

Morphologic Analysis of Artifacts in Human Fetal Eyes Confounding Histopathologic Investigations

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PURPOSE. Human fetal eyes are an excellent source for studies of the normal ocular development and for examining early ocular changes associated with various syndromes in the context of a pediatric pathologic or prenatal sonographic diagnosis. However, artifacts caused by different factors often render an exact interpretation difficult. In this study, the frequency and extent of artifacts in human fetal eyes were investigated with the aim of distinguishing more precisely these artifacts from real findings, allowing also for a more diligent forensic interpretation.

METHODS. The cohort included 341 fetal eyes, ranging in age from 8 to 38 weeks of gestation, that were investigated macroscopically and by light microscopy.

RESULTS. In most specimens, artifacts such as pigment spillage and autolytic changes of the retina were noted. Nearly all specimens showed changes of the lens with remarkable similarities to cataractous lenses in adult eyes. Structural ocular changes associated with systemic syndromes were also observed and in most instances could be distinguished from artifacts.

CONCLUSIONS. Morphologic changes in fetal eyes should be classified in artifacts caused by way of abortion, mechanical effects from the removal of the eyes, delayed fixation with autolysis, and the fixative itself and should be distinguished from genuine structural abnormalities associated with ocular or systemic disease. This classification can be fairly difficult and requires experience. In addition, lens artifacts are often misleading, and the diagnosis of a fetal cataract should not be made based on histopathologic examination alone. (*Invest Ophthalmol Vis Sci.* 2011;52:2712-2718) DOI:10.1167/iops.10-6301

The investigation of human fetal eyes collected after abortion or postnatal death is important with regard to a complete pediatric pathologic investigation, on the one hand to describe prenatal ocular involvement in malformations and syndromes with and without chromosomal aberrations (which may allow for prenatal sonographic detection) and on the other hand to provide help (if needed) in finding the pediatric pathologic diagnosis.¹

Because of certain variables, different artifacts involving various structures of the eye to a varying extent can render an exact interpretation difficult. Some of these influences, such as delayed fixation causing autolysis or mechanical trauma during

the removal of the eye, are known. Others are more speculative, such as the influence of potassium chloride used during the abortion procedure. Finally, the fixative itself may also induce tissue artifacts.^{2,3}

Postmortem artifacts already known in the literature were also considered. Lange folds usually occur in the ora serrata region of fetal eyes (but not in adult eyes) as macroscopically and microscopically detectable circular folds. Longitudinal folds next to the Lange folds are called meridional folds. In the developing macular region similar folds, called plica centralis, can be detected. The origin of these folds is speculative, but the lack of vasculature at these particular sites is said to contribute to their development and may explain why they are not observed in adult eyes.^{4,5}

Here, we evaluated the frequency and extent of all artifacts we encountered in our cohort of human fetal eyes with regard to the analysis of possible causative factors. The question of clinical relevance is addressed regarding the distinction between artifacts and true findings, thus providing reliable results with which to complete the pediatric pathologic investigation and allowing for an interpretation of forensic cases (e.g., in battered child/shaken infant syndrome, usually revealing many autolytic changes) with higher certainty. To provide reliable data allowing for a positive distinction from true pathologic findings, their localization is described as accurately as possible.

MATERIALS AND METHODS

Materials

A total of 341 eyes from 180 human fetuses ranging in age from 8 to 38 weeks of gestation (WoG; median, 20 WoG) were collected (Fig. 1, Table 1). In some abortions of fetuses up to 14 WoG, only one eye could be investigated because of accidental laceration during removal of the specimen ($n = 19$). All eyes were obtained routinely during the pediatric pathologic autopsy. In all cases, written informed consent was signed by the parents. The research followed the tenets of the Declaration of Helsinki.

Methods

Most of the fetal eyes ($n = 261$) were fixed in 4% formaldehyde (in 0.075 M phosphate-buffered saline). Eighty eyes were fixed in Karnovsky's fixative (2% paraformaldehyde and 2% glutaraldehyde in 0.1 M Sørensen phosphate buffer; pH 7.3).

After fixation, all eyes were measured (anteroposterior diameter of the globe and corneal diameter) and inspected macroscopically, including transillumination. The horizontal plane was identified, and the specimen was bisected horizontally next to the optic nerve. Thereafter, all eyes were examined with the dissecting microscope, with particular emphasis on the lens (for identifying a cataract), the hyaloid artery, the optic nerve, and retinal/choroidal defects (to detect, for example, colobomas).

