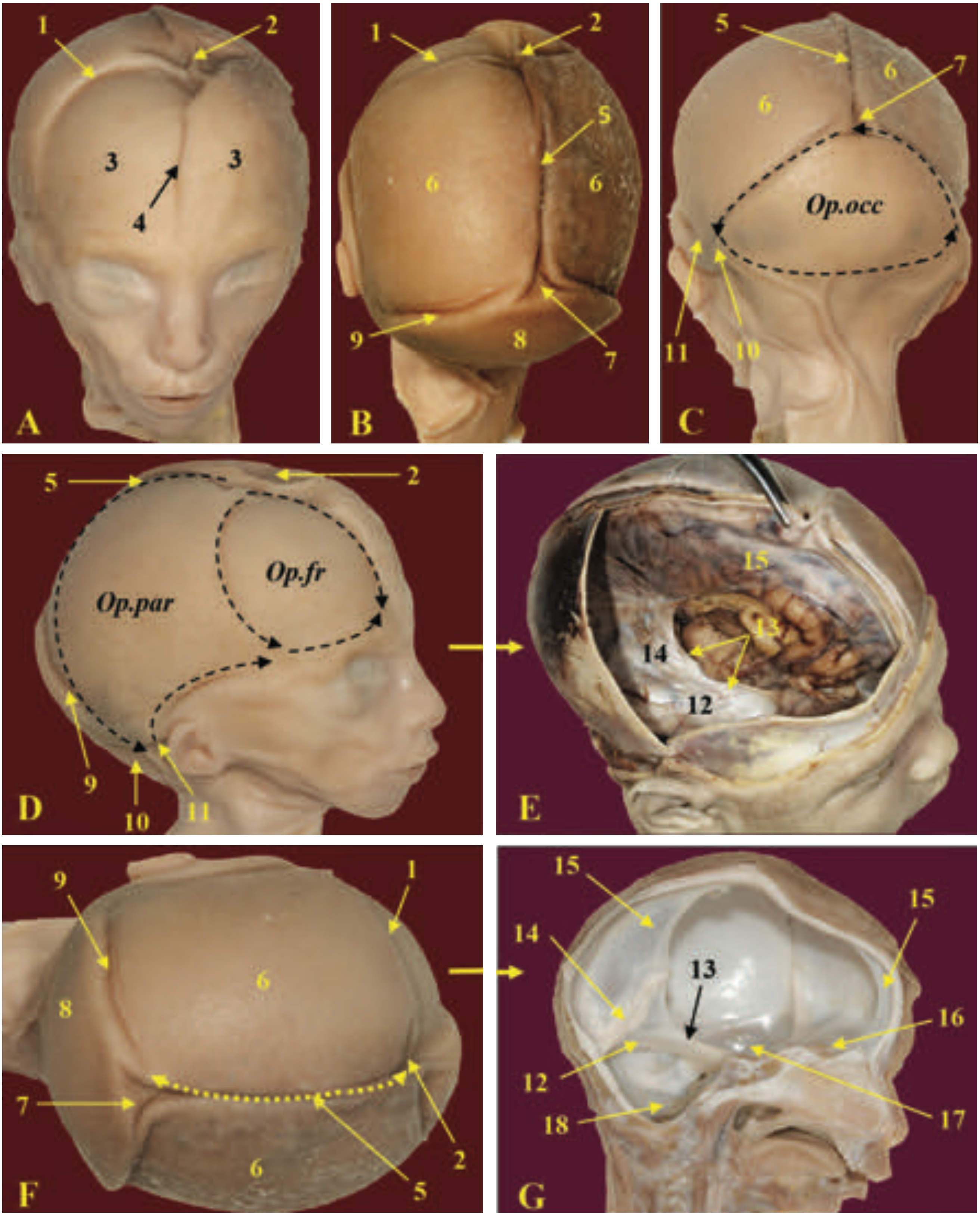

Figure 1. Access pathways into the brain box through: a). Cutting the calvaria opercula (A-F); b). Cutting in midsagittal plane through the cephalic extremity (G). 1.Site of future the coronal suture. 2.Anterior fontanelle. 3.Squama part of the frontal bone. 4. Frontal part of the sagital suture or the metopic suture. 5.Parietal part of the sagital suture. 6.Parietal bone. 7.Posterior fontanelle. 8. Squamous part of the occipital bone. 9.Site of future the lambdoid suture. 10.Site of future the occipitomastoid suture.11.Site of future the parietomastoid suture. 12.Tentorium cerebelli. 13.Tentorial notch or Incissura tentorium. 14. Falx cerebelli. 15. Falx cerebri. 16.Anterior cranial fossa. 17.Middle cranial fossa. 18.Posterior cranial fossa. Op.occ:Operculum occipital; Op.fr: Operculum frontale; Op.par:Operculum parietale.

