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ORIGINAL ARTICLE

Retinae of Anencephalic Fetuses: Quantitative Analysis and Comparison with Fetuses Without any Malformations

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Abstract Anencephaly is a common congenital malformation characterised by absence of a major portion of the brain. The sequence of brain degeneration starts around 20 days post fertilization which overlaps with the appearance of optic grooves from the forebrain. A number of ocular malformations associated with anencephaly have been reported. Based on the assumption that eyes, which are derived from the brain, are likely to be abnormal since the organ of origin undergoes degeneration, studies have been carried out on anencephalic retinae. Some of the results have indicated that retinae of anencephalic fetuses are abnormal with absence or diminished presence of ganglion cell layer compared to the fetuses without such a brain malformation. We undertook the present study with an aim to objectively and quantitatively evaluate retinal thickness and numbers of ganglion cell in anencephalic fetuses and fetuses without any malformations. Six anencephalic and six fetuses without malformations were included, all of them from 18 to 21 weeks of gestation. Histopathological evaluation of retinae was done and findings compared. We did not find any atrophy or reduced number of ganglion layer cells in anencephalic fetuses. This evaluation assumes importance since retinae from anencephalic fetuses can be a potential source for retinal tissue or stem cell transplant. Moreover, the contention that normal brain development is essential for retinal development appears to be invalid.

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Introduction

In the normal human embryo, ocular development begins in the 5th week as a pair of shallow grooves on the sides of the diencephalon. With simultaneous closure of the neural tube, these grooves form outpouchings of the forebrain to develop into optic vesicles by the 4th week of gestation [1]. Anencephaly is the most common congenital anomaly of the central nervous system. The incidence of anencephaly is 1:1000–1:20,000. It is a congenital developmental defect associated with absence of major portions of the brain, skull and scalp which begins in the 1st month of gestation. The abnormalities occur due to defective closure of the cranial part of the neural tube, due to which neural tissue is not invested with the skull covering and therefore the neural tissue is exposed to the amniotic fluid resulting in destructive changes like degeneration and hemorrhage. Anencephaly is also associated with other congenital developmental anomaly of the vertebral column, abdominal wall, diaphragm and other parts of the human fetus [2]. Anencephaly has been associated with anophthalmia, microphthalmia, cystic eye ball, cyclopia, synopthalmos, uveal colobomas, corneal dermoids, thin corneal epithelium, pupillary vascular membrane, early cataract changes, posterior pole lens concavity, ectopic lacrimal tissue in the episclera and retinopathy of prematuriy [3–7]. Since the eye develops from the forebrain (diencephalon), it is reasonable to assume that malformation of brain particularly diencephalon should affect ocular development resulting in defects that can be discerned either by gross or microscopic examination.

The literature so far does not offer an equivocal verdict regarding retinae of anencephalic fetuses. Whereas Hendrickson et al. along with Boniuk et al. have concluded that the ganglion cell layer is completely absent in retinae of anencephalic fetuses from 24 weeks onwards, other investigators have found the same to be present although atrophic and/or hypoplastic [1, 7–13].

We have attempted to fill the gap in this knowledge because of dearth of systematic and quantitative studies on retinae in such cases. Retinal evaluation in anenecephalic cases might be important given the therapeutic potential of either full thickness retinal transplant or a part, including stem cell transplantation [14–16]. In the context of transplantation it is important to demonstrate that anencephalic retinae are similar to fetuses without any congenital malformations. So far the opinion is divided in the literature.

Material and Methods

Fetuses from 18 to 21 weeks of gestation were evaluated after consent of one of the parents on a standard consent form. Ethical approval was taken from the institutional committee. Autopsy was carried out in all the fetuses. Six cases of anencephaly and six cases of intrauterine death without any congenital malformations were included. The six fetuses used for control were selected on the basis of similar gestational age to that of the anencephalic group. It was ensured that none of the control group had gross or microscopic findings consistent with any congenital malformation or defect. None of the anencephalic fetuses had any other malformation. Fetuses without congenital malformation but showing evidence of maceration on gross examination were excluded. Eye dissection of fetuses was carried out and globes were injected with 10% neutral buffered formalin using a 25 gauge needle. The eyes were kept in a container with 10% neutral buffered formalin for at least 3 days. After fixation of the specimen the sections were taken from each eye and were processed in cassettes for embedding in paraffin and block making. Five micron thick sections of the eye were taken and the slides were subjected to routine Hematoxylin and Eosin staining.