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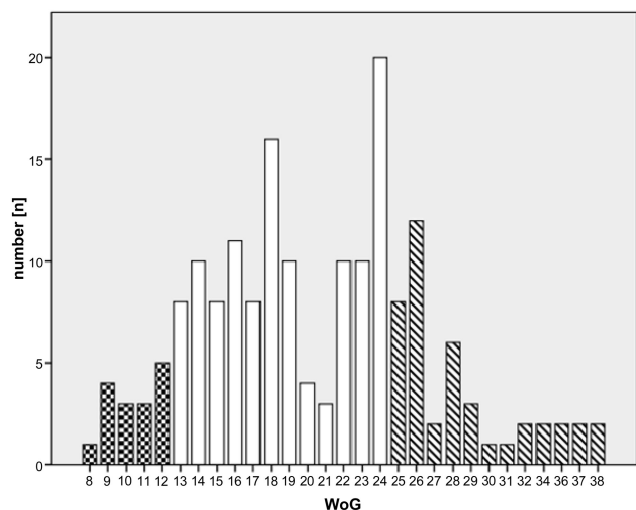


FIGURE 1. Cohort of 180 human fetuses ranging in age from 8 to 38 WoG. Checked bars; first trimester; white bars: second trimester; striped bars: third trimester.

Specimens were dehydrated in increasing concentrations of ethanol (70%–99%) and embedded in paraffin (McCormick Scientific, Richmond, IL) at 60°C. Step sections at a thickness of 4 μm were cut and floated on deionized water at 45°C and mounted as single sections on glass slides (Superfrost Plus; Menzel-Gläser, Braunschweig, Germany). Slides were subsequently dried at 60°C for 24 hours. Step sections were stained with hematoxylin and eosin (H&E; Merck, Darmstadt, Germany) and periodic acid-Schiff reaction (PAS; Merck).

Clinical Data

In 131 of 180 fetuses, a syndrome or malformation was diagnosed by the pediatric pathologist. The most frequent syndromes with possible ocular involvement were trisomy 21 ($n = 20$), monosomy X ($n = 5$), trisomy 13 ($n = 7$), cat eye syndrome ($n = 2$), trisomy 17 ($n = 1$), trisomy 18 ($n = 2$), various chromosomal aberrations ($n = 4$), and Goldenhar's syndrome ($n = 1$). In the remaining 49 cases, maternal problems such as placental insufficiency and chorioamnionitis led

to termination of the pregnancy. In these cases, syndromes and malformations were excluded by autopsy (Table 1).

In 115 cases abortion was induced, mostly because of a prenatal diagnosis of a specific syndrome by sonography or biopsy of chorionic villi. Fifty-six fetuses died in utero, predominantly because of placental insufficiency but also because of hitherto unknown fetal chromosomal aberrations. Nine fetuses died postpartum.

RESULTS

Artifacts According to Their Localization

Dimensions and structures of our specimens are presented in a low-power micrograph (Fig. 2) of a fairly regular eye (from a 14-week-old fetus with trisomy 21) with an anteroposterior diameter of 7 mm, a corneal diameter of 4 mm, and an artificial retinal detachment. A prominent tunica vasculosa lentis representing a transient finding that usually disappears before birth can be detected surrounding the lens. Its anterior part, the remnants of which may sometimes persist as pupillary membrane, can be confused with an iris neovascularization (e.g., occurring in advanced early-onset retinoblastoma) in premature children.

Conjunctiva and Cornea

Rarely, the conjunctiva showed artifacts such as altered epithelium. With regard to the cornea, artifacts were found more frequently, predominantly involving the epithelium and endothelium. In many eyes the epithelium or the endothelium, or both, was partially damaged or completely absent, whereas the underlying basement membranes (basement membrane of the epithelium, Descemet's membrane of the endothelium) remained intact (Fig. 3A). A swollen cornea was seen only in eyes with severe autolytic changes. In a few fetal eyes, artificial synechiae were detected between the conjunctiva and the cornea (Fig. 3B).

TABLE 1. Overview of the Cohort of Fetal Eyes Listing the Fixative, the Presence of Syndromes, and the Termination Mode

Cohort	n
Fetuses	180
Eyes	341
Fixation (eyes)	
Formalin	261
Glutaraldehyde	80
Fetuses with syndromes/malformation	131
Monosomy X	5
Trisomy 13	7
Trisomy 17	1
Trisomy 18	2
Trisomy 21	20
Other chromosomal aberrations	4
Cat eye syndrome	2
Goldenhar's syndrome	1
Fetuses without syndrome	49
Termination mode (fetuses)	
Feticide	115
Intrauterine death	56
Postpartum death	9



FIGURE 2. Eye from a fetus of 14 WoG without obvious abnormalities (H&E). Note the artificial retinal detachment (arrow) after fixation, embedding in paraffin, and sectioning. The lens is surrounded by a prominent tunica vasculosa. Parts of the hyaloid artery can be found next to the lens (arrowhead).