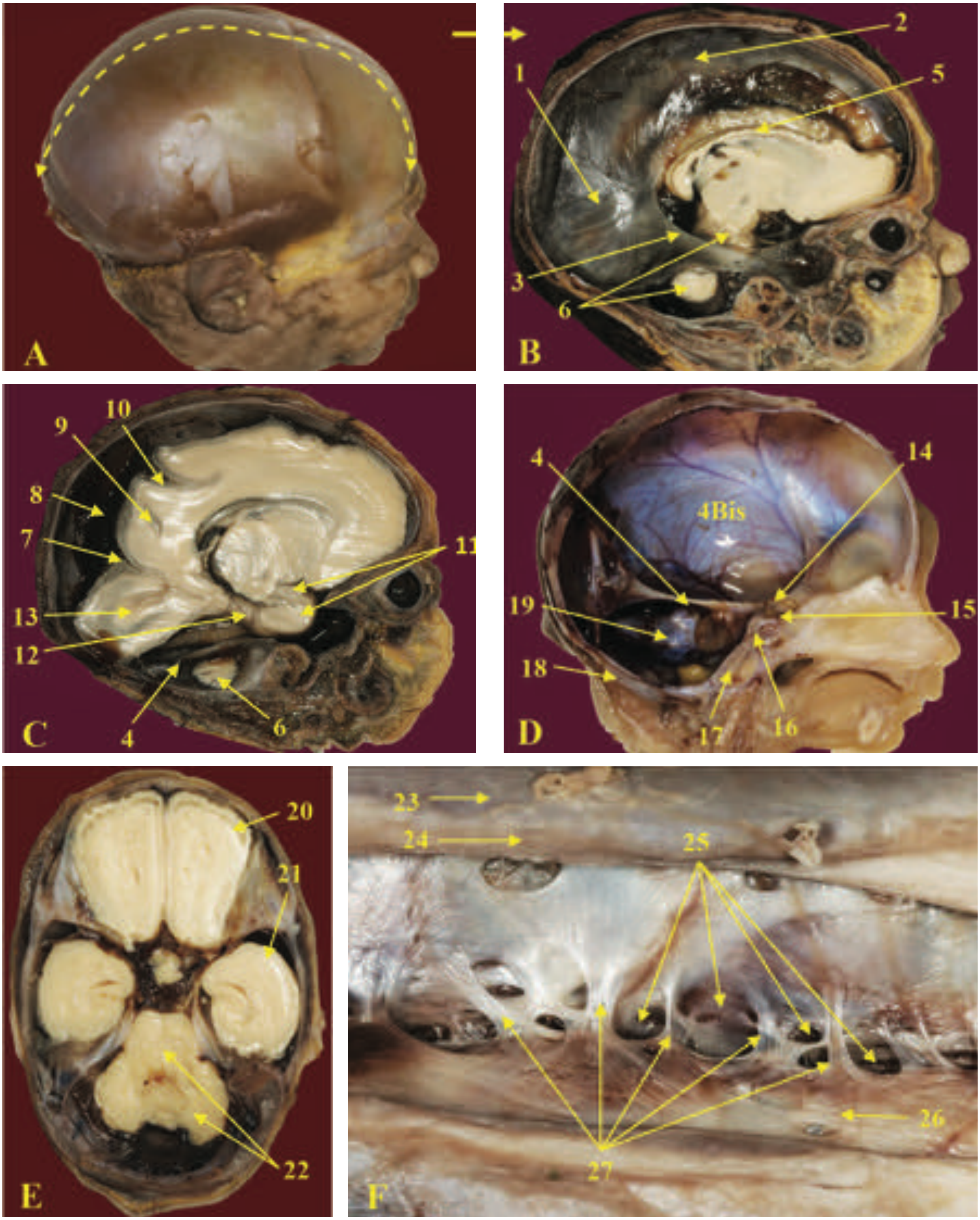

Figure 2. Localization of the conjunctive structures within the brain box: a). Lamellar structures of the cranial dura mater (B-E): b). Trabecular structures in the cranial arachnoid matter (f). 1. Falx cerebelli. 2. Falx cerebri. 3. Tentorial notch, or Incisura tentorium. 4.Tentorium cerebelli. 4.Bis Supratentorial space of the brain box containing mainly the left cerebral hemisphere. 5.Corpus callosum. 6.Superior cerebellar peduncles and and Cerebellum. 7.Parieto-occipital sulcus. 8.Subdurale space. 9. Marginal sulcus of Sulcus cinguli. 10.Central sulcus. 11.Temporal pole and Uncus. 12. Parahippocampal gyrus. 13.Occipital lobe. 14. Diaphragma sellae. 15.Hypohysial fossa. 16. Dorsum sellae. 17. Basilar part of the occipital bone. 18. Squamous part of the occipital bone. 19. Infratentorial space of the brain box containing cerebellum and part of the brain stem. 20. Frontal lobe in the frontal cranial fossa. 21.Temporal lobe in the middle cranial fossa. 22.Metencephalon in the posterior cranial fossa. 23. Cranial dura mater and subdural space. 24. Cranial arachnoid mater. 25. Subarachnoid space. 26. Cranial pia mater. 27. Arachnoid trabeculae.