Using Leica microsystem framework version 4.3, total thickness of the retina was measured and in the same area the numbers of ganglion cells were counted in a given length of 100 μm.

In retinal examination only those sections with linear retinal segments and with no significant autolytic changes were included. Sections of retina with tangential cuts were excluded from evaluation.

The comparison was made between features seen in anencephalic fetuses and those in fetuses without any congenital malformations.

Results

12 fetuses were evaluated out of which six were anencephalic and six were without any gross malformations. Five were female and seven male. The gestational age ranged from 18 to 21 weeks.

Figures 1 and 2 show representative pictures of acceptable retinal sections and Figs. 3 and 4 show unacceptable sections because of autolytic changes and tangential section respectively.

The numbers of cases were small for robust statistical evaluation.

Discussion

Eye development starts around 22 days of gestation when two small grooves develop on each side of the forebrain in the neural folds, called optic grooves or sulci and later, as the neural tube closes these are called optic vesicles. The optic vesicles extend from the forebrain towards the surface ectoderm. As the vesicles grow, these are attached to the brain via the optic stalk which later develops into optic nerves [1]. Therefore, it is natural to presume that if the brain development is compromised to begin with, the eyes, which are extensions of brain as outlined above, are likely to show changes. Therefore, the first step in analysis would be to examine the time at which brain begins to degenerate in anencephaly.

Acrania which is absence of the skull along with skin and meningeal coverings represents the first stage of this mal-development sequence that takes place 18–20 days post-fertilization [17] and the progression from exencephaly which is exposed brain as a result of lack of skull and meninges with skin, to anencephaly was first described

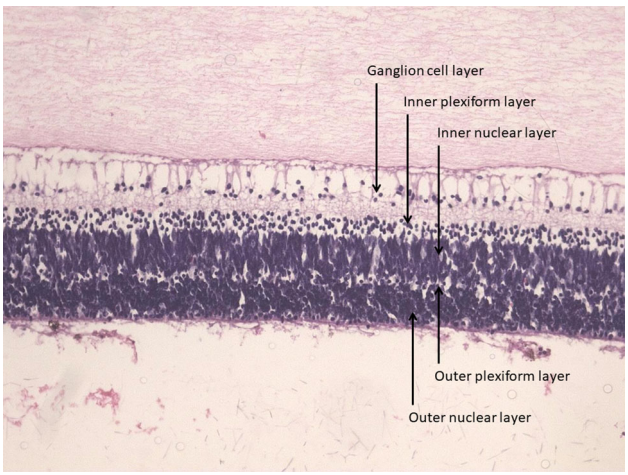

Fig. 1 Retinal section from normal fetus. (H&E) 10x with different layers labeled