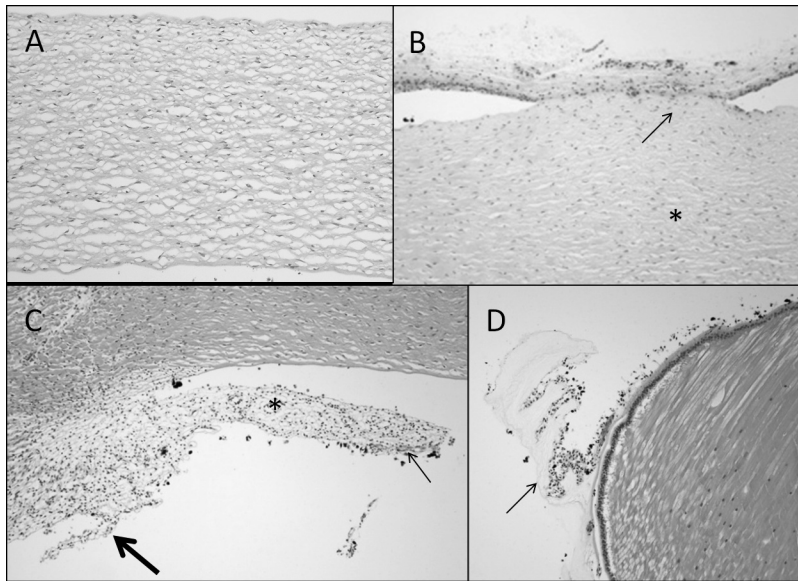


FIGURE 3. (A) Cornea with loss of epithelium and endothelium and consequently swollen corneal stroma (24 WoG, H&E). The latter, however, is still relatively unaffected by artifacts with regard to its structure. (B) Fusion (*arrow*) of conjunctiva and cornea (*asterisk*) with absence of the corneal epithelium (22 WoG, H&E). (C) Iris (*asterisk*) with artificial loss of the pigment layer (22 WoG, H&E). The sphincter muscle is detectable (*arrow*). To a varying degree, parts of the ciliary body are also absent (*bold arrow*) and are adherent to the lens capsule (D, *arrow*, H&E).

Anterior Chamber and Chamber Angle

In some of the fetal eyes, retinal fragments or spilled pigment from uveal structures were present in the anterior chamber. The different developmental stages of the chamber angle and Schlemm's canal could be retraced in most cases. However, there were also eyes with a closed angle caused by what appeared to be synechiae or, as in most instances, autolytic damage of the involved ocular structures, namely the iris. An Axenfeld, Peters, or Rieger anomaly could not be identified, but an embryotoxon posterius was unequivocally detected in one specimen.

Iris, Ciliary Body, and Ora Serrata

In early developmental stages, the iris and the ciliary body were very small, making evaluation challenging. The main confounding artifact was the spillage of pigment from the pigmented epithelium, predominantly of the iris and the pars plicata of the ciliary body (Figs. 3C, 3D).

Because of the frequent presence of Lange folds, the ora serrata and the pars plana of the ciliary body were often obscured for detailed investigation. (Some authors, therefore, recommend the removal of this fold to gain insight into the peripheral retina and the pars plana region.⁵) A further complication is the fact that, together with the Lange fold, the ciliary body and especially the pars plana were detached in some of the older eyes. Macroscopically, a white circular band was present, microscopically verified by a loss of "adhesion" between parts of the ciliary body/peripheral retina and the underlying pigment epithelial layer.

However, despite those artifacts, involvement of the iris and the ciliary body in one case of persistent hyperplastic primary vitreous (PHPV) could be detected unequivocally.

Lens

Multiple lens changes were present in most of our fetal eyes. The lens capsule was fractured and the lens epithelium was damaged. Small globules, subcapsular accumulation of material and clefts could be found to a different extent in nearly every lens (Figs. 4A-C). The distinction from cataractous changes was extremely challenging because the lens morphology in our formalin-fixed fetal eyes was similar to that in adult eyes with cataract. In older lenses, an indentation of the posterior pole was observed frequently macroscopically and microscopically.

Among all fetal eyes, however, only one specimen with PHPV in trisomy 13 showed more severe lens changes next to the PHPV lesion that was interpreted as a localized cataract-like opacification (Fig. 4D). Retention of cellular nuclei in the lens nucleus (as described in rubella) as a hint for a congenital cataract was not detected in our cohort.

Vitreous

Usually, the vitreous looked clear and transparent by macroscopic investigation, and the hyaloid artery was embedded in its cavity. Microscopically, only parts of the vitreous were visible, especially its base, that were related to the tertiary vitreous being in contact with the zonules (Fig. 5A). Four pairs of fetal eyes presented with a macroscopic opacification of the vitreous that revealed no cellular elements but appeared condensed on microscopic investigation.

Microscopically, the hyaloid artery was difficult to detect and was visible in only a few sections because of the nature of a vessel to be thin and curled (Fig. 2). In general, it could be best observed next to the optic nerve.

