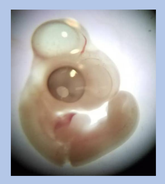

Expression of HMGN3 in the Developing Retina of *Gallus gallus* (Domestic Chicken)

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Introduction

- ~405.1 million people live with vision impairment; 36 million of them are blind (Bourne, 2017). Vision impairment can be caused by retinal damage.
- Ganglion cells, cones and horizontal cells are developed in the retina first, later followed by amacrine cells, rod photoreceptors, bipolar cells, and Müller glial cells (see Figure 1).
- Previous research has found the expression of HMGN3 within the inner nuclear and ganglion cells of the retinas of adult mice (Lucey et. al. 2008).
- The model used in this study was *Gallus gallus* due to its large retina as well its quick retinal development starting 3 days post fertilization (dpf) and continuing to develop until 18 dpf (Doh, 2010).
- Although HMGN3 has been found to be expressed in the retinas of mice, it is currently unknown where it is expressed in the retina of *Gallus gallus* and its function and expression during retinal development are still unclear.
- Determining the expression of HMGN3 in the retinas of *Gallus gallus* embryos allows for a better understanding of how the retina develops, as well as providing others with beneficial information that can be used to further retinal therapies that can treat people with vision impairments.

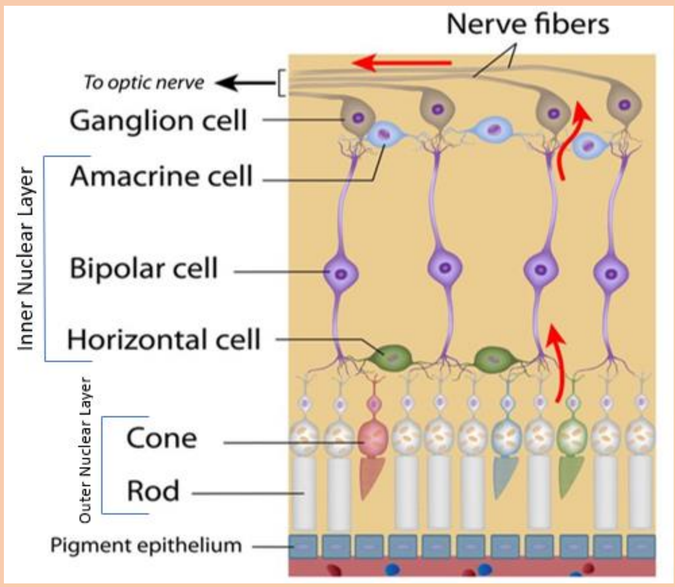

Figure 1. Retinal cell types (DeRemmer, 2016).

Objective

- **We aimed to elucidate the temporal and spatial expression of HMGN3 during retinal development in *Gallus gallus*.**

Methods

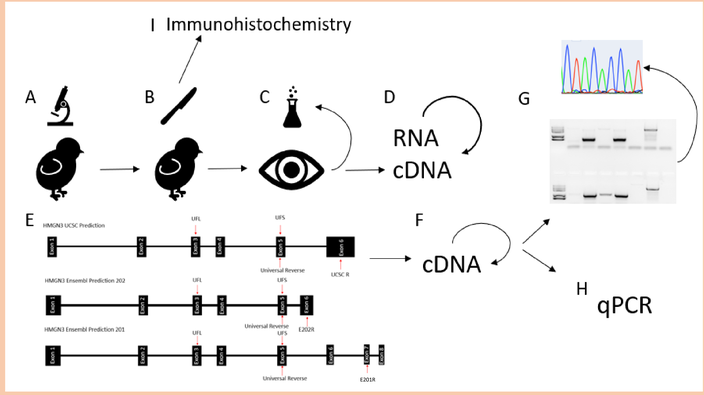

Figure 2. A) *Gallus gallus* embryos were staged using Hamburger Hamilton staging method (Hamburger and Hamilton, 1951). B) Retinas were dissected at stages 23, 27, 30, 35, 40. C) Retinas were suspended and dissolved in Trizol and RNA was isolated. D) RT-PCR was performed in order to synthesize cDNA. E) Primers were created to amplify different splice forms. F) cDNA was amplified using Taq DNA PCR. G) 1.5% agarose gels were created to ensure primer amplification and purity of product before they were cut out and sent for sequencing. H) qPCR determined expression of HMGN3 at different stages of development. I) Retinas were fixed, embedded, and sectioned before immunohistochemistry staining to visualize expression pattern of HMGN3 in retinal cell types during development.

All research activities in this study were approved by the York College of Pennsylvania Institutional Animal Care and Use Committee (IACUC).