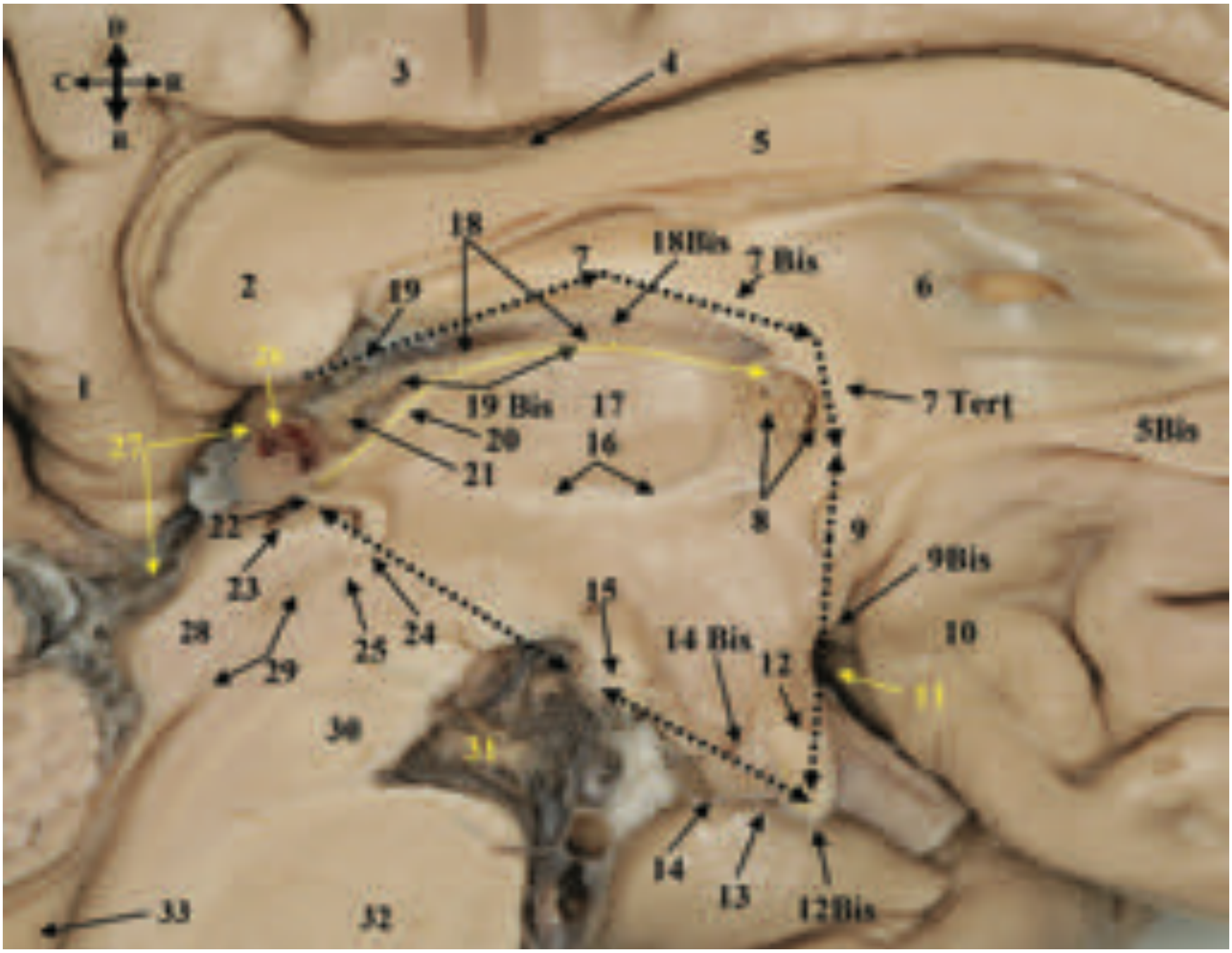

Figure 3. Midsagittal section through the adult brain. Relief forms of the medial face of the left cerebral hemisphere at the border between the telencephalon, diencephalon și mesencephalon. 1. Isthmus of the cingulate gyrus. 2. Splenium of the corpus callosum. 3. Cingulate gyrus. 4. Sulcus of the corpus callosum. 5. Trunk of the corpus callosum. 5Bis.Rostrum of the corpus callosum. 6. Septum pellucidum. 7.Crus of the fornix. 7 Bis. Body of the fornix. 7 Terț. Columna of the fornix. 8.Interventricular foramen and Choroid plexus of third ventricle. 9.Anterior commissure. 9Bis. Lamina terminalis 10.Frontal lobe. 11.Anterior (Rostral) perforated substance. 12. Supraoptic recess. 12Bis.Optic chiasma.13.Chiasmatic cistern 14.Median eminence of the tuber cinereum.14Bis.Infundibular recess. 15.Mammillary body. 16. Hypothalamic sulcus (Sulcus of Monro) .17.Interthalamic adhesion. 18. Space between the fornix and Epithalamus (Cistern of the transvers fissure or Cistern of the velum interpositum);18 Bis. Choroid membrane-foița superioară. 19. The internal cerebral vein. 19 Bis. Choroid membrane-foița inferioară). 20. Habenula. 21. Choroid plexus of the third ventricle. 22. Habenular commissure. 23. Pineal recess. 24. Posterior commissure. 25. Opening of aqueduct of the midbrain. 26. Pineal gland. 