Retina

Most of the fetal eyes presented with autolytic changes within the retina. Even eyes assumed to have a fairly regular retinal structure on macroscopic investigation presented rather severe autolytic changes when analyzed microscopically. These changes included retinal edema with dispersed and thinned retinal layers, an extremely folded retina, and fragmentation to a varying extent (Fig. 5B). The photoreceptors (and their precursors) especially were often separated from the entire retina and, therefore, could be detected as clumped accumulations next to the retinal pigment epithelium (Fig. 5C). Nevertheless, specific retinal changes could be detected in areas that were only slightly affected by autolysis or other interfering factors. In the present cohort of fetal eyes, two findings were of particular interest; these are described further in Probably True Histopathologic Findings.

Retinal Pigment Epithelium

The single-layered retinal pigment epithelium (RPE) is one of the intraocular structures rarely affected severely by artifacts. In some of the more autolytic fetal eyes, the RPE was disrupted, slightly dispersed, or folded (Fig. 5D).

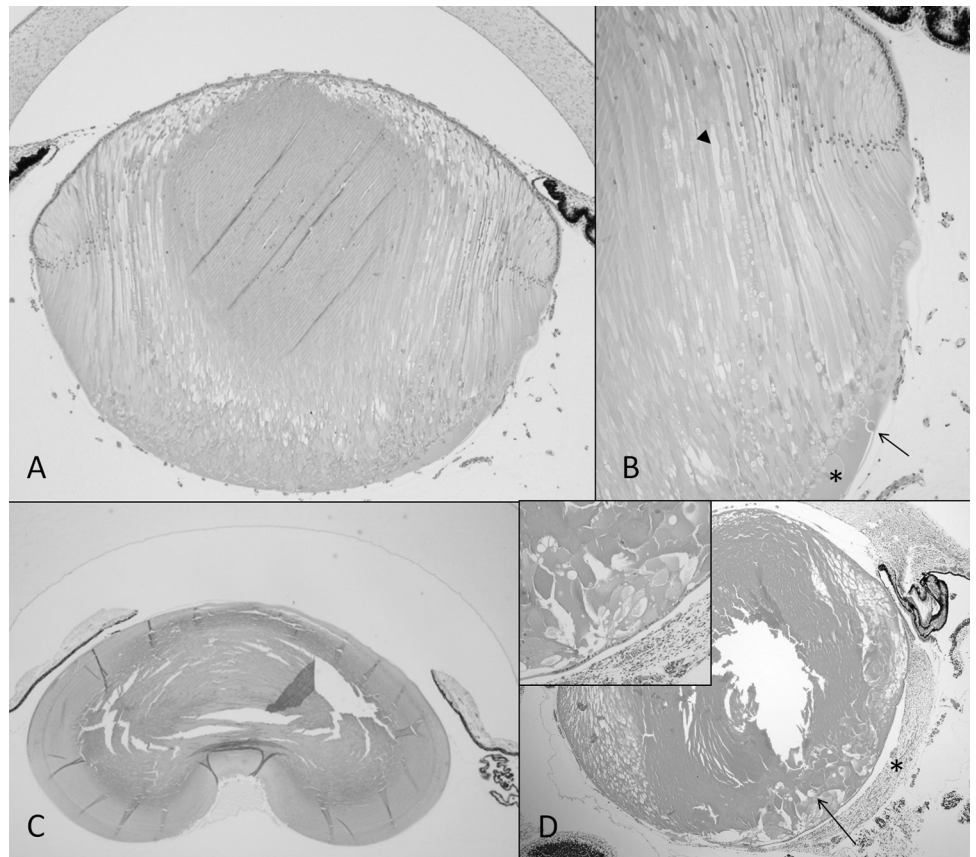


FIGURE 4. Common artificial changes of the fetal lens that appear similar to cataractous lenses in adults (15 WoG, H&E). (A) Low power. (B) Higher magnification showing clefts (*arrowhead*), globules (*arrow*), and subcapsular material. (C) Indentation of the posterior pole (37 WoG, H&E). (D) More obvious cataract-like changes (*arrow, inset*) associated with PHPV (*asterisk*) are present in a lens of a fetus with trisomy 13 (17 WoG, H&E).

Choroid

The choroid was usually not the main target of autolysis. In some fetal eyes choroidal hemorrhage was present but presumably attributed more to certain perinatal circumstances, including iatrogenic interventions (Fig. 5E). Rarely, the choroid was swollen, and small “vacuoles” were present next to hemorrhagic vessels corresponding to transudation. Usually there was no doubt with regard to making the diagnosis of colobomatous lesions affecting the choroid and RPE because artificial loss or damage of the choroid alone was not observed (in contrast to the RPE).

Optic Nerve

In general, the optic nerve did not reveal many autolytic changes. Because of manipulations during the removal of the globe, the optic nerve head could appear swollen, if not completely absent, macroscopically. The microscopic view then displayed edematous glia and nerve fibers protruding into the vitreous cavity (Fig. 5F).

