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Review Article

Albumins and Carotenoids of the Human Fetal Vitreous Body and their Morphogenetic Role during Midgestation

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Abstract

The vitreous is a transparent, highly hydrated gel located between the lens and the retina. In the adult vitreous carotenoids are absent. At 15–28 weeks of gestation the molecular composition of human fetal vitreous is characterized by twenty fold increase in the albumin content as compared to that in the adult vitreous, and by the presence of two transitory molecules – lutein and alpha-fetoprotein. A strong correlation between the albumin and lutein concentrations was found. The organizing molecule of this complex is albumin. The morphogenetic roles of albumin, alpha-fetoprotein and lutein in the human eye development and physiology are discussed.

Keywords: Human Fetal Vitreous; Albumin; Lutein; Alpha-Fetoprotein; Embryonic Intraocular Pressure; Macula Lutea

Abbreviations

WG: Weeks of Gestation;

3,3'-Di-(gamma-sulfopropyl)-4,5,4',5'-dibenzo-9-EthylthiacarboCyanine betaine pyridinium salt;

SDS PAGE: Sodium Dodecyl Sulfate PolyAcrylamide Gel Electrophoresis;

HPLC: High Performance Liquid Chromatography;

UV: Ultra Violet;

FAZ: Fovea Avascular Zone;

PEDF: Pigment Epithelium-Derived Factor;

NPPB: Natriuretic Peptide Precursor B;

Eph-A6: Ephrin type-A receptor 6

Introduction

The vitreous body of the adult eye is a transparent highly hydrated gel-like extracellular matrix of a special type. It consists almost entirely of water (98–99.7%) and occupies the posterior cavity of the eye, being located between the retina and the lens. The gel structure of the vitreous is maintained by a diluted network of thin unbranched collagen fibrils comprising type II, V/XI and IX collagens. In the mammalian vitreous, glycosaminoglycan hyaluronan is the major component that fills the space between collagen fibrils. Other components of the adult vitreous are inorganic salts, albumin, globulin, opticin, coagulation proteins, complement factors, and low-molecular-weight proteins [1–5]. In contrast with other eye tissues, in the adult vitreous carotenoids are absent [6, 7].

Studies of molecular composition of the human fetal vitreous are important not only for comprehending its morphogenetic role in the normal eye development, but also for understanding the etiology of inborn eye defects. The latter aspect sides with the interest of ophthalmologists to the problem of retinopathy of prematurity.

A new approach to molecular studies of the human fetal vitreous has been stimulated by embryologists [8–18]. It has been experimentally proven that after closure of the choroid fissure, the developing vitreous creates intraocular pressure. Tangential forces arising within the eye walls under this influence control the total growth of the eye and coordinated growth of the retina and retinal pigment epithelium, as well as cell differentiation and normal morphogenesis of all parts of the eye. Disturbances in the vitreous development provoke different inborn ocular abnormalities. The results obtained attracted attention to the morphogenetic function of the vitreous [8–18]. In this review, we have undertaken an attempt to decipher this function at the molecular level. Classification of the developmental stages of the human vitreous was proposed by Ida Mann [19].

Despite the considerable knowledge of the macromolecular structure of adult vitreous, only few studies were devoted to the fetal human vitreous. Type III collagen was found in the primary and early second vitreous, as well as type II collagen, chondroitin-6-sulfate-proteoglycan and hyaluronic acid in the secondary and tertiary vitreous, and chondroitin-4-sulfate-proteoglycan [20, 21] and opticin [22, 23] in the tertiary vitreous (Fig. 1). Undoubtedly, these molecules are a part of the system responsible for the prenatal intraocular pressure. The same molecules, except type III collagen, take part in the maintenance of the adult vitreous volume. However, whereas the volume of the normal adult vitreous retains its *status quo*, the volume of the fetal vitreous increases permanently and is characterized by the period of intensive growth between 16 and 24 weeks of gestation (WG) (Fig. 2) [24], in parallel to the intensive growth of the total eye [25]. The search for molecules capable of securing such intensification of the vitreous growth attracted attention to albumin. Alpha-fetoprotein and lutein were first found in the human fetal vitreous in the course of these studies. This review is focused on the cooperative morphogenetic and metabolic role of albumin, alpha-fetoprotein and lutein of the human fetal vitreous in the eye development.