27. Space between the splenium, the tectum of midbrain and the cerebellum (Partea mediană a fisurii cerebrale Bichat). 28. Mesencephalic tectum. 29. Aqueduct of midbrain. 30. Cerebral peduncle. 31. Interpeduncular fossa. 32. Pons. 33. Fourth ventricle. Topography marks: D=Dorsalis (Synonym-Superior); B=Basalis (Synonym-Ventral;Inferior); R=Rostralis (Synonym-Anterior); C=Caudalis (Synonym-Posterior).

spaces of the brain box, (Fig. 1G-Index number:13;Fig. 2D-Index number:4); 2. to analyze the relief forms of the medial face of brain hemispheres in adults, at the frontier between the telencephalon, diencephalon and mesencephalon (Fig. 3); and 3. to analyze the localizations of leptomeningeal hemorrhages in their morphogenesis dynamics (Fig. 4).

b2) Cutting in parasagittal planes:

Cutting the brain box in two parasagittal planes at 5 and 15 mm, reported to the midsagittal plane, respectively, made it possible to analyze the relatioships between the brain and the lamellar structures of the cranial dura mater(Fig. 2B-Index numbers:1; 2; 3; 5; 6 and Fig. 2C-Index numbers:13; 12; 11; 6).

b3) Cutting in horizontal plane

Cutting the brain box in horizontal plane was carried out through the antropometric points glabela and inion. This section reveals the localization of the brain lobes in the fossettes of the internal surface of cranial (Fig. 2E-Index numbers: 20; 21; 22).

