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Original Investigation

Fetal and Perinatal Brain Autopsy: Useful Macroscopic Techniques Including Agar In-situ and Pre-Embedding Methods

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Abstract
Fragile perinatal and fetal brains are the rule rather than the exception for developmental neuropathologists. Retrieving the fresh brain from the skull and examining early fetal, macerated or severely hydrocephalic brains after fixation can be a challenge. Textbooks on neurodevelopmental pathology mention these challenges to macroscopic examination of the developing central nervous system only in passing, but many perinatal pathologists recognize this diagnostic problem. We reviewed protocols and publications on the removal, fixation, slicing and sampling of these fetal- and perinatal brains. In addition, we describe a technique to facilitate the removal of severely hydrocephalic brains with very thin cerebral walls from the skull by replacing the intraventricular fluid with agar *in-situ*. Furthermore, we present a method for post-fixation pre-embedding in agar to facilitate slicing, macroscopic examination and sampling of fragile and macerated brains.

Keywords
neurodevelopmental pathology, brain, perinatal, agar, autopsy, techniques

Introduction

Examination of a perinatal brain is not only an intellectual- but also a technical challenge. An easy to follow protocol for examining the central nervous system in stillbirths is published by Pinar et al.[1] The technical difficulties of handling the immature brain are caused by the high water content and a lack of myelination or a significant reticulin framework. These features cause general weakness and faster maceration of the developing central nervous system compared to other organs.[2] Hypoxia and ischemia make the tissue even more vulnerable to damage. Damage of the brain during the retrieval and slicing is often difficult to prevent, and may compromise analysis of stillbirths and developmental abnormalities such as ventriculomegaly.[3–5] In this article we summarize published techniques to prevent artefactual damage of the brain. Additionally, we describe an *in-situ* technique to facilitate removal of hydrocephalic brains from the skull by replacing the intraventricular liquor with agar. We also describe a method to maintain the integrity of the fragile fetal- and perinatal cerebrum during sectioning by pre-embedding in agar.

Agar, a hydrocolloid derived from seaweed, gelatinizes between 32 and 37°C and stays solid till 60 to 85°C. Because of this feature microbiologists have used it as a culture medium since 1882.[6] Routine tissue processing in pathology does not exceed the melting temperature of agar. Therefore, agar is a perfect pre-embedding medium and has been used to maintain orientation of small tissue biopsies and to make cell blocks in

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molecular pathology.[7,8] In experimental neuropathology, agar has also been used to make molds for examining small animal brains or to support brain tissue when sectioning with a macrovibratome.[9,10] We describe two other uses of agar to aid in the examination and diagnosis of the extremely soft fetal and perinatal brain. We also summarize other practical techniques that may help the neurodevelopmental pathologist.

Methods

Removal of the Brain

After external inspection of the skin and the shape of the skull, an incision is made in the scalp from ear to ear over the posterior fontanelle. The skin is reflected anteriorly over the cranium to the level of the eyebrows and posteriorly, to the occipital level. The diameter of the frontal fontanelle is measured. Incomplete ossification with soft sutures and open fontanelles allow opening of the cranium with scissors. The cranial bones can then be folded open like the petals of a flower to expose the cerebrum. Prahlow et al.[11] provide a step by step description of this procedure with photographs. A posterior approach is used when abnormalities of the cerebellum, brainstem or cervical spine are suspected. In this case, the ear to ear scalp excision is extended to the midline and caudally to the mid cervical region resulting in a 'question mark' incision. After removal of the occipital bone it is possible to examine the contents of the posterior fossa and cervical spine.[12]

If the brain is relatively firm it can be removed like an adult brain. However, if the brain is soft and macerated it is almost impossible to avoid artefactual damage during removal. Several methods have been described to maintain the integrity of the fetal brain prior to and during removal. These include *in-situ* fixation and an immersion technique whereby the brain is removed under water. Using the *in-situ* fixation method described by Cimmino et al.[13] fluid is aspirated from the ventricles with a syringe and replaced with a fresh mixture of 30% glacial acetic acid, 30% formalin and 40% distilled water till the ventricles and subarachnoid space are filled. The amount of aspirated cerebral fluid is an indication of the presence and the severity of ventriculomegaly or hydrocephalus. After fixation for 20 hours, the brain is removed from the skull and placed in ethanol (80%) for three to five hours before sectioning. The glacial acetic acid stabilizes the lipids and the formalin makes cross links between proteins. Postfixation in ethanol enhances the effects of the acetic acid and formalin and causes some dehydration of the tissue. However this technique has several disadvantages. It is difficult to perform if the ventricles are not enlarged; glacial acetic acid results in loss of haemoglobin staining in erythrocytes and some loss of iron from the tissue so it may compromise assessment of haemorrhage; and prolonged post-fixation in ethanol may result in excessive shrinkage and also make the tissue brittle and difficult to cut on the microtome.