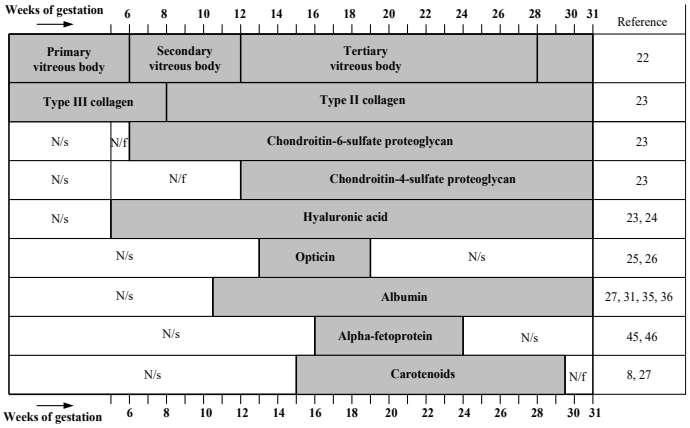

Figure 1. Scheme summarizing the data on molecular composition of the human fetal vitreous. Stages of the human fetal vitreous development according to Ida Mann's classification [19] are specified in the first upper horizontal bar. Other horizontal bars show molecules found in the vitreous at different stages of development of human fetuses. N/s, not studied; N/f, not found.

Albumin

Albumin was chosen as the first molecule for the study due to its several properties. Albumin possesses a high ability to bind water, thus creating viscosity and controlling the osmotic pressure of solutions. In particular, 1% albumin solution creates colloid-osmotic pressure of 75 mm H_2O, whereas the corresponding globulin solution creates only 19 mm H_2O [26]. This property of albumin is realized in the blood stream, where it is responsible for creation of the oncotic pressure and regulation of the circulating blood volume [27]. Based on these data, a hypothesis was suggested on possible involvement of albumin in creation of the prenatal intraocular pressure [28]. Albumin is also the main transport protein in mammals and humans [27] and possesses antioxidant properties [29–31]. To understand the role of albumin in the human eye development, it was necessary to study the dynamics of its content in the fetal vitreous throughout most of the prenatal development.

As a new method for the eye studies, the spectral-fluorescent probe, polymethine (cyanine) dye 3,3'-di-(gamma-sulfopropyl)-4,5,4',5'-dibenzo-9-ethylthiacarbocyanine betaine pyridinium salt (DEC) was used to measure the concentration of albumin in the human fetal vitreous [32–34]. DEC possesses unique

properties: it can specifically recognize molecules of human serum albumin, as well as permits working with small aliquots of material under study in a state close to native [28, 33, 35, 36]. DEC did not specifically interact with albumins of animals such as rat, cattle, or rabbit [36]. The data on human serum albumin obtained by DEC were confirmed by the results obtained on a biochemical analyzer Synchron CX4-CE (unpublished data), by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE) and Western blotting [33].

Studies of albumin content in the human fetal vitreous has revealed regular changes in its concentration. At 9.5–12.5 WG, albumin was not detected by DEC absorption spectra. However, the presence of trace albumin at 10.5, 11 and 12.5 WG was shown by DEC fluorescence spectra [33]. Thereafter, the albumin concentration steadily increased and, according to the measurements of DEC absorption spectra, was retained at the highest level between 16 and 24 WG with a maximum during 17 WG (2.11 x 10^{-4} mol/L), that is, about twenty-fold higher than the albumin content in the adult vitreous. To 28 WG, the vitreous albumin concentration decreased and fell down to (0.029–0.12) x 10^{-4} mol/L at 30–31 WG [24, 33] (Fig. 3), that is, to the albumin level in the adult vitreous (0.013–0.29) x 10^{-4} mol/L) [37].

The coincidence in time of the high concentration of the fetal vitreous albumin with the intensive growth of the vitreous volume (Figs. 2, 3) [24] and of the total eye growth [25] has confirmed the suggestion that albumin is a part of the molecular system ensuring the oncotic intraocular pressure, but only during the second trimester of pregnancy. In this case albumin may be considered as one of the main components of the vitreous molecular system responsible for the intensive growth of the eye in the tertiary vitreous.