RESULTS

The analysis and evaluation of extraventricular localizations of dissemination pathways and collection spaces for leptomeningeal hemorrhages was performed in 3 stages: 1. compartmental analysis of the brain box in fetus through the lamellar structures of the cranial dura mater; 2. the analysis of the intra- and extraventricular relief forms of the diencephalon and the brain stem in adults; and 3. identification and evaluation of leptomeningeal hemorrhage collection in feti.

A. Compartmental analysis of the brain box in feti through the lamellar structures of the cranial dura mater

Macroanatomic visualization of lamellar structures of the cranial dura mater was performed when the brain box was opened either by sectioning the calvaria opercules (Fig. 1D; E) or by cutting in midsagittal plane (Fig. 1G; Fig. 2D), in parasagittal planes (Fig. 2B;C) or in a horizontal plane (Fig. 2E).

The analysis of the relationships between the falx cerebri, the falx cerebelli and the tentorium cerebelli, on the one hand, and the compartmental limiting in the brain box on the other, were carried out through cutting the calvaria oopercules (Fig. 1. E-Index numbers; 15; 14; 12 and Fig. 1 G-Index number:13). Falx cerebri appears like a septal, conjunctive structure of the cranial dura mater, that deeply penetrates the longitudinal cerebral fissure, close to the corpus callosum (Fig. 1E – Index number 15; Fig. 2B-Index number 2). At its free margin we identified the inferior sagittal sinus, while at the connection site to the parietal dura mater we visualized the superior sagittal sinus. At the meeting place between the falx cerebri and the tentorium cerebelli, the straight sinus appears. Still in sagittal plane, we easily identified the falx cerebelli that separates the hemispheres of the cerebellum (Fig. 1E-Index number 14). Tentorium cerebelli was easily identified as a septal conjunctive structure of the cranial dura mater, resembling a arched lamella that penetrates the transverse cerebral fissure and separates the cerebrum from the cerebellum (Fig. 1E-Index number 12). It makes up the posterior cranial fossa (Fig. 2D-Index number 4).

Visualization of supra- and sub-tentorial compartments of the brain box was made possible by using the midsagittal plane cutting (Fig. 1G (Index number 13; and Fig. 2D Index numbers: 4; 4Bis; 19). Analysis of the relationships between the lamellar structures of the cranial dura mater and the brain was performed by cutting on parasagittal planes (Fig. 2B-Index numbers: 1; 2; 3 and Fig. 2C-Index number: 4).

Visualization of frontal lobes localization at the level of the anterior cranial fossa, of the temporal lobes in the middle cranial fossa and of the metencephalon at the level of the posterior cranial fossa , on the one hand and the analysis of the relationships between the structures of the cranial dura mater and the brain, on the other, become visible when cutting on horizontal plane is performed (Fig.2E-Index numbers: 20; 21; 22).

In the subarachnoidian space we also identified the arachnoid trabelulae that connects the two structures of the the leptomeninx: the arachnoid mater and the pia mater (Fig. 2I – Index number 27).

B. Analysis of intra- and extraventricular relief forms of the diencephalon and the brain stem in the adult human

The identification and evaluation of intra- and extraventricular relief forms at the level of the diencephalon and the brain stem was made possible through the macroscopic examination of the medial face of brain hemispheres in adults.

1. The diencephalon

The diencephalon, as part of the forbrain, is of special interest given its relief forms, the presence of choroidian structures, of the intraventricular communication pathways, of the dissemination paths and of the leptomeningeal hemorrhage collection spaces (Fig. 3). The delimitations of the diencephalon were traced by means of the reference marks established by morphogenesis criteria within the structures the brain, i.e: 1. the primitive brain vesicle generates the cerebral hemispheres, evolving from the caudal to the rostral mark; 2. the lamina terminalis is the basic morphogenesis of the reference marks: a. the corpus callosum evolving from the rostral to the caudal (Fig. 3-Index numbers: 2; 5; 5Bis); b. the anterior commissure, having transversal distribution for the connection of the temporal lobes (Fig.3-Index number:9) and c. the fornix, as an associative system between the hippocampus and the mammillary body (Fig.3 - Index numbers: 7; 7Bis; 7third; 15).