Less time-consuming is the immersion technique as illustrated by Prahlow et al.[11] where the brain is removed under water. This can be done by immersing the head, or, in the case of a small fetus, the whole body, in water (Figure 1). Then the brain is removed using the same technique as described above. Since the density of the fetal or perinatal brain is similar to that of water the upthrust of the water keeps the brain floating while it is removed from the skull. The brain is removed after transecting the cranial nerves, vessels, falx, tentorium and cervical spinal cord under direct vision. Since the brain is essentially suspended in water, it is often easier to use small scissors rather than a scalpel blade for this, since a scalpel blade tends to push the tissue away from the operator. Similarly, rapid movements should be avoided to prevent damaging the corpus callosum, the cerebral hemispheres by impinging on the edge of the reflected cranial bones, or the brainstem by rotation at the junction with the cerebrum. After removal of the brain, the pituitary gland can be extracted from the cartilaginous sella.

Examination of the fresh brain. After opening of the cranium, visual inspection of the two cerebral hemispheres is performed and special attention is paid to the space between brain and the dura. If this is excessive, destruction of brain tissue or a subdural effusion should be considered. If hemorrhage is noted the amount and type should be specified e.g. subgaleal, subdural, subarachnoid or intracerebral. Further examination is performed after removal of the brain. Since blood clots are very firm after fixation and difficult to section without

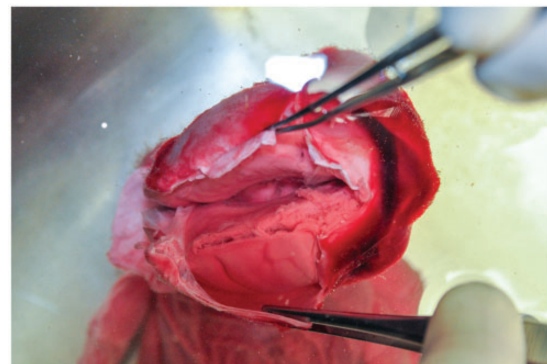

Figure 1. Removal of the brain of a fetus delivered at 25 + 6 weeks gestation after intrauterine death at 25 + 4 weeks gestation. With the body including the head fully immersed, the upthrust from the water makes the brain easier to remove and prevents artefactual tissue damage.

damaging the brain, it may be preferable to carefully remove large clots from the surface of the brain and fix them separately. The brain is weighed, preferably prior to fixation. For fetal or very soft brains this is most easily achieved by weighing the brain in a pre-weighed container of formalin and subtracting the weight of the container and formalin. When using the immersion technique only the container has to be pre-weighted. After the water is drained, the brain in the container can be weighed and the weight of the empty container can be subtracted to calculate the weight of the brain. Draining the water can result in collapse or damage in very immature or soft brains. In these situations, transfer the brain to a pre-weighed container of formalin, either using a spoon or a small container with a concave base. The fresh brain weight is compared with normal values.[14]

Removal of severely hydrocephalic brains after in-situ stabilization with agar. Pre- and postnatal ultrasound and MRI images are useful to estimate the severity of the hydrocephalus. Although using the immersion technique can prevent collapse of hydrocephalic brains in many cases, removal of the brain is particularly challenging and often results in post mortem damage. In our hands, *in-situ* replacement of the intraventricular fluid by agar supports the thin cerebral wall and facilitates removal of the brain from the skull without damage. Using a 60-200 cc syringe filled with liquid agar at 45- to 50°C and fitted with a 16- or 18 gauge needle, the intraventricular fluid is replaced by liquid agar. The correct position can be confirmed by careful aspiration while inserting the needle into the brain. If cerebral fluid is aspirated, the ventricle can be filled with the agar. The intraventricular fluid is drained by a second needle inserted at a lower level. Prior imaging of the cerebrum can help to estimate the location and size of the ventricles. If necessary, this procedure is repeated with the other ventricle. Manipulating the head helps to distribute the agar within the ventricles. If a full autopsy is allowed, agar injection should be performed at the beginning of the postmortem to allow the agar time to solidify for two hours prior to removal of the brain. The brain can then be removed as described above and the result is shown in Figure 2(A). The protocol is described in detail in Table 1.