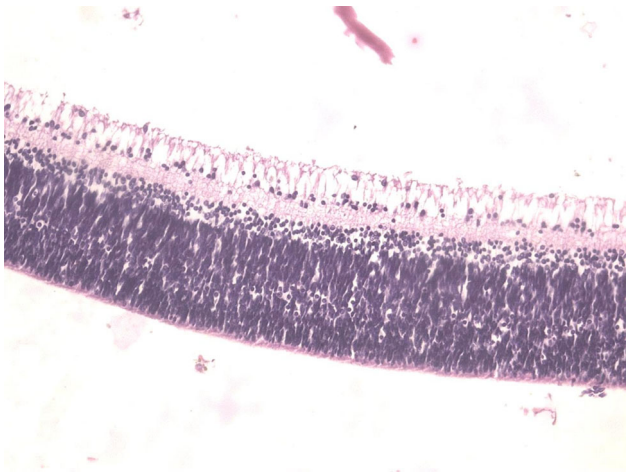

Fig. 2 Retinal section from anencephalic fetus (H&E) 10x

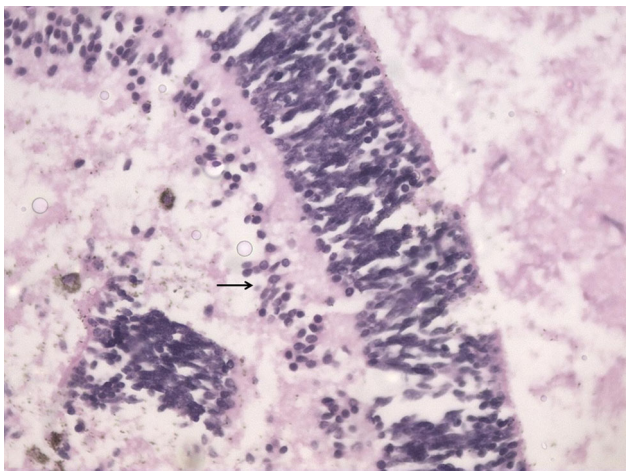

Fig. 3 Retinal section: autolytic changes showing irregular ganglion cell distribution (Arrow) (H&E) 10x

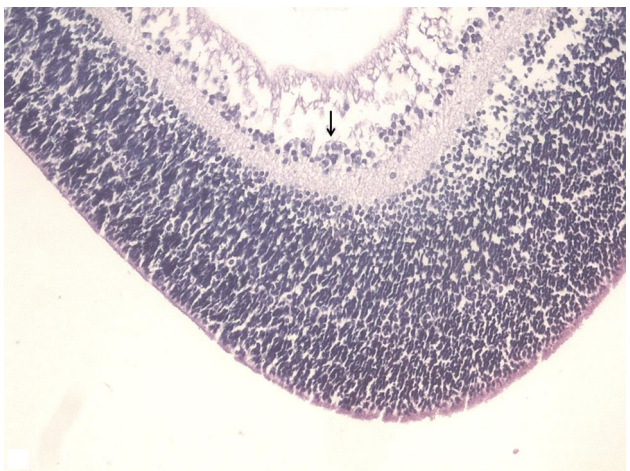

Fig. 4 Retinal section: tangential cut artifact with clustering of ganglion cells (Arrow) (H&E) 10x

by Wilkins et al. [18]. When acrania is seen at an early stage, disorganized brain tissue (vasculo-membranous area) is detectable above the orbits, a process called exencephaly [19]. Anencephaly evolves as a result of failed closure of the midbrain and forebrain, but with normal fusion at the level of the hindbrain and the cervical cord region [20]. Since the sequence and, therefore, exposure of brain and subsequent deterioration starts around 18 to 20 days and optic grooves also make their appearance around 22 days, it stands to reason that future development of eyes might have developmental issues if the forebrain is degenerating. The basic underlying assumption is that eye development is contingent on intact brain development. The assumption may not be true, particularly in light of studies including the present one, wherein retina in anencephalic fetuses is demonstrated to be similar to that of normal fetuses [11]. In the present study, not only was no difference seen between the two groups but there was slight increase in the number of ganglion cells as well as the thickness of retina.

This may be a little difficult to reconcile with the studies where investigators have found the number of ganglion cells to be less and to that end it the extremely important to pay attention to the fundamentals of 'normal ', 'more than normal ' and 'less than normal '.

The first basic requirement for comparison is to examine the 'normal' and compare that against the abnormal. And the second one is that the evaluation needs to be as objective as possible.

The results of a study conducted by Addison J et al. [7] showed that not only were ganglion cells reduced, but that, there was also increased vascularity And according to the authors sequence of events leading to the vascular proliferation begins with virtual absence of ganglion cells in anencephalic fetal retinae. The authors make a mention of 'decreased' or 'atrophy' or 'diminished' without offering any comparison against which such judgments are made. Similarly, Mayer et al. [9] concluded that the main findings in anencephalic babies are atrophy of the retinal ganglion cells of the nerve fiber layer and the optic nerve. Once again the comparison between normal fetal retinae and anencephalic retinae are wanting. Therefore, diagnosis of atrophy doesn't appear to hold much water.

Li et al. concluded that there is no significant difference between ganglion cells of anencephalic and normal fetal retinas and they have clearly mentioned that comparison was made between retinas of normal and anencephalic fetuses. However, the method of cell count is not described clearly in the article [11].

We had taken precautions to keep the comparison as objective as possible. The numbers of ganglion cells were counted in each retina along 100 μm length and then

comparison was made between normal and anencephalic cases.