Consequently, the delimitations of the encephalon were traced on the bases of the previously standardized reference marks, i.e.: the dorsal delimitation lies between the splenium of the corpus calosum and the crus and the body of the fornix (Fig. 3-Index numbers: 2; 7Bis); the rostral delimitation is between the columna of the fornix and the optic chiasma (Fig. 3-Index numbers:7;12Bis); the basal delimitation is situated between the optic chiasma and

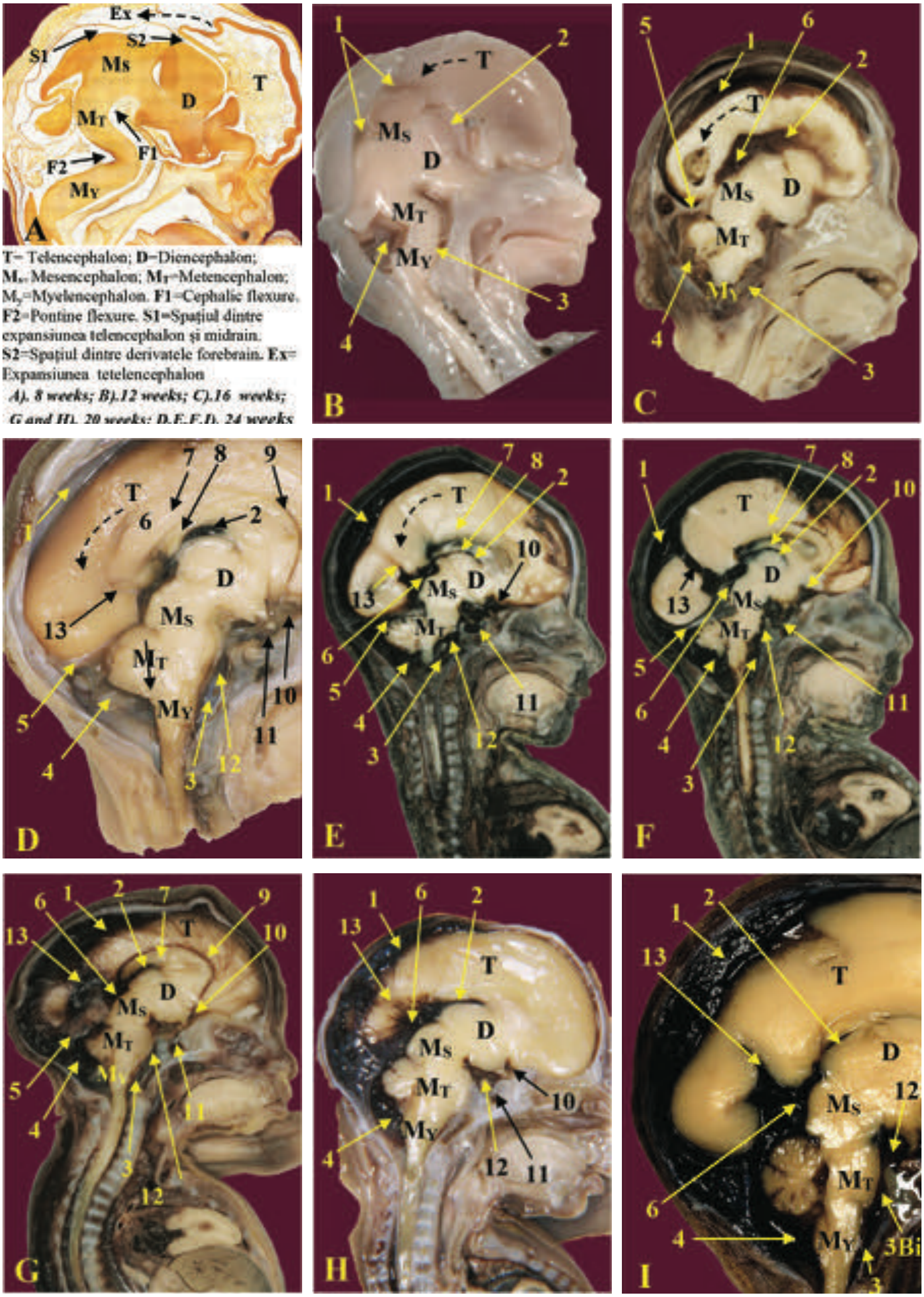

Figure 4. Localizations of leptomeningeal hemorrhages in fetus; morphogenetic dynamics o the brain. 1. Spatium subdurale; 2. Space between the fornix and thalamus (Cistern of transvers fissure or The cistern of the velum interpositum); 3. Space between the medulla oblongata and the clivus of the occipital bone (The premedullary cistern). 3Bis. Space between the anterior surface of the pons and the clivus of the occipital bone (The prepontin cistern) 4. Space between the cerebellum, the medulla oblongata and the squamous part of the occipital bone (Posterior Cerebellomedullary cistern; or Cisterna magna); 5. Tentorium cerebelli; 6. Space between the splenium the corpus callosum, the tectum of the midbrain and the cerebellum (Quadrigeminal cistern or Cistern of the great cerebral vein) ; 7. Corpus Callosum; 8. Fornix; 9. Artera pericallosa; 10. Space pre-glandula pituitaria between the Chiasma opticum and Frontal lobe (The Chiasmatic cistern); 11. Fossa hypophysialis; 12. Space between the cerebral peduncles and the dorsum sellae of the sphenoid bone (The interpeduncular cistern) 13. Sulcus parietooccipitalis.

the mammillary body (Fig. 3-Index numbers:12Bis;15); the caudal delimitation is between the caudal extremity of the mammillary body and the habenular commissure (Fig. 3-Index numbers:15;22).

At macroanatomic level, between the delimitations of the diencephalon we identified, its 3 major components: the epithalamus, the thalamus and the hypothalamus, of which two correspond to the lateral wall of the third ventricle (Fig. 3).