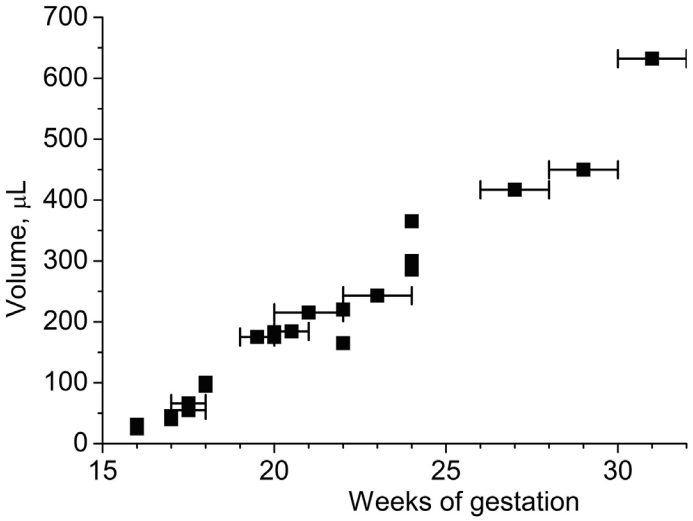

Figure 2. Average volume of the fetal vitreous during 16–31WG [24]. Horizontal bars denote intervals of fetal ages (WG) determined by an obstetrician.

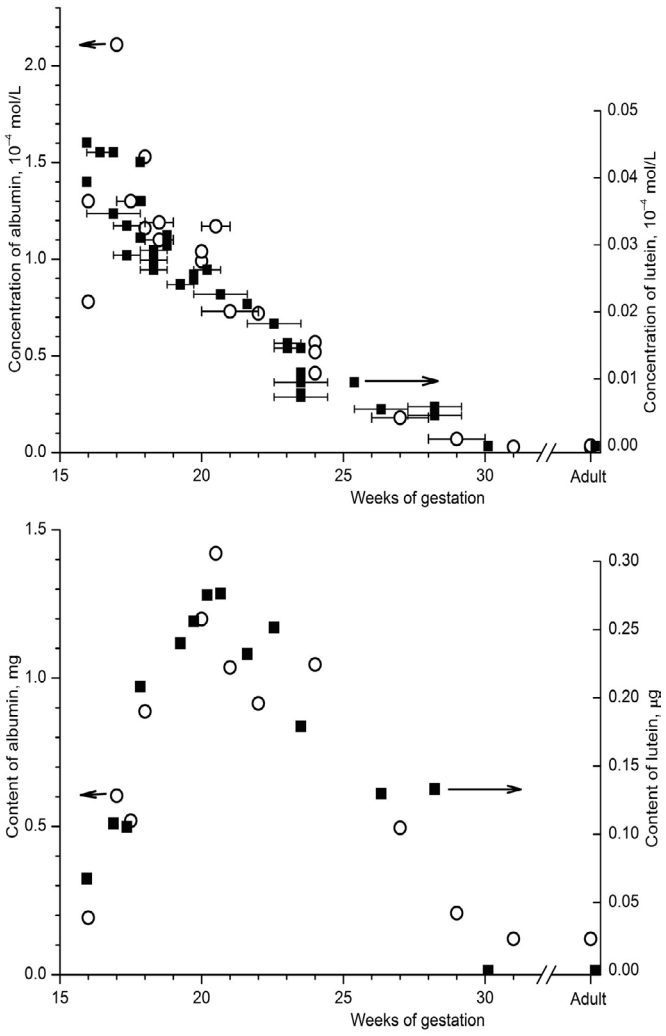

Figure 3. Concentrations (A) and total content (B) of albumin (open circles, left-hand scale) and lutein (filled squares, right-hand scale) in the human fetal vitreous during 16–31 WG [24]. Horizontal bars denote intervals of fetal ages (WG) determined by an obstetrician. Correlation between albumin and lutein concentrations and content in the human fetuses vitreous can be clearly seen.

Between 28 and 31 WG, when the albumin concentration in the human fetal vitreous decreases to that in the adult vitreous, a change in the mechanisms maintaining the intraocular pressure takes place. From the beginning of 28 WG, the intraocular pressure becomes dependent upon the secretory activity of the ciliary body and development of the anterior chamber of the eye with the system of Chlemm canal, as in adults [18, 38, 39].

Alpha-fetoprotein

Alpha-fetoprotein belongs to the albumin family and is similar in its structure and functions to serum albumin [40, 41]. The

study of alpha-fetoprotein, a specific blood protein of human embryos and fetuses, was carried out on the human fetal vitreous during the period of the highest albumin concentration (16–24 WG). Using the diagnostic chemiluminescent method with antibodies to alpha-fetoprotein, the presence of alpha-fetoprotein in the human fetal vitreous was shown [42] (Fig. 1). Alpha-fetoprotein did not interact with DEC [43] indicating a high selectivity of DEC for human albumin.