Liquid and very fragmented brain tissue. Limited information may be derived from macroscopic examination of very soft or fragmented brains. Marked softening and fragmentation may be due to immaturity and is a significant problem after fetal death in early pregnancy with subsequent maceration, where there is a prolonged post mortem interval prior to autopsy, or following antepartum KCL injection. In macerated early pregnancies liquified brain tissue may be squeezed through the foramen magnum during delivery. The extruded tissue can be found in the spine, in the paravertebral soft tissue, beneath the skin and even in other organs. In these cases, as much of the tissue as possible should is collected and weighed although significant loss of tissue may be unavoidable and should be noted. If possible, cortical tissue, white matter, basal nuclei, choroid plexus and

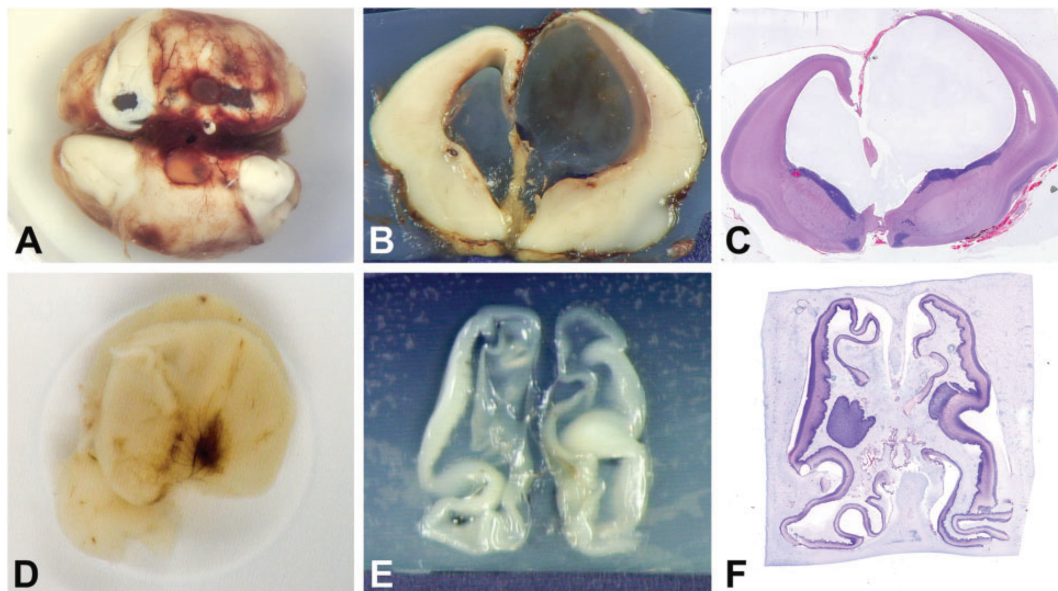

Figure 2. A, A severely hydrocephalic brain removed from the skull after filling the enlarged ventricles with agar photographed under water. B, A central coronal slice after pre-embedding in agar. The enlarged ventricles have not collapsed. C, H&E staining of a large slide from B after processing. D, Brain (1,5 cm length) after termination of pregnancy at 13 weeks gestation for mosaic trisomy 16. E, A central slice after embedding in agar. F, H&E stained of the slide depicted in E. The agar is amphophilic this does not hamper microscopic examination.

Table 1. *In-situ* Agar Stabilization Protocol for Fetal and Perinatal Brains With Ventriculomegaly.

	Step	Explanation
1	Make a 3% w/v solution of agar in tap water.	Weigh the amount of agar powder (3 gram agar per 100 mL water) into an microwavable flask and add warm tap water.
2	Stir to mix.	
3	Heat the agar mixture in a microwave till the boiling point.	At intervals of half a minute remove the flask and stir the contents to mix well. Repeat until the agar has completely dissolved. Be careful because eruptive boiling can occur. Alternatively use a heat plate with a stirrer or a Bunsen burner. Heat an extra 30 seconds to evaporate small air bubbles.
4	Fill a 60-250 cc syringe with agar and attach a 16- or 18-gauge needle.	
5	Let the agar cool to 45–50°C.	At this temperature it is comfortable to hold it in your hands. A higher temperature can cause thermal damage to the tissue.
6	Puncture the frontal fontanelle with the needle and attached syringe, aiming for the left lateral ventricle.	The proper placement can be confirmed by slightly pulling the plunger. If fluid is aspired the needle is most likely in the ventricle.
7	Insert a needle at a lower level in the left lateral ventricle.	This needle is placed to drain the intraventricular fluid.
8	Inject the agar slowly into the left ventricle.	
9	Repeat step 4 to 7 on the right side.	
10	Let the agar solidify for one to two hours.	
11	Remove the brain as usual and fix for a few days to two weeks in 10% neutral buffered formalin.	
12	If needed, the brain can be pre-embedded in agar following fixation.	

meninges should be sampled. Often structures in the posterior fossa are difficult to recognize, although in cases of cerebral hypoperfusion, the cerebellum may be relatively preserved.