Results

HMGN3 (Universal) qPCR

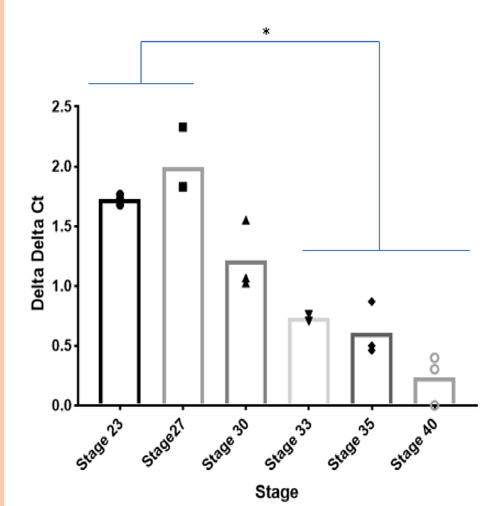

Figure 3. qPCR comparison of HMGN3 gene at different stages of development in *Gallus gallus* embryos using ULF primer and Universal Reverse primer. Delta Delta Ct calculated using GAPDH as reference gene. Means with asterisk are significantly different based on a Tukey HSD (P<0.05) after a one-way ANOVA (F(5,11)=28.32, P<0.0001).

Immunohistochemistry

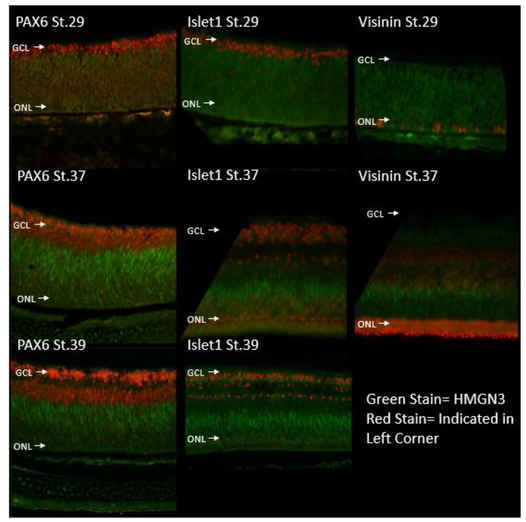

Figure 4. Immunohistochemistry of *Gallus gallus* retinas at different stages. Green stain indicates HMGN3 antibody while red stain is indicated in the upper left-hand corner (PAX6, Islet1, or Visinin). HMGN3 is seen to stain progenitor cells, while PAX6 stains amacrine and progenitors, Islet1 stains for ganglion, amacrine, and bipolar cells, while Visinin stains for bipolar cells and photoreceptors. Ganglion Cell Layer (GCL) is located towards the top and Outer Nuclear Layer (ONL) is towards the bottom of each image.

HMGN3 (UCSC) qPCR

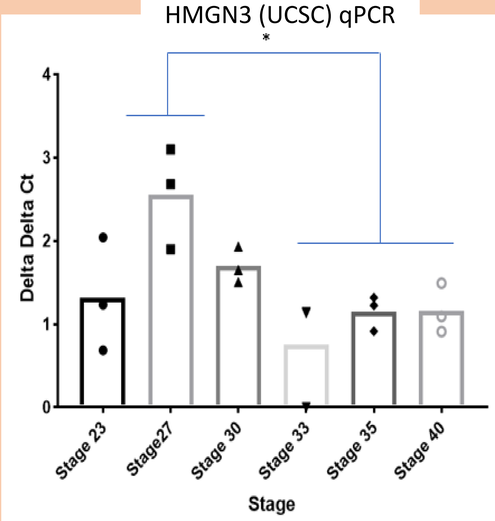

Figure 5. qPCR comparison of HMGN3 gene at different stages of development in *Gallus gallus* embryos using USF primer and UCSC reverse primer. Delta Delta Ct calculated using GAPDH as reference gene. Means with asterisk are significantly different based on a Tukey HSD (P<0.05) after a one-way ANOVA (F(5,12)=4.859, P=0.0116).

HMGN3 (E202A) qPCR

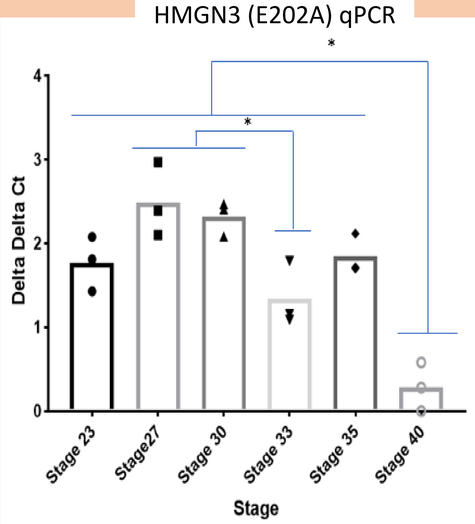

Figure 6. qPCR comparison of HMGN3 gene at different stages of development in *Gallus gallus* embryos using ULF primer and E202A reverse primer. Delta Delta Ct calculated using GAPDH as reference gene. Means with asterisk are significantly different based on a Tukey HSD (P<0.05) after a one-way ANOVA (F(5,12)=17.79, P<0.0001).

Conclusions

- **Through this study we were able to successfully elucidate the expression of HMGN3 during retinal development in *Gallus gallus*.**
- **Through qPCR the expression of HMGN3 in the retina of *Gallus gallus* was found to be expressed greater during early development (stages, 23 and 27) compared to later in development (stages 35, 40).**
- **It was also supported, via immuno-histochemistry, that HMGN3 is expressed in the progenitor cells of developing retinal tissue and not amacrine, ganglion, bipolar or photoreceptor cells.**
- **Future studies may include determining the function of HMGN3 within the retina of *Gallus gallus*, or studying the expression over time after hatching.**

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