Since the distribution of ganglion cells is not uniform along the retina, for counting, the areas of maximum ganglion cell cellularity were included. Moreover, areas of sections with tangential cuts were excluded since that would have artificially increased the cell count.

By employing uniform and objective criteria we were able to compare the cell counts in both the groups. The comparison showed mean ganglion cell count and retinal thickness slightly more in cases of anencephaly than in fetuses with no congenital malformations. The numbers of cases are too small to reach a definitive conclusion and statistical significance. Our findings appear to be along the line of results of Li et al. [11].

It is pertinent to mention the study by Hendrickson et al. [8] who claimed that almost all ganglion cells are absent in anencephalic cases by 19–20 weeks gestation and that ganglion cells were absent across retinae of 24, 34 and 36 weeks fetuses. These findings are at odds with rest of the studies concluding that ganglion cell layer is either atrophic or hypoplastic or both [7, 9–12]. The presence of ganglion cells has also been shown in retinae of anencephalic 36–41 week old fetuses [13]. The sole publication, apart from that by Hendrickson et al. [1] stating that ganglion cells are absent, is a single case evaluation.

It must be realized that there is fundamental difference between complete absence and present but with reduced quantity. Atrophy and or hypoplasia imply presence. The present study employs uniform quantitative appraisal of areas with maximum ganglion cells concentration in all the cases without bias. At first it may seem counterintuitive to note that anencephalic fetuses in the present study show more number of cells (21.66) compared to normal fetuses (17.66). The normal fetuses were all cases of intrauterine death and the fetuses were delivered after varying intervals with autolytic changes. In contrast, all anencephalic cases were abortions and the fetuses showed comparatively less autolysis. The cellular details are obscured with the extent of post mortem autolysis and that appears to be the reason why anencephalic retinae show more ganglion cells (Table 1).

Given the results of our study, it would be safe to conclude that presence of ganglion cell layer and retinal thickness does not appear to be influenced by anencephaly. It has been speculated that on the whole, the survival and integrity of most layers of the retina might have no bearing on the survival and integrity of the brain during prenatal development [11]. It is possible that by the time the brain degenerates in this case, the retina has already been fully developed. This assumption, however, contradicts the report by Wilkins-Haug and Freedman that the brain actually started to degenerate as early as 16 weeks of gestation or the first trimester [21, 22]. Only by 23–25 weeks, the retina of the normal fetus had developed the visual cell layer [23]. Keeping in mind the time of beginning of brain degeneration and the time at which the retina acquires the visual cell layer, there are two possibilities (Table 2):

(a) The retina either completely or partially fails to develop the ganglion cell layer by 23 weeks because the brain is degenerated by this time. The studies showing less numbers of ganglion cells by different authors appear to agree with this possibility that there is partial but not complete failure [7, 9, 10, 13].
(b) The retina acquires ganglion cell layer regardless of cerebral hemispheres and once the retinal differentiation sets in, it becomes independent of brain development even if eye and retina bud off from the brain to begin with. Our results appear to support the second possibility. In addition, results by Li et al. have also indicated that there is no significant difference between the two groups [11].

The implications of this kind of study assumes importance given the fact that quite a lot of effort has been made in retinal transplantation. By the end of the last decade, phase 2 clinical trials involving fetal retinal pigment epithelium retina transplant in patients with retinitis pigmentosa and age related macular degeneration had been carried out. The encouraging outcomes in the majority of patients provide clinical evidence of the safety and beneficial effect of retinal implants and corroborate results in animal models of retinal degeneration [14]. In a pilot study of human neural retinal transplantation undertaken to investigate feasibility of transplantation, the authors concluded that transplantation of fetal retinal photoreceptor suspensions into the subretinal space was achieved safely. Although a definite positive effect on visual function could not be demonstrated, the apparent high tolerance for graft tissue is promising for future efforts in the field of neural retinal transplantation [15]. Another promising advantage of the fetal eye is that it can be a potential source of stem cells. The eyes of anencephlic fetuses can be used as a source, in future, studies regarding stem cells as a potential treatment option for ocular diseases. It has been claimed that there are 10,000 stem cells in the retina and these are capable of producing all the different retinal cell types. Retinal stem cells require no growth factors and grow easily and rapidly even in completely defined serum free media. They could lead the way for stem cell ocular therapy such as implanting photoreceptors grown in culture into the blind eye of an individual with retinitis pigmentosa or other retinal degenerative disorders [16]. One of the problems associated with retinal stem cell transplantation has been that it requires a large amount of tissue and