Removal of the spinal cord. The spinal cord should be examined if abnormalities are suspected, such as neural tube closure defects or arthrogryposis multiplex. When a Chiari abnormality is suspected the posterior approach is advised to allow adequate assessment of the posterior fossa structures and the spinomedullary junction.[12] In these cases, the usual coronal incision to remove the brain can be extended caudally in the midline resulting in a question mark-shaped incision. After carefully loosening and undermining the skin, the paravertebral muscles are dissected laterally to expose the vertebral column. The pedicles are sectioned on both sides and the posterior elements are removed en bloc to expose the dura. Prior to ossification, this is best accomplished using sharp scissors. In older perinatal autopsies, the laminae rather than the pedicles can be sectioned as is the standard procedure in adults. The spinal nerves are sectioned and the cord is removed within the intact dura. Care should be taken not to acutely angulate the cord during removal to avoid artefactual damage (so called "toothpaste artefact"). The spinal nerves should be sectioned as far laterally as possible to include some of the lower dorsal root ganglia. Although the posterior approach allows greater exposure of the cord, especially in the cervical and lumbosacral regions, it does require a separate skin incision. If this is not permitted and consent for a full autopsy has been obtained or if the entire spinal cord does not need to be examined, a ventral approach can be used after the thoracic, abdominal and retroperitoneal organs have been removed. After entering the spinal canal by cutting through the lowermost intervertebral disc, the pedicles can be sectioned on both sides and the vertebral bodies removed en bloc, exposing the spinal cord within the dura.

Fixation

To prevent artificial deformation of the cerebrum due to flattening against the bottom of the container during fixation, sodium chloride (NaCl) can be dissolved in the formalin until the brain begins to float. Alternatively, the brain can be placed in a 'hammock' of gauze and strings or a disposable hairnet, although this can also cause deformation in macerated brains and results in a characteristic microscopic artefact in the superficial cerebral cortex. Usually the whole perinatal brain is immersed in 10- or 20% neutral buffered formalin (3.7-8% formaldehyde in sodium phosphate buffer) for two weeks.

The macromolecular crosslinks formed during the initial 24- to 48 hours of fixation are largely reversible with antigen retrieval techniques. Prolonged fixation should be avoided since methanoic- or formic acids, generated by

formalin oxidation, acidifies the tissue. This reduce antigenicity and causes degradation of nucleic acid, compromising immunohistochemistry and molecular analysis.[15–17] Although specimens should be fixed in at least ten times their volume of fixative,[1] in reality, containers for fixation are often small and the brain is often only fixed in two- or three times its volume of formalin. Refreshing the formalin after one week or fixing in 20% rather than 10% neutral buffered formalin are alternatives.

Even after formalin fixation, fetal and perinatal brains are considerably softer than adult brains, in part due to their increased water content (newborn 80%; adult 77%[16]). Consequently, a number of other fixatives have been described including 20% buffered formalin,[18] zinc-formalin ethanol, 20% buffered formalin with glacial acetic acid added until the brain remains suspended, or mixtures of formalin, ethanol and acetic acid. The latter also partially dehydrates the tissue.[16] While this may render the tissue firmer and facilitate macroscopic examination and dissection, prolonged fixation in solutions with a high concentration of ethanol may result in significant shrinkage and may make the tissue brittle, hampering microtomy, and acidic fixatives may cause unacceptable DNA degradation.[15] Changes in fixation may require reoptimization of antigen retrieval protocols for immunohistochemistry. For rapid dissection of pediatric brains heat-accelerated fixation in a microwave is described by Barrett et al.[19]

Slicing the Brain

After external examination, including assessment of gyral development and measurement, including the transverse cerebellar diameter,[20] and prior to sectioning, the brain should be photographed for documentation (Figure 3(A)–(C)). Gestational age is estimated based on standard external landmarks.[21] To section the cerebrum without fixation, Wyatt-Ashmead divides the corpus callosum to separate the hemispheres, places each hemisphere on its medial, flat, surface and sections each coronally,[22] while Paradiso cuts the fresh brain into 1 cm thick coronal slices prior to fixation to facilitate penetration and accelerate fixation.[16] The fixed brain can be sectioned after overnight fixation in 20% formalin with added glacial acetic acid, after two days fixation in 20% neutral buffered formalin or after two weeks fixation in 10% neutral buffered formalin. The brainstem and cerebellum are removed from the cerebrum with a scalpel by cutting through the mesencephalon perpendicular to the long axis of the brainstem with the blade immediately rostral to the oculomotor nerves and caudal to the mammillary bodies (Figure 3(D)). The contents of the posterior fossa are weighed separately, and their weight, as a percentage of the total brain weight is calculated and compared with normal values.[14] Depending on the firmness of the brain, the cerebral hemispheres can be slice at between 0.5 and 1 cm intervals, either free hand or with the aid of 0.5