In two pairs of fetal eyes, leukocytes were present in the optic nerve. Most likely these were genuine because both fetuses died of inflammatory encephalopathy.

Sclera and Extraocular Muscles

The sclera and extraocular muscles of the fetal eye are structures affected less by artifacts. Scleral involvement in fetal pathology was rare but manifested as staphyloma with a thinned and ectatic sclera. These findings could not be mistaken for artificial lesions.

Postmortem Artifacts Associated with Fixation

Some artifacts, such as Lange folds and retinal detachment, are well known and typically occur after fixation. In addition, we

observed artificial folds in the developing macular region in many eyes.

In our cohort, Lange folds (and associated meridional folds) were found in fixed fetal eyes from the beginning of the second trimester, occurring as small macroscopically and light microscopically detectable folds in the ora serrata region (Fig. 6A). Many of our eyes showed these folds regardless of whether they were fixed in formalin or in glutaraldehyde.

Folds called plica centralis, similar in shape to Lange folds, were found in the developing macular region in the second and third trimesters⁴ (Fig. 6B). The structure of the retina involved in these folds was not altered compared with the surrounding retina except for thinned and more “separated” retinal layers, indicating a more advanced differentiation. These macular folds were also detectable macroscopically and light microscopically. Because all eyes were opened horizontally, it was possible to localize this fold to the area of the developing macula.

The phenomenon of retinal detachment after fixation (Fig. 2) is well known and is related to the fact that there are no cellular adhesions between the neuroretina and the RPE; retinal attachment requires the pumping mechanism of the RPE. In most fixed eyes the retina seems to be more or less attached after the globe is bisected. Microscopically, however, the retina was detached after fixation in formaldehyde and in Karnovsky’s solution. In general, as expected, Karnovsky’s solution provided a slightly better preservation of the ocular structures than formalin fixation.

Probably True Histopathologic Findings

In many cases the diagnosis of true histopathologic findings was confounded by artifacts. The following four examples are presented to illustrate the difficulty and problems of differentiating between artifacts and real findings.