Alpha-fetoprotein may be considered as the second molecule responsible for the fetal intraocular oncotic pressure, based on the fact that alpha-fetoprotein, as well as albumin, provides oncotic pressure in the blood stream of the fetus [40]. Both albumin [29–31] and alpha-fetoprotein [44] are effective antioxidants. The presence of both molecules in the fetus vitreous should enhance its antioxidant protection.

Albumin and alpha-fetoprotein possess the properties of transport proteins. They bind and carry various biomolecules such as growth factors, hormones, vitamins, lipids, and polyunsaturated fatty acids, which are necessary for growth and differentiation of various tissues [27, 40]. Albumin and alpha-fetoprotein provide the embryo and fetus cells with polyunsaturated fatty acids necessary for the construction of cell membranes, but the affinity of alpha-fetoprotein for polyunsaturated fatty acids is much higher than that of albumin [45]. In the mammalian fetus, cell membranes of the retina and the brain are characterized by a high content of polyunsaturated fatty acids [46] and contain numerous receptors to alpha-fetoprotein [47]. Hence, one of the main functions of vitreous alpha-fetoprotein during midgestation might be transport of polyunsaturated fatty acids from mother to fetus [48, 49] and, subsequently, from the fetal blood into the fetal vitreous. The functional significance of alpha-fetoprotein in the fetal vitreous deserves further research.

Carotenoids

Various carotenoids circulate in the blood; however, lutein and zeaxanthin are the only carotenoids found in the eye [6, 50]. Lutein and zeaxanthin were found in all eye parts, but they are absent in the adult vitreous [6]. In the human fetal vitreous in the state close to native, a yellowish color of the vitreous was noticed. The assumption that this color depends on the presence of carotenoids in the fetal vitreous was confirmed. Using UV spectroscopy and high performance liquid chromatography (HPLC) with lutein as a standard, lutein was first revealed in the human fetal vitreous [7]. Oxidized forms of lutein were also identified in the fetal vitreous and in the lens [51, 52]. Using HPLC and UV spectroscopy, lutein in the human fetal vitreous was detected in the range of 15–28 WG [7]. At 16–28 WG, the content of lutein was measured by UV spectroscopy, and the highest values of lutein concentration were found at 16–17 WG ($4.5x10^{-6}$ mol/L), that is, during the time of the highest concentration of vitreous albumin. Then lutein concentration gradually decreased (Fig. 3), and at 30–31 WG lutein was absent in the fetal vitreous, as well as in the adult one [7, 24]. Hence, during the second trimester of pregnancy, lutein enters into the human vitreous composition as a transient molecule.

The finding of lutein in the human fetal vitreous is of great importance. The striking correlation of lutein and albumin concentrations at 16–28 WG (Fig. 3) should be considered as a regular phenomenon since the ability of albumin to bind carotenoids with formation of a water-soluble complex is known [53]. Excess of albumin over lutein content by more than 40 times [24] can provide better binding of carotenoids with albumin. It seems that fetal vitreous albumin could be regarded as an organizing molecule for lutein in the processes of its uptake, storage, transport and deposition to the target eye tissues. If so, then the level of the lutein concentration in the vitreous should depend on the concentration of albumin.

During postnatal development, carotenoids as antioxidants and a filter absorbing blue light protect the macula against damages [54–59]. *In utero* the filter function, of course, is of no use. But the antioxidant role of carotenoids in the fetal tertiary vitreous is very important for protection of the retina, lens and vitreous itself against damaging impact of reactive oxygen species in a situation of the hyaloid system regression and development of the definitive retina blood system. It is possible that albumin in a complex with lutein enhances its antioxidant capability, as shown for the complex «albumin–bilirubin» [29].

The role of the fetal vitreous lutein is not exhausted by its protective function. If vitreous albumin is an organizing molecule with respect to carotenoids, carotenoids themselves play a direct morphogenetic role in the differentiation of various parts of the visual system, as discussed in the comprehensive review of Zimmer and Hammond [58]. In particular, lutein interacts with tubulin in the formation of microtubules and plays a crucial role in the directed growth of axons forming the ocular nerve and in regulation of gap junction activity between neurons and glia [59–61]. We assume that vitreous lutein, located so close to the ganglion cells and their axons, which express beta-III tubulin [62], might be considered as an "inductor" of presumptive macula and, in any case, as one of the important factors of macular differentiation throughout midgestation. To approach this problem, it is of interest to compare the early events of macula formation with the dynamics of lutein concentration in the fetal vitreous.