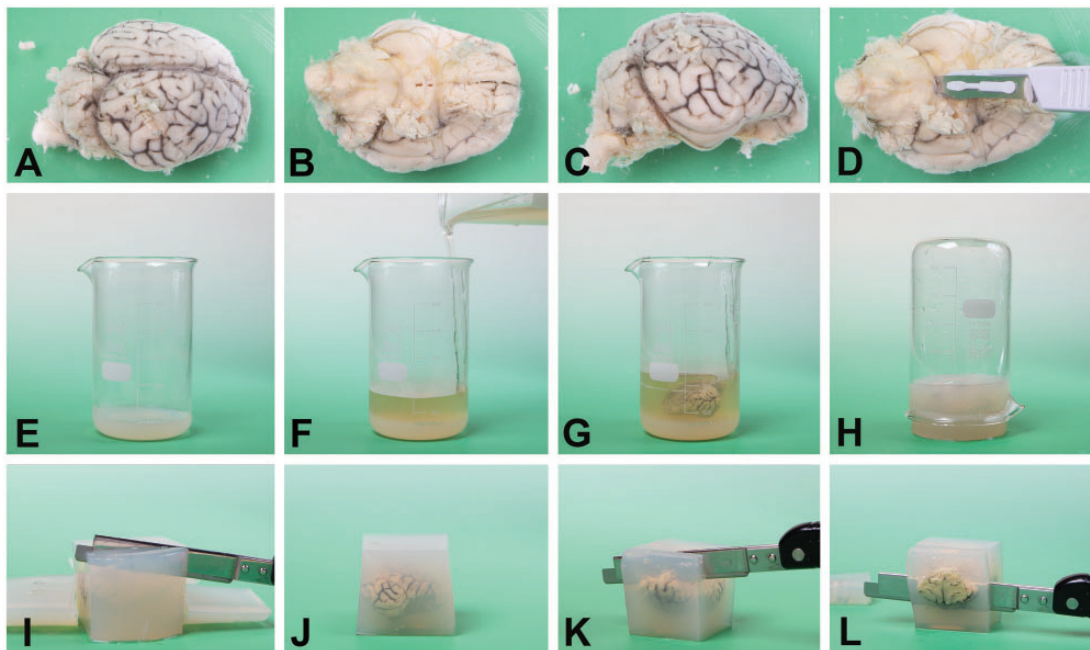

Figure 3. A–C, Photographic documentation of a pig brain under water. D, Removal of the brainstem and cerebellum at the level of the mesencephalon. E, 1 cm solidified agar base in a beaker large enough to contain the brain. F, Agar (45-50°C) poured on top of the solidified layer of agar. G, The brain placed in the liquid agar. H, The agar is released from the beaker. I, Trimming of the block to remove surplus of agar. J, Block after trimming and prior to slicing. K and L, Slicing the brain embedded in agar.

or 1 cm cutting guides. Although considered as "training wheels" by some, the latter ensure slices of uniform and reproducible thickness in each case. The number of slices depends on the size of the brain and the slice thickness but a mature perinatal brain yields up to eleven slices (Table 2). Although macroscopic examination of fragmented or partly liquefied brains is often unrewarding, histological detail is often better preserved than expected in autolyzed central nervous system tissue.[23] Although the diagnostic yield in these cases is limited, sometimes calcification following ischemia, infarction or infection, viral inclusions such as CMV, or haemosiderin, suggesting previous hemorrhage, can be found. Depending on the gestation, the approximate age of a hypoxic event or other insult can be suggested by identifying activated microglia or macrophages and/or gliosis by immunohistochemistry. An immunohistochemical investigation on the gradation and localization these findings and of the expression of antigens that could be correlated with an hypoxic-ischemic damage is published by Riezzo et al.[24]

Agar pre-embedding. Pre-embedding in agar is useful for brains that are more or less intact but remain very soft after formalin fixation or are macerated. This technique prevents loss of loosely attached fragments and maintains their spatial relationship. Additionally, agar pre-embedding is helpful in cases with ventriculomegaly or hydrocephalus or where there are cystic lesions of the posterior fossa. In these situations, it prevents disruption and, for posterior fossa lesions, maintains the spatial relationships between the vermis, the brain stem and any cystic lesion. In the latter situation the combined brainstem and cerebellum are embedded in agar and sectioned sagittally. This allows assessment of the vermis and the shape of the brainstem and is especially helpful for demonstrating Dandy Walker and associated malformations. Subsequently, the specimen can be sliced parasagittally to investigate the aqueduct if not demonstrated in the initial sagittal section. Alternatively one half of the specimen can be sectioned parasagittally and the other transversely to assess the brainstem.

Table 2. Landmarks Used for Slicing a Perinatal Cerebrum.

