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The postnatal presence of human chorionic gonadotropin in preterm infants and its potential inverse association with retinopathy of prematurity

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Abstract

Background: Human chorionic gonadotropin (hCG) and luteinizing hormone (LH) are pro-angiogenic gonadotropic hormones, which classically target the reproductive organs. However, hCG, LH and their shared CG/LH receptor are also present in the human eye. The possibility that a deficiency of these hormones may be involved in the pathogenesis of retinopathy of prematurity (ROP), during its early non-proliferative phase, hasn't been explored.

Methods: We conducted a cross-sectional study of Michigan-born preterm infants utilizing dried blood spots. We analyzed hCG and LH blood levels at 1 week and 4 weeks of age from 113 study participants (60 without ROP; 53 with non-proliferative ROP). We utilized electrochemiluminescence assays on the Mesoscale Discovery platform.

Results: Similar levels of hCG are found in preterm infants at both 1 week and 4 weeks after birth. Preterm infants with non-proliferative ROP, after adjusting for sex and gestational age, have 2.42 [95% CI: 1.08–5.40] times the odds of having low hCG at 4th week of age.

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Conclusion: We found that hCG is present postnatally in preterm infants and that a deficiency of hCG at 4 weeks of age is potentially associated with non-proliferative ROP. This provides novel evidence to suggest that hCG may participate in human retinal angiogenesis.

Introduction:

If preterm birth interrupts *in utero* retinal angiogenesis, retinopathy of prematurity (ROP) can occur. Vascular endothelial growth factor (VEGF) is the main driver of angiogenesis in the eye.(1–3) Preterm infants must maintain appropriate levels of ocular VEGF for normal retinal vascularization to continue ex utero. ROP, a disorder of dysregulated VEGF, has two distinct phases which we will refer to as non-proliferative (NP) and proliferative. If ocular VEGF levels fall too low, retinal vessel formation halts or regresses, thereby initiating non-proliferative ROP (NP-ROP). (4–6) Thus, NP-ROP represents under-vascularization of the retina (compared to that of other similarly aged preterm infants); it is observed clinically as a line or ridge demarcating vascularized from non-vascularized retina. Most of the time, non-proliferative ROP regresses and retinal angiogenesis resumes.(7) However, if ocular ischemia overstimulates VEGF expression, then retinal neovascularization (an aberrant, collateral microvascular network) forms in response to poor perfusion, resulting in proliferative ROP.(4,6) In this manuscript, we define proliferative ROP as disease with documented retinal neo-vascularization and NP-ROP as ROP without clinical documentation of such (further elucidated upon under methodology).

Indisputably, oxygen levels influence VEGF production in the eye; hyperoxia negates VEGF expression and retinal ischemia stimulates it. This explains why the exposure of premature infants to fluctuating O_2 levels is associated with ROP.(6,7) However, identifying the factors, besides oxygen, involved in both physiologic and pathologic VEGF regulation in the developing eye is an important undertaking. To date, physiologic hypoxia is seen as the main regulator of VEGF and angiogenesis during eye development. (8–10) However, it is unlikely that oxygen levels alone are responsible for choreographing all ocular VEGF production. The potential contribution of hormones to ocular VEGF and retinal angiogenesis has not been fully explored.

Human chorionic gonadotropin (hCG) and luteinizing hormone (LH) are related pro-angiogenic hormones which share the same CG/LH receptor in the body.(11–14) Moreover, these hormones, as well as their shared receptor, are present in the eye.(15–17) In light of this, we have been investigating the potential role of hCG and LH in retinal angiogenesis. (16–19). We have recently shown that genetically-modified neonatal mice without any CG/LH receptors, and raised under identical, normoxic conditions to unmodified mice, have ~15% reduction in ocular VEGF levels with a corresponding reduction in retinal vascular density.(17) This experiment provides evidence that CG/LH receptor signaling, independent of oxygen, plays a role in retinal angiogenesis during mouse eye development.

Building upon our prior work, we hypothesized that (a) gonadotropins, namely hCG and/or LH, contribute to retinal angiogenesis during human eye development and that (b) low gonadotropin levels in preterm infants may prevent retinal vascularization from proceeding ex utero, thereby contributing to ROP initiation (NP-ROP). During pregnancy, the placenta

produces large amounts of hCG; hCG is a component of fetal blood at all gestational ages.(20–22) Premature birth leads to the early separation of infants from the placenta; thus, preterm infants are deprived of placental hCG exposure. In light of all this, we reasoned that (a) to avoid ROP, sufficient stimulation of the retinal CG/LH receptor must occur postnatally; and that (b) preterm infants compensate for loss of placental hCG exposure by increasing their gonadotropin levels.

To support our above hypotheses, we aimed to show that preterm infants with NP-ROP (a proxy for deficient retinal angiogenesis) would have greater odds of having lower postnatal levels of hCG and/or LH than their preterm counterparts without the disorder. To accomplish this aim, we utilized pre-collected, de-identified dried blood spots (DBS) to measure hCG and LH levels in very preterm infants at high risk for ROP; DBS are whole blood specimens which are collected from newborns and dried on filter paper for the purpose of newborn screening. In adult studies, hCG and LH have been shown to be stable on DBS with high rates of recovery.(23,24)

Materials and Methods:

Study Approval:

In the State of Michigan, DBS that remain (after newborn screening is completed) are archived at the Michigan Neonatal Biobank (MNB). For very preterm infants, DBS are usually collected twice, once within the first week of life and again at approximately 4 weeks of age. The residual DBS from both timepoints are stored at the MNB. The research use of these specimens is overseen by the Michigan BioTrust for Health program at Michigan Department of Health and Human Services (MDHHS). Approval for this study was granted by MDHHS Institutional Review Board and the Michigan BioTrust for Health Scientific Advisory Board. Before the start of the study, MNB requested the names of all individuals who would have access to the original data. Because of this, the dataset for this study will not be made publicly available.