Carotenoids of the fetal vitreous and development of the macula

By the birth, all parts of the eye have been formed, except the macula, whose differentiation will be completed only by four years of postnatal life [63]. Lutein remains a predominant

carotenoid of the macula up to 1 year and 7 months, whereas zeaxanthin becomes the dominant form by 3 years [64]. In the adult eye, on the average, 70% of carotenoids are concentrated in the macular area of the retina with the highest concentration in the inner layers of the fovea [65].

At the morphological level, the first ganglion cells are detected in the dorso-temporal sector of central retina at 6 WG, and their axons are detected at 6–8 WG [19, 66]. Using antibodies to beta-III tubulin, tubulin was detected in the ganglion cells and in the layer of nerve fibers in the area of presumptive macula at 8.5–10 WG [62]. Since tubulin is a structural carotenoid-binding protein of axon microtubules and neurons [59–61], one may suggest that the interaction of fetal vitreous lutein with beta III tubulin of the first ganglion cells is responsible for the localization of presumptive macula. However, it is unknown whether lutein is present in the vitreous at these early stages. But in view of the fact that albumin, a carotenoid-carrier protein, is present in the fetal vitreous in trace amounts at these stages, it cannot be excluded that lutein may also be present in the vitreous in such amounts.

The concentration of cones increases in the area of future macula at 10–11 WG [63, 67]. Traces of carotenoids were found in the presumptive macula at 17–20 WG, with the content of lutein being 2.5 times that of zeaxanthin [64]. During this important period, the vitreous is characterized by the highest concentration of lutein (Fig. 3) [24]. At 20 WG, the total content of carotenoids in the retina is 3 ng [64] and 276 ng in the vitreous (Fig. 2D) [24].

A prerequisite of fovea development in the center of macula is the presence of fovea avascular zone (FAZ). The formation of FAZ begins at 25–27 WG [68, 69], and development of the fovea begins at 26 WG [68, 69]. The factors that impede the spreading of vessels to FAZ include negative regulators of angiogenesis. These are carotenoids [70, 71], PEDF, NPPB, type IVα2 collagen, and Eph-A6 [66, 71–73].

The lutein content in the human fetal vitreous becomes negligible by 28 WG, and it is no longer detected at 30–31 WG [24], whereas differentiation of the macula continues. This means that when the definitive retinal blood circulation is close to completion [74], the source of carotenoids delivery to the macula is changed. By the birth, lipoproteins are known to be carriers of carotenoids from blood to macula [53, 75–78].

A long-term accumulation of lutein in the fetal vitreous precedes the appearance of the first traces of carotenoids in the presumptive macula at 17–20 WG. Hence, the early macula differentiation proceeds against the high level of vitreous lutein concentration. By contrast, the period of FAZ formation is realized against the background of a low concentration of vitreous lutein. Nothing is known about vitreous lutein at the earliest stages of macula development. These data raise a problem of dose-dependent relationship between vitreous carotenoids and the early stages of normal macula differentiation.

The strong dependence of the lutein content on the albumin concentration in the fetal vitreous put forward the aspect of full-value of the food formula for pregnant women with respect to optimal doses of not only food carotenoids [79, 80], but also of protein components.

A reduction of the albumin concentration in the tertiary vitreous would entail also serious morphogenetic consequences. These can be a drop in the oncotic intraocular pressure with disturbances of the retina structure such as arising retina rosettes and folding, as well as a shift of the visual axis and a decrease in the lutein concentration, which may affect the macula differentiation. Substantiation of these ideas is a task for future studies.

Conclusions

It seems that the results summarized in this review will enhance the interest to the problem of developmental function of carotenoids in the visual system. Furthermore, the studies of the human fetal vitreous carotenoids will inevitably contribute to the problems of macular dystrophy of alimentary etiology and food formula for women during pregnancy and lactation [78–83].

To answer the question whether the prenatal vitreous carotenoids are prerogative of the human eye, extensive comparative studies of prenatal vitreous of different species are necessary. These might be primates, whose retina has the macula, and any other animals, whose retina is devoid of this structure. So, if a causal relationship between fetal vitreous carotenoids and early stages of macula differentiation does exist, vitreous carotenoids should be found in primates and should be absent in other species of animals.

The second task for future studies is a search for mechanisms that underlie a possible influence of fetal vitreous carotenoids on the early stages of macula differentiation. We hope that this review will be useful for future studies.

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Conflict of interests

There are no conflicts of interests, and no relationships that would in any way influence or bias this review.

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