Size of the brain		Explanation
<3 cm	1	Midway between frontal and temporal poles.
	2	Through the center of the lateral fossa.
	3	Occipital.
3–7 cm	1	Midway between frontal and temporal poles.
	2	Just behind the temporal pole.
	3	At the level of the optic chiasm.
	4	Mammillary corpus.
	5	Between corpora ruber and aqueduct.
	6	Occipital pole.
>7 cm	1	Midway between frontal and temporal poles.
	2	Frontal edge temporal pole.
	3	1 cm behind temporal pole.
	4	Anterior of the chiasma.
	5	Posterior of the chiasma.
	6	Through mammillary corpora.
	7	Through nucleus ruber.
	8	Through aqueduct.
	9	Posterior edge of mesencephalon.
	10	Occipital.

Any type of agar or agarose can be used for the pre-embedding although they vary in their transparency. We use 1.5- to 3% food grade agar (Unique Products Schuurman, Nieuwegein, Netherlands). For a small brain (24 week of gestation or less) 200 ml agar solution is sufficient. For larger brains the amount is doubled. The agar is mixed with tap water and brought to a boil on a hot plate with a magnetic stirrer or in a microwave oven. A thin (approximately 1 cm) layer is poured into a container and allowed to solidify (15 minutes in a refrigerator; Figure 3(E)). Rectangular plastic takeaway food containers with flexible sides are an ideal shape and volume. The remaining agar is removed from the hot plate and allowed to cool to 45-50°C. Continued stirring will prevent a skin of solidified agar forming on the surface. The cooled agar is then added to the container. The cerebrum is then immersed in the agar (Figure 3(F) and (G)). If the cerebrum floats, gentle manipulation to expel intraventricular air may be necessary so that the cerebrum remains completely submerged. If necessary, needles can be inserted into the base layer of solidified agar to keep the hemispheres in the correct orientation. The agar solidifies after one to two hours at room temperature. This process can be accelerated by putting the container into the refrigerator. After solidification the container is inverted to remove the agar (Figure 3(H)). If a flexible plastic container has been used, gentle squeezing will release the vacuum between the agar and the container. This is facilitated by covering the surface of the agar with water. If a rigid container such as a beaker was used, a knife or probe can be slid between the wall and the agar to release the vacuum. After removal, the agar block is trimmed so the brain remains covered on all sides by at least 1 cm of agar. Since the brain may have moved before the agar had solidified, the block should be trimmed to maintain the correct orientation for slicing. The brain within the agar is then sliced (Figures 3(I)–(L) and 4). During slicing, sampling and histologic processing the agar continues to support the tissue and does not impair the quality of the stained sections (Figures 2, 5, and 6). The agar does not need to be removed before processing, cutting or staining.

Table 3. Agar Pre-Embedding Protocol for Fragile Fetal and Perinatal Brains.

	Step	Explanation
1	Select a vessel that is large enough to contain the brain.	A beaker with a diameter from 5 to 7 cm is sufficient to contain immature brains. More mature brains can be placed in a 2 to 15 cm diameter plastic disposable takeaway food or a container for surgical specimens. Estimate the amount fluid that is needed to fill the space that is left between the brain and vessel. Typically, 200 ml for brain up to 24 weeks of gestation.
2	Make a 1.5–3% w/v solution of agar in tap water.	Weigh the amount of agar powder (1.5 to 3 gram agar per 100 mL water) into a microwavable flask and add warm tap water. The percentage of agar that is adequate for supporting the brain varies with type of agar used. Lower concentrations of agar result in a more transparent block and renders the brain more visible, facilitating correct orientation prior to slicing.
3	Stir to mix.	
4	Heat the agar mixture in a microwave till the boiling point.	At intervals of half a minute remove the flask and stir the contents to mix well. Repeat until the agar has completely dissolved. Be careful because eruptive boiling can occur. Alternatively, a heat plate with a stirrer or a Bunsen burner can be used. Heat an extra 30 seconds to evaporate small air bubbles.
5	Pour a 1 cm baselayer of agar in the vessel or beaker. Allow this layer to set at 4°C.	This takes 15–20 minutes. Let the rest of the agar cool at room temperature to 45–50°C. Continue stirring while the agar cools to prevent a skin forming on the surface
6	Pour the remaining agar (cooled to 45–50°C) into the container on top of the solidified base layer.	
7	After removing the brainstem and cerebellum, place the cerebrum in the melted agar.	Ensure that the brain is completely submerged in the agar. If the brain floats, intraventricular air can be expelled by gentle manipulation. Pins inserted into the base layer can be used to keep the cerebrum in place.
8	Let the agar solidify at room temperature (overnight) or at 4°C (one to three hours).	When the agar is completely solidified it turns opaque and feels cold and firm.
9	Turn the vessel upside down on to the cutting board to remove the brain embedded in agar from the vessel.	If necessary, a knife can be run between the agar and the container to help break the vacuum between the agar and the vessel.
10	Excess agar can be trimmed away.	Transilluminating the block may be help to identify the position of the brain within the agar prior to initial trimming.
11	Cut the brain within the agar block into 0,4–1cm thick coronal slices.	This can either be done “freehand”, cutting the agar block vertically, or with the aid of 0.5 cm wide cutting guides with the agar block cut horizontally. The slices can be easily manipulated using a ruler, spatula or flexible metal blades. Filling blades used for spreading plaster work well (Figure 4B).
12	Take the samples for microscopic examination.	There is no need to remove the agar from these samples. The agar supports the tissue during processing and embedding and does not interfere with routine processing, cutting and staining.