The lateral wall of the third ventricle cavity was visualized by cutting in midsagittal plane. This wall is an irregular polygon, its margins oriented dorsally, rostrarly and caudally. The (upper) dorsal margin is like an arch, its concavity oriented towards the base (lower part) and a horizontal trajectory on the trace of the habenula, between the habenular commissure and the interventricular foramen (Fig. 3- dotted yellow line). The (anterior) rostral margin is vertical and has a slight inclination towards the base and the rostrum and was traced between the columna of the fornix and the supraoptic recess (Fig. 3-Index numbers: 7 and 12). The (lower) basal margin is horizontal, and has a slight inclination towards the base and the cauda and was traced between the supraoptic recess and the infundibular recess (Fig. 3-Index numbers:12 and 14bis). The (posterior) caudal margin is oblique at 450 from the vertical, on the trace between the opening of aqueduct of midbrain and the infundibular recess (Fig. 3-Index numbers: 25 and 14bis). The margin of the mesodiencephalic jonction (Pretectum) is situated at the border between the diencephalon and mesencephalon on the trace between the habenular commissure and the opening of midbrain aqueduct (Fig. 3-Index numbers: 22 and 25).

The internal surface of the lateral wall of the third ventricle is separated into two levels by the hypothalamic sulcus: a dorsal or thalamic one, ovalar in shape, situated between the habenula and the hypothalamic sulcus and a quadriform, hypothalamic or basal one, situated between the hypothalamic sulcus and basal margin of the third ventricle between the supraoptic recess and the infundibular recess (Fig. 3-Index number: 16; 12; 14Bis).

Choroid structures were easily localized at the level of the diencephalon under the form of a choroid membrane of the third ventricle (TNA Latin: Tela choroidea) and choroid plexus of the third ventricle (TNA Latin: Plexus choroideus).