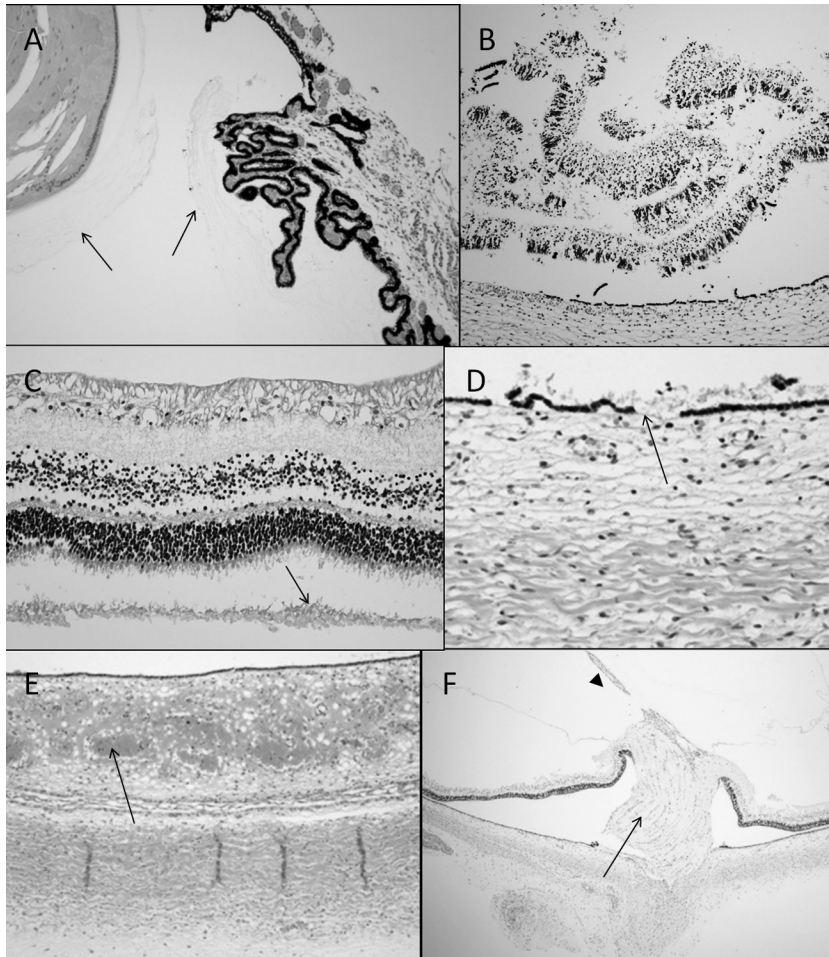


FIGURE 5. (A) Only parts of the vitreous (*arrows*) are present after the fixation and embedding process and are usually found next to the ciliary body and lens as zonules/tertiary vitreous (18 WoG, H&E). (B) Fragmentation of the retina impairing the ophthalmopathologic investigation. The RPE and the choroid remain largely intact (23 WoG, H&E). (C) Regular retina of a mature newborn with separation and accumulation of photoreceptor material (*arrow*, H&E). (D) Damage of the RPE (*arrow*) with regular choroid and choriocapillaris (20 WoG, H&E). (E) Hemorrhage (*arrow*) of the choroid and presence of small vesicles (22 WoG, H&E). (F) Swollen and displaced optic nerve head (*arrow*; 19 WoG, H&E). Note parts of the hyaloid vessel (*arrow-head*).

Example 1. A fetus with Goldenhar's syndrome (23rd week of gestation) revealed one normal-sized and one microphthalmic eye with a chondromatous scleral choristoma accompanied by a coloboma and a staphyloma (Fig. 7A). Although there were many obvious autolytic changes, especially involving the retina, the diagnosis of this congenital colobomatous defect of the RPE and choroid could be verified by histopathologic investigation.

Example 2. A fetus with trisomy 13 (29th week of gestation) had retinal anomalies that consisted of knob-like excrescences (Fig. 7B) in both eyes. Typical autolytic artifacts, such as the slightly detached retina and some retinal "breaks" and folds, could clearly be distinguished from these retinal changes and were also different from so-called pseudorosettes that have been found in other eyes with trisomy 13.

Example 3. A fetus (23rd week of gestation) that died in utero because of chorioamnionitis without any evidence of a malformation, a chromosomal aberration, or another syndrome was macroscopically found to have a peripapillary loss of RPE. Histopathologic investigation confirmed this finding (Fig. 7C), revealing a lack of RPE next to the optic nerve with what appeared to be an obvious direct connection between the neuroretina and Bruch's membrane. All in all, there were many autolytic alterations, especially of the retina, but the observed adhesion seemed to be more than an accidental attachment.

Example 4. In an otherwise healthy fetus (24 WoG) a circumscribed defect in the retinal midperiphery was detected by macroscopic routine examination. Light microscopy showed the retina to be absent in a small area (Fig. 7D). The RPE was intact, as was the choroid. Nevertheless, this areolar defect

might have been caused by an extrinsic force, such as rough removal of the globe.

DISCUSSION

Artifacts were observed frequently in our cohort of fetal eyes. Different influence factors such as autolysis, fixation, the cause of death (namely, termination of pregnancy by feticide vs. intrauterine death), and mechanical trauma have to be considered (Herwig MC, et al. *IOVS* 2009;50:ARVO E-Abstract 4814). In most cases, the artifacts can be interpreted as a result of a combination of all influence factors. An exact differentiation between these factors is, therefore, impossible, but an approach to describe some specific characteristics is provided.

Autolysis

As described, numerous artifacts can be observed in fetal eyes, and most of them are the result of autolysis, either intrauterine or predominantly postpartum. Artifacts in tissues of the cornea, conjunctiva, sclera, choroid, or RPE do not have a huge impact on evaluation quality. In contrast, other ocular structures such as the retina and the lens are more severely affected by artifacts and thus are more difficult to evaluate histopathologically. In particular, the evaluation of the fetal lens is challenging because of autolytic changes (such as globules, clefts, and subcapsular exudates) that mimic cataractous changes in adult eyes. These variations of the lens structure should not be used for the diag-

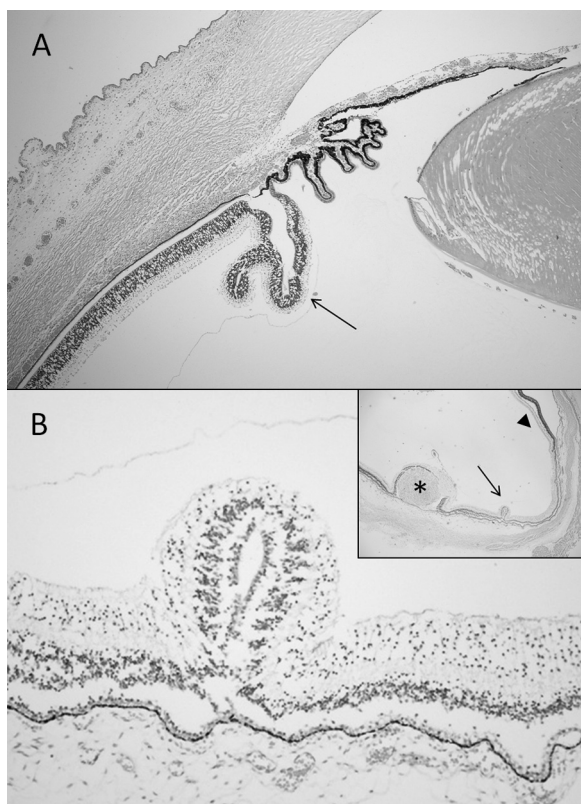


FIGURE 6. Well-known postmortem artifacts including Lange folds (A, arrow; 23WoG, H&E) in the ora serrata region and the plica centralis (B, arrow; plica centralis; asterisk, swollen optic nerve head; 18 WoG, H&E), both of which occur characteristically in fixed fetal eyes. Note the different morphology/architecture of the peripheral retina (inset, arrowhead) in contrast to the developing macular region in (B).