Discussion

The development of the central nervous system is far from complete by the time of birth. Even after conventional formalin fixation, due to immaturity, maceration and/or tissue damage the fetal or perinatal brain often remains very soft and difficult to examine. Several methods have been described facilitate the examination of these specimens. However, in our hands we cannot always make perfect slices after fixation and for autolyzed or macerated brains, even prolonged fixation does not improve the consistency to allow examination without marked fragmentation since penetration of formalin into autolyzed tissue is poor.[23] Maceration cannot be reversed no matter what fixative or duration of fixation is used. Therefore, we experimented with several protocols to facilitate examination of these brains without delaying the autopsy or further neuropathological examination and without compromising standard processing, staining and immunohistochemical protocols. We found that pre-embedding fetal and perinatal brains in agar provided support during slicing, allowing detailed macroscopic assessment and the preservation of spatial relationships during block selection and sectioning. In addition, by injecting agar into the enlarged cerebral ventricles *in-situ*, we facilitated the removal

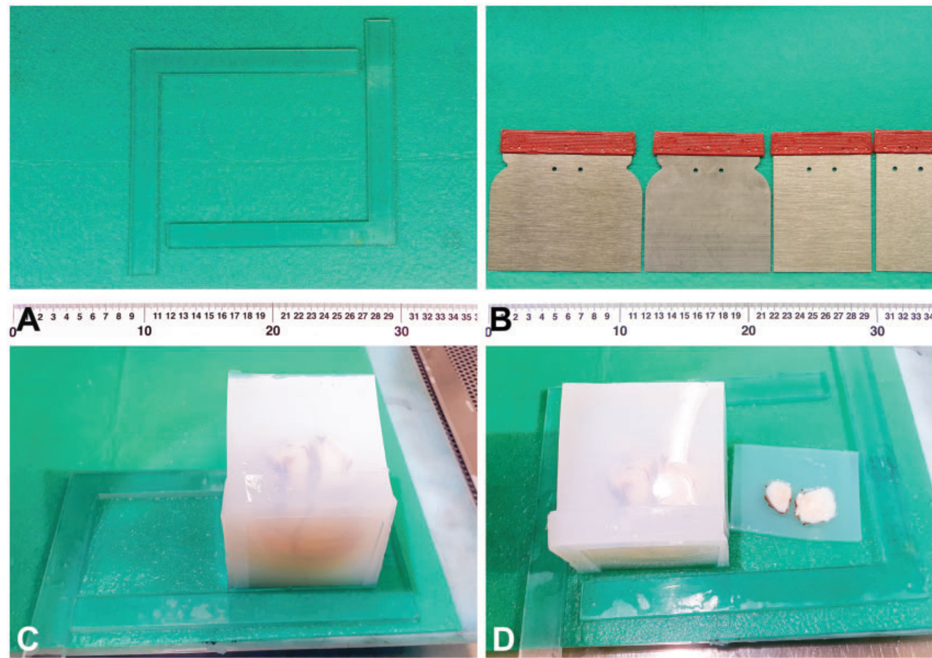

Figure 4. A–C, By using cutting guides (0.4 to 0.5 cm thick), all slices are of uniform thickness. Cutting guides can be easily fabricated from Perspex sheets. When guides are used the agar block is sectioned horizontally. This may reduce the tendency for fragments of severely macerated brains to dislodge if the agar block is sectioned vertically. D, Using metal filling blades facilitates manipulation of the slices. Filling blades are flat, thin, flexible and inexpensive and can be purchased from hardware stores.

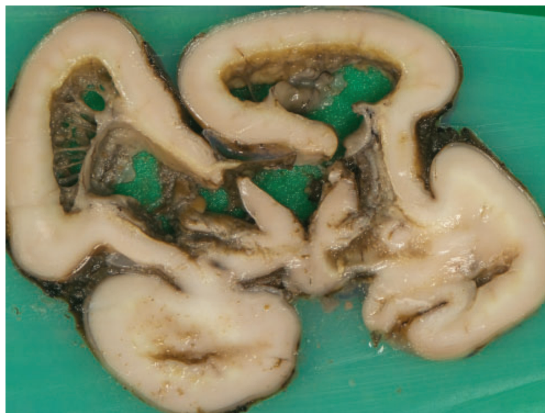

Figure 5. A brain with severe periventricular necrosis. Cutting this without agar preembedding would be unlikely to show the residual ependyma and intact ventricular wall in the left.

and examination of hydrocephalic brains with very thin cerebral walls.