Table 1 Retinal thickness and ganglion cell count in anencephalic fetuses

S. no.	Gestation weeks + days	Sex	Thickness retina (μm)	No. ganglion cells per 100 micro meter
1	20 + 4	M	161.2	18
2	18 + 0	F	157.7	33
3	20 + 4	F	182.4	17
4	20 + 0	F	108.5	23
5	20 + 3	F	194.3	24
6	20 + 0	M	137	15

Range ganglion cells count/100 μm: 15–33. Mean Ganglion cells count: 21.66

Range Retinal thickness: 108.5–194.3 micro meters. Mean Retinal thickness: 156.85 micro meters

Table 2 Retinal thickness and ganglion cell count in normal fetuses

S. no.	Gestation weeks + days	Sex	Thickness retina (μm)	No. ganglion cells per 100 (μm)
1	19 + 0	M	200.1	21
2	20 + 5	M	143.3	19
3	19 + 0	M	97.2	12
4	19 + 4	M	155.6	10
5	20 + 0	M	137	17
6	20 + 0	F	130.3	27

Range of Ganglion cells count/100 μm: 17–27. Mean ganglion cells number: 17.66

Range of retinal thickness: 97.2–200.1 micro meters. Mean retinal thickness: 143.91 μm

therefore most researchers have been working on in vitro expanded multipotent stem cells [24]. Human fetuses and/or neonates that do not survive could be an adequate source of these stem cells at least for research initially Table 3.

Our study has shown that regardless of lethal congenital anomaly such as anencephaly, the retinae remain unaffected and are like the ones from fetuses without any malformation. Most of the times, unfortunately, fetuses either selectively aborted or those that die soon after birth are not utilized for scientific endeavors. This needs to be changed, because so far research on transplantation of retinal stem cells has shown that this method is potentially a practical strategy for novel therapy for some of the retinal disorders [25]. Utility of retinal stem cells harvested from human fetuses has been convincingly demonstrated by Zhou et al. The team isolated retinal progenitor cells from

Table 3 Facilitates a concise look at various relevant publications

References	No. of cases	Gestation age	Hypoplasia	Complete absence
[7]	6	> 24 weeks:5 Not stated: 1	Atrophy	
[8]	8	2 Cases: 19 and 20 Weeks 6 Cases: > 20 Weeks		Total absence
[9]	6	NA	Hypolasia	
[10]	4	Stillborn > 20 weeks	Hypoplasia and/or atrophy	
[11]	3	NA	Ganglion cells present	
[12]	9	Stillborn > 20 Weeks	Hypoplasia and/or atrophy	
[13]	7	36 to 41 weeks	Atrophy	
[21]	1	34 Weeks		Total absence
Present Study	6	All cases: < 21 weeks	Ganglion cells present	

human fetal retinas (12–14 weeks old). c-Kit+cells were found in the inner part of the fetal retina. Injection of these c-Kit+/ SSEA4−cells into the subretina of Royal College of Surgeons (RCS) rats showed that the cells survived for at least 3 months after transplantation into host subretinal space where they exhibited the ability to self- renew and differentiate into retinal cells [26].

Therefore, in conclusion although anencephaly is a lethal malformation, the retinae of anencephalic fetuses appear to be similar to normal ones. However, given the lack of sufficient quantitative studies in the literature, more detailed investigations with larger number of cases is required to confirm the conclusions of our study. Given the fact that most of the studies, with an occasional exception, attests to the presence of ganglion cells in anencephalic retinae, with the advances in retinal transplants these fetuses can be a practical source of retinal tissue.

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Compliance with Ethical Standards

Conflict of interest None.

Ethical Approval Obtained from institutional ethical committee.