nosis of a fetal cataract. This diagnosis should only be established in the presence of a pattern of definitive cellular variations. The anterior chamber and the ciliary body can also be affected by autolysis, making an evaluation of these delicate structures with respect to developmental aspects extremely difficult.

Fixation

In this study, fetal eyes were fixed either in formalin or in glutaraldehyde. Although glutaraldehyde is known to preserve the tissue morphology slightly better than formalin, immunohistochemical investigations are less reliable. It is well known that fixation, apart from conserving tissues, also has a negative impact on the specimen itself and, therefore, induces artifacts.^{2,3} In the late 19th century, the impact of different artifacts on the lens and its capsule had been described.^{6,7} In general, however, these changes in tissue integrity cannot be avoided because fixation is needed as a standard procedure in pathology.

Cause of Death

Type of termination can also influence the extent of artifacts. Fetuses that died in utero can remain up to weeks in the maternal uterus, leading to a higher extent of autolysis. Inflammatory processes as in chorioamnionitis or other causes for placenta insufficiency lead to digestive changes within the fetus.

In contrast, feticide is performed by injecting potassium chloride into the fetal umbilical vein. This poison may also

have an influence on ocular tissues through the blood supply, especially the ophthalmic and the hyaloid arteries.

Mechanical Trauma

Artifacts caused by mechanical trauma occur in most cases in relation to the actual autopsy. They are less frequent than the previously discussed influence factors and are dependent on the technician. Examples of mechanical alterations are perforation and compression of the globe, complete removal of the optic nerve with vitreous extrusion, and corneal scratches. In Example 4 the circumscribed absence of the peripheral retina most likely can be attributed to mechanical trauma as well. Hence, careful and more diligent autopsy may prevent the frequency of these kinds of artifacts.

Postmortem Artifacts

Well-documented postmortem artifacts that occur in fixed fetal ocular tissue are Lange folds (with associated meridional folds) and the plica centralis. They have been previously described by Lange, Daicker, and others.^{5,7,8} The folds of the peripheral fundus also impair the investigation of the ora serrata region.⁵

The postmortem occurrence of retinal detachment is a phenomenon not only in fetal eyes; it can also be observed in adult eyes because of the lack of cellular adhesion between the neuroretina and the RPE. Usually, this phenomenon occurs during the process of fixation.

Examples

Although artifacts may impact the histopathologic investigation, distinct findings can be diagnosed even in eyes with autolytic changes.

The fetus with Goldenhar's syndrome (Example 1) revealed tremendous autolytic damage to the retina, but the coloboma (with lack of the choroid and RPE) and the choristoma and staphyloma could be diagnosed without any doubt. By assuming the presence of a choroidal coloboma macroscopically, one can easily detect it microscopically with appropriate sectioning because the involved ocular structures are relatively insensitive to autolysis. This must be considered in fetuses with cat eye syndrome that usually reveal colobomata.⁹ In contrast, the detection of an iris coloboma is much more difficult macroscopically and microscopically because of impaired corneal transparency postmortem and a higher sensitivity of iris structures to autolysis.

In addition, the knob-like excrescences of the retina in a fetus with trisomy 13 (Example 2) are difficult to interpret. Retinal findings have been described in 55% of fetuses with trisomy 13.⁹ One of our 7 fetuses with trisomy 13 had pseudorosettes in combination with PHPV. These knob-like retinal changes are not likely to have been pre-stages of rosettes because of the fetal age (29 weeks of gestation) and the different morphology. Although they might have been regarded as true lesions, a (cutting) artifact cannot be completely excluded.