Agar, a polysaccharide extracted from seaweed by boiling, forms an edible jelly-like substance that has been used for centuries in Asian desserts. It is also used as a culture medium in microbiology and, in agarose gels, to separate DNA fragments in molecular biology. In pathology, agar is sometimes used to maintain the correct orientation of small tissue biopsy specimens and for embedding cytological specimens for immunohistochemistry. These reports confirm that agar pre-embedding does not hamper tissue processing or staining, or DNA extraction.[8,25,26] This has also been our experience with human central nervous system tissue. Since the agar is not removed by processing, pre-embedding also reduces fragmentation during subsequent manipulation, in particular when the processed tissue is transferred from the cassette to the embedding mold. Although we only used pre-embedding after fixation, fresh tissue biopsies can also be embedded in agar because formalin penetrates the agar.[7]

The costs of the pre-embedding are low because food grade agar can be used instead of electrophoresis grade agar. Compared to electrophoresis grade agar, food grade agar has only a slightly lower clarity, but the brain is still sufficiently visible within the agar block after trimming to correctly position for coronal slicing. Although the method takes a little more time than routine examination, most of this is waiting for the agar to solidify. Boiling, cooling to 45–50°C and injecting the agar into the ventricles adds an additional 15 minutes to the autopsy. The rest of the autopsy can be performed while the agar solidifies and the subsequent removal of the brain is faster because the brain is firmer and the ventricles do not collapse. The pre-embedding process also takes 15 to 20 minutes of additional hands-on

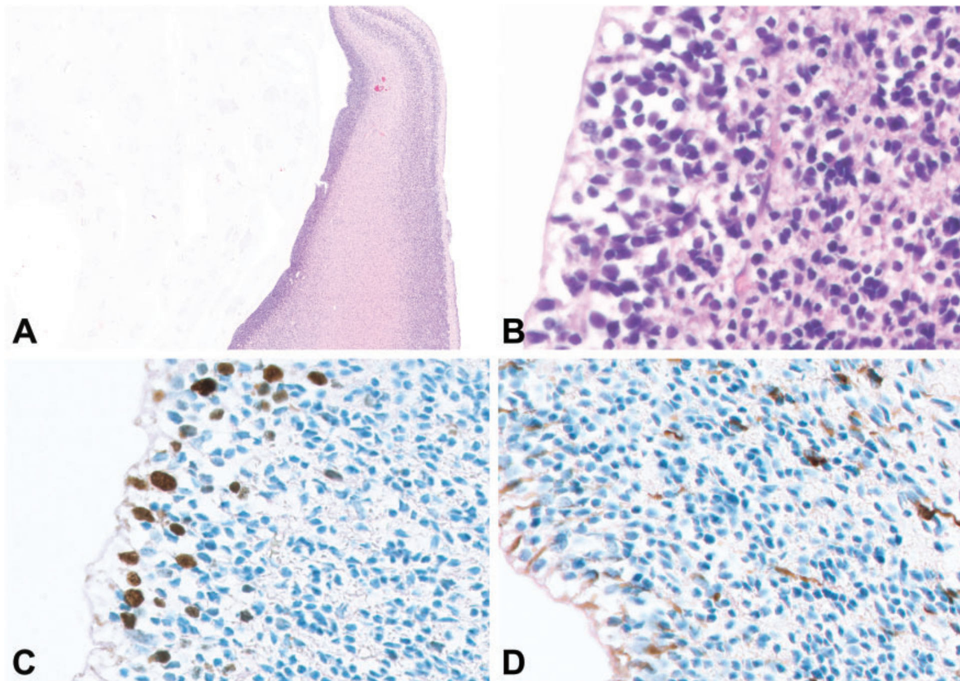

Figure 6. A, H&E stained slide of the brain of a fetus with symmetric ventriculomegaly delivered at 19 + 1 weeks gestation. The ventricular space is filled with agar. B, A higher magnification image showing the histology of agar-embedded brain tissue. Cell detail is well preserved. C, Ki-67 stain and D, GFAP stain. This demonstrated that nuclear and cytoplasmic immunoreactivity is retained after agar embedding.

time and one to two hours of waiting time while the agar solidifies. Slicing the brain is faster and manipulation of the sections is easier after pre-embedding. The pre-embedded brain can also be kept in the refrigerator overnight. The surface of the agar block should be kept wet and the container should be covered to prevent evaporation.

In conclusion, we describe two rapid and cost effective techniques using *in-situ* injection of agar into enlarged cerebral ventricles and post fixation pre-embedding in agar to improve the examination of the fetal and perinatal central nervous system.

Declaration of Conflicting Interests

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