The choroid membrane of the third ventricle was identified ouside the third ventricle cavity, at the level of the walls of the transverse cerebral fissure (Bichat). It is made up of folds of the cranial pia mater that appear like two superimposed layers: an upper one and a lower one. The upper layer is convex and is placed between the splenium of the corpus callosum and the fornix to which is attached through conjunctive tracts as the arachnoid trabeculae (Fig. 3-Index number:18Bis). The lower layer adheres to the epithelial lamella situated at the internal top of the third ventricle (Fig. 3- Index number: 19Bis). The two layers are fused at their rostral extremity and move apart at their caudal extremity.

The choroid plexus of the third ventricle has a strange trajectory. Initially, it is situated extraventricularly in the caudal part of the transverse cerebral fissure and becomes intraventricular at the anterior part of this fissure and then enters the foramen interventriculare where it is continuous with the choroid plexus of lateral ventricle (Fig. 3-Index numbers: 21; 8).

2. The Brainstem

The brainstem, through its structures in the midbrain (the mesencephalon) and the hindbrain (the rhombencephalon) ensure the functional and anatomic continuity between the diencephalon and the medulla spinalis. The former also contributes to the delimitation of the dissemination pathways and the collection of leptomeningeal hemorrhages.

C. Identification and evaluation of leptomeningeal hemorrhages in feti

After analyzing the cuts performed in midsagittal plane in the brain boxes of feti aged between 8 and 32 weeks, we easily identified the phenotypical changes that occurred in the telencephalon, diencephalon and brainstem in the antepartum morphogenetic stages and their participation in delimiting the spaces of leptomeningeal hemorrhages (Fig. 4).

We have identified and evaluated the following 6 spaces: 1. the space between the fornix and the thalamus that make up the cistern of transverse fissure or the cistern of the velum interpositum (Fig.4:Index number:2); 2. the space between the splenium of the corpus callosum, the tectum of the midbrain and the cerebellum called the cistern of great cerebral vein, or Quadrigeminal cistern (Fig.4: Index number: 6); 3. the space between the cerebellum, the medulla oblongata and the squamous part of the occipital bone identified as the posterior cerebellomedullary cistern (Synonym:the cisterna magna) (Fig. 4. Index number:4); 4. the space between the medulla oblongata and the clivus of the occipital bone that correspond to the premedullary cistern (Fig. 4: Index number: 3); 5. the space between the pons and the clivus of the occipital bone identified as the prepontin cistern (Fig. 4: Index number 3Bis); 6. the space between the cerebral peduncles and the dorsum sellae of the sphenoid bone that make up the interpeduncular cistern (Fig. 4: Index number: 12).

DISCUSSION

Leptomeningeal hemorrhages are part of the group of intracranial hemorrhages. According to the site of bleeding we have identified 5 main types of intracranial hemorrhages: epidural, subdural, subarachonidian – primary or leptomenigeal – intracerebelous and intraventricular ependimara [6-14].

Anatomic localizations of leptomeningeal hemorrhages depend on the morphogenesis of the dissemination pathways and of the collection by the difference of neuronal tube derivatives, on the one hand and the structures of the pachymeninx (the dura mater) and the leptomeninx (the arachnoid mater and pia mater, on the other [15-18].

On the basis of our observations on leptomeningeal hemorrhages in feti, in the dynamics of brain morphogenesis and considering the concurrence of the triggering factors antepartum, intrapartum and postpartum, including the growth restrictions regarding the fetus, we have come to the conclusion that it is very difficult to evaluate the primary source, the volume and the dissemination of the bleedings unless a strict anatomic protocol is observed [19]. This is useful for the accuracy of visualization and evaluation of lamellar conjunctive structures of brain box compartmental division, of the relief forms made by the neuronal structures of the diencephalon and the brain stem, and last but not least, of the dissemination pathways and the collectios of the leptomeningeal hemorrhages that are frequently associated to epidural, subdural and/or intraventricular ependimara hemorrhages.

The forensic evaluation in feti suffering from intracranial hemorrhages deceased at variable degrees of prematurity, raises quite a number of questions associated to the identification of the hemorrhage site in the spaces formed by the pahmeningeal and/ or leptomeningeal structures, to the evaluation of the triggering factors in such bleedings, to the magnitude of the obstetrical trauma, and to the causes of the lesions, viability or death of the fetus antepartum, intrapartum or postpartum [20-25].

Conflict of interest

The authors declare that they have no conflict of interest.

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