There are also findings of unknown origin. The direct connection between the neuroretina and Bruch's membrane in the absence of RPE (Example 3) is difficult to interpret. On one hand, this finding looks like a true adhesion, but on the other hand it cannot be explained from a developmental point of view.¹⁰ In peripapillary atrophy, a similar phenomenon can occur next to the optic nerve, but in our lesion there was a small space between the optic nerve and the assumed adhesion. In addition, in contrast to colobomata that occur in the region of the previous embryonic fissure,

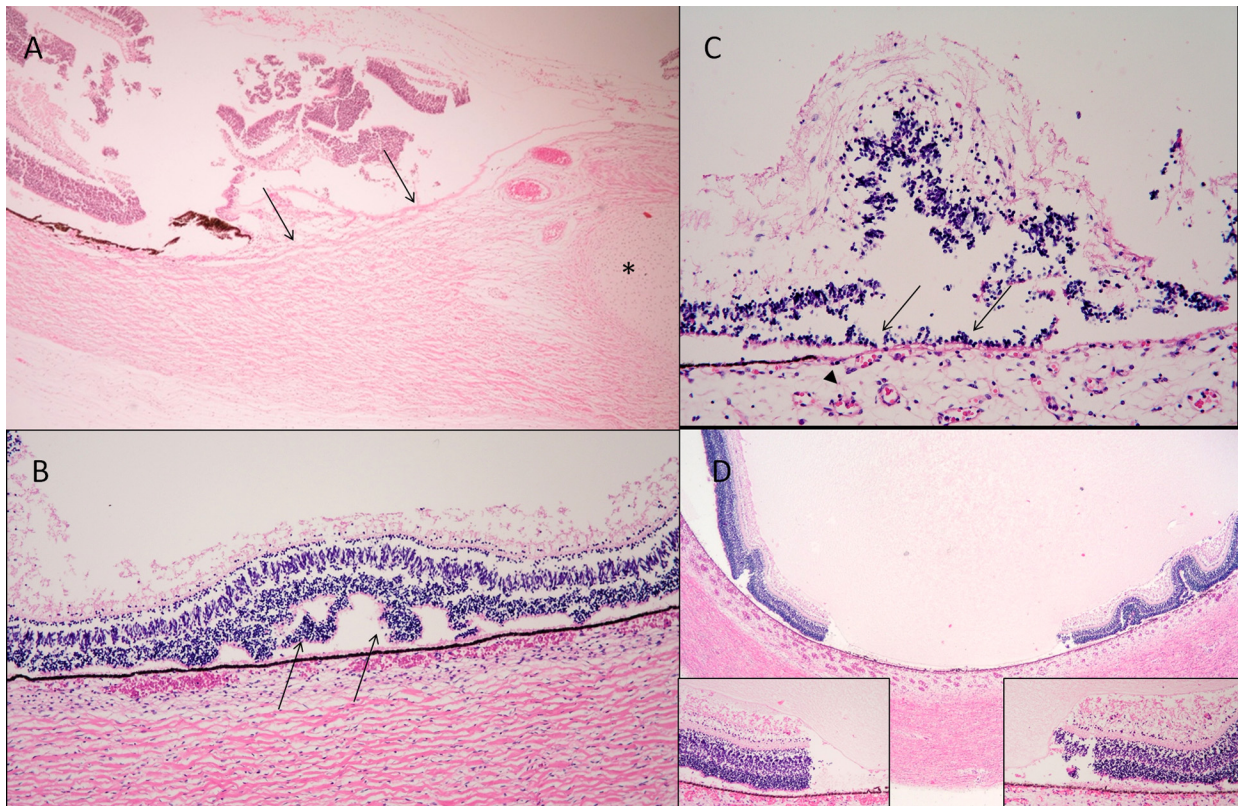


FIGURE 7. (A) Microphthalmic eye of a fetus with Goldenhar's syndrome revealing a chondromatous choristoma of the sclera (*asterisk*) and a coloboma (*arrow*) in the region of a staphyloma (23 WoG, H&E). (B) Knob-like excrescences (*arrows*) in a fetus with trisomy 13 (13 WoG, H&E). (C) Peripapillary loss of RPE (*arrowhead*) with adhesion between neuroretina and Bruch's membrane (*arrows*; 23 WoG, H&E). (D) Circumscribed defect in the retinal periphery in an otherwise healthy fetus (24 WoG, H&E).

our lesion revealed only a lack of RPE cells and was circular (around the optic nerve). Therefore, an artificial origin cannot be excluded.

Despite the diverse artifacts that can occur in fetal eyes, a reliable differentiation between artifacts and true findings is possible in most instances, provided that the investigating ophthalmic pathologist analyzes the findings thoroughly and critically.

Forensic Aspects

The analysis of fetal eyes is important not only for a complete pediatric pathologic investigation or to diagnose prenatal ocular findings that allow for a sonographic correlation prenatally. Under forensic aspects especially, the evaluation of artifacts in fetal eyes is of great importance to warrant a reliable evaluation of postmortem eyes of fetuses, newborns, and infants. With regard to the circumstances of a forensic autopsy, artifacts caused by autolysis or mechanical trauma must be considered.

Considering the variety of artifacts, the interpretation of fetal eyes is challenging. Obvious findings need a thorough and critical analysis to distinguish ocular abnormalities from artifacts. Hence, experience is required to determine the presence not only of subtle but also of more advanced prenatal ocular abnormalities.

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