References

1. Boniuk V, Ho PK. Ocular findings in anencephaly. Am J Ophthalmol. 1979;88:613–7.
2. Sadler TW. Langman's medical embryolopgy. Eye 12 Ed. Wolters Kluver-Lippincott Williams and Wilkins; 2012. pp. 329–38.
3. Duke-Elder S. Normal and abnormal development; congenital deformities. Syst ophthalmol. 1964;3:1085–90.
4. Sharma V. Ocular abnormalities of the fetus with special reference to cyclopia. Br J Ophthalmol. 1963;47:193–202.
5. Wolter JR, Knoblich RR, Ravin JG. Synohthalmos and anencephaly. J Pediatr Ophthalmol. 1968;5:217.
6. Andersen SR, Brp Rasmussen F, Tygstrup I. Anencephaly related to ocular development and malformation. Am J Opthalmol. 1967;64:559–66.
7. Addison DJ, Font RL, Manschot WA. Proliferative retinopathy in anencephalic babies. Am J Ophthalmol. 1972;74:967–76.
8. Hendrickson A, Djajadi H, Erickson A, Possin D. Development of the human retina in the absence of ganglion cells. Exp Eye Res. 2006;83:920–31.
9. Meyer E, Navon D, Zonis S, Sova I. Eye pathology in anencephalic babies. Ophthalmic Genet. 2009;1:219–26.
10. Rootman J, Carvounis EP. Vasculature of the optic nerve anencephaly. Br J Opthalmol. 1979;63:188–90.
11. Li WW, Lu G, Pang CP, Lam DS, Yew DT. The eyes of anencephalic babies: A morphological and immunohistochemical evaluation. Int J Neurosci. 2007;117:121–34.
12. Andersen SR, Bro-Rasmussen F, Tygstrup I. Anencephaly related to ocular development and malformation. Am j ophthalmol. 1967;64(3):559–66.
13. Bernado AI, Krisch LS, Brownstein S. Ocular anomalies in anencephaly: a clinicopathological study of 11 globes. Can J Ophthalmol. 1991;26:257–63.
14. Radtke ND, Aramant RB, Petry HM, Green PT, Pidwell DJ, Seiler MJ. Vision improvement in retinal degeneration patients by implantation of retina together with retinal pigment epithelium. Am J Ophthalmol. 2008;146:172–82.
15. Humayun MS, de Juan E Jr, del Cerro M, Dagnelie G, Radner W, Sadda SR, del Cerro C. Human neural retinal transplantation. Invest Ophthalmol Vis Sci. 2000;41:3100–6.
16. Levin LA, Ritch R, Richards JE. Stem cell therapy for ocular disorders. Arch Ophthalmol. 2004;122:621–7.
17. Müller F, O'Rahilly R. Development of anencephaly and its variant. Am J Anat. 1991;190(3):193–218.
18. Wilkins-Haug L, Freedman W. Progression of exencephaly to anencephaly in the human fetus—an ultrasound perspective. Prenat Diagn. 1991;11:227–33.
19. Cox GC, Rosenthal SJ, Holsapple JW. Exencephaly: sonographic findings and radiologic-pathologic correlation. Radiology. 1985;155:755–6.
20. Gilbert-Barness E, Kapur RP, Oligny LL, Siebert JJ. Potter's pathology of the fetus. Chicago (IL): Infant and child. Mosby; 2007.
21. Wilkins-Haug L, Freedman W. Progression of exencephaly to anencephaly in the human fetus—an ultrasound perspective. Prenat Diagn. 1991;11:227–33.
22. Kennedy KA, Flick KJ, Thurmond AS. First-trimester diagnosis of exencephaly. Am J Obstet Gynecol. 1990;162:461–3.
23. Wu Y, Lorke DE, Lai H, Wai SM, Kung LS, Chan WY, Yew DTW. Critical periods of eye development in vertebrates with special reference to humans. Neuroembryology. 2003;2:1–8.
24. Meta WD, Yvan A. Retinal stem cells: promising candidates for retina transplantation. Cell Tissue Res. 2008;331:347–57.
25. Canola K, Angenieux B, Tekaya M, Quiambao A, Naash MI, Munier FL, Schorderet DF, Arsenijevic Y. Retinal stem cells transplanted into models of late stages of retinitis pigmentosa preferentially adopt a glial or a retinal ganglion cell fate. Invest Ophthalmol Vis Sci. 2007;48:446–54.
26. Zhou PY, Peng GH, Xu H, Yin ZQ. c-Kit+ cells isolated from human fetal retinas represent a new population of retinal progenitor cells. J Cell Sci. 2015;128(11):2169–78.