Study Design:

Our study is a cross-sectional study of Michigan-born preterm infants with birthweights (BW) less than ≤1500 g and gestational ages (GA) of ≤30 weeks. Even though our study contains elements of a case-control study, it is not characterized as such for the following reason. The dates of ROP diagnoses (from the hospital records) were not available to us. Thus, determining the time order between ROP diagnosis and blood sampling is not straightforward. DBS obtained in the 1st week of life would have clearly preceded a diagnosis of ROP. The DBS obtained at approximately 4 weeks of life, would have precededROP diagnosis most of the time . However, it is possible that the events occasionally occurred the other way around. Studies, such as this, in which time order is not definitively determined, are usually referred to, in epidemiologic terminology, as cross-sectional (and not as case-control).

Sources of Data:

Three databases were utilized: (a) Live birth data from Division of Vital Records and Health Statistics at the Michigan Department of Health and Human Services (MDHHS) (b) Michigan Inpatient Hospital Database (MIDB), which contains inpatient-level data for hospitalizations at all acute care hospitals in Michigan. (c) Michigan Newborn Screening (NBS) database containing unique codes for residual DBS samples from Michigan-screened newborns. Linkage between the first two databases was performed by the Office of the Registrar of the State of Michigan; DBS were then linked with birth records and inpatient hospitalization records by NBS staff.

Selection of the study population:

Use of ICD codes: ICD 9 codes from the MIDB dataset were used to identify cases (NP-ROP) and non-cases (no-ROP). NP-ROP cases were defined as any ROP without documented retinal neovascularization, identified by ICD 9 codes: ICD 362.20, 362.23, 362.24. These codes represent unspecified ROP, ROP Stage 1, and ROP Stage 2. To maximize the potential number of NP-ROP cases with available DBS for research purposes, we included the diagnostic code for unspecified ROP (further elaborated upon in discussion). We also inadvertently included, among the NP-ROP cases, code 362.21 (retrolental fibroplasia), a diagnostic code rarely used in recent decades. It is unlikely that this code was utilized at all (or at least to any significant degree) within the recent birth years included in this study. Infants with ICD 9 codes representative of ROP Stages 3–5 (362.25, 362.26, 362.27) on their medical records were excluded from this particular investigation, but will be utilized in a separate investigation.

All DBS were de-identified before their release for research use. Because of this, we were not provided with any individual diagnostic codes; instead, each DBS was classified as either NP-ROP or no-ROP. The specific ICD9 ROP codes for study participants were not made available to us, and thus, we could not differentiate non-specific ROP from ROP Stage 1 or from ROP Stage 2. Because the U.S. standard of care is to provide ROP screening for infants born at ≤1500 g and/or ≤30 weeks of gestational age (i.e. our study population) (7), it is likely that nearly all of our study participants would have received in-hospital ROP screening. Therefore, absence of an ROP diagnostic code on the medical record is a reasonable proxy for the absence of ROP during the hospital stay.

Selection of cases and non-cases: For this particular study, we utilized archived blood from infants who fulfilled all of the criteria below:

- Birth took place in a hospital in the state of Michigan between 8/1/12 and 3/15/15. (During this time frame, all archived DBS were stored at −20°C at the MNB.)
- Infant born at ≤30 weeks of gestation
- Infant weighed less ≤ 1500 grams at birth

- Infant had available archived DBS at two time points, once within the 1st week of life (we refer to as Week 1 DBS) and again between the 3rd and 5th weeks of age (we refer to as Week 4 DBS)
- Infant had no ICD 9 codes representative of ROP Stages 3–5

The pool of infants satisfying all criteria totaled 479. From these 479 infants, using an approximate systematic sampling procedure, we selected 53 study participants with NP-ROP, and a slightly larger number with no-ROP (n=60). Thus, a total of 113 study participants, 226 DBS (one DBS from each of two time points for each infant) were selected for this particular study. The size of this study was limited by budgetary constraints.

No matching of cases to non-cases were undertaken, and it is assumed that the selected infants were representative of the cases and non-cases in the source population available to us. Because we did not match non-cases to cases, we refer to the no-ROP group as the non-case group, and not as a control group. Though our goal was to achieve fairly equal division between cases and non-cases, we mildly oversampled the non-cases. The reason for this was to ensure adequate representation of no-ROP at lower GAs (where NP-ROP cases are more prevalent). The only additional variables provided were sex (male, female), BW category (<500, 500–749, 750–999, 1000–1249, 1250–1500 grams) and GA category (≤24, >24–26, >26–28, >28–30 weeks).

DBS extraction: 3 mm punch from each neonatal DBS was soaked in 120 μl of PBS buffer containing 0.05% Tween-20 and 0.08% sodium azide for 4 hours in ice, as previously described(25). Samples were vortexed intermittently at every 30 minutes to ensure the extraction. After 4 hours, samples were centrifuged at 13000 rpm for 10 min at 4°C. Supernatants were immediately transferred to −80°C freezer until further analysis.

Electrochemiluminescence (ECL) assay development on the Mesoscale Discovery (MSD) platform: The methodology for ECL assay development on the MSD platform has previously been described by us and others.(16,26,27)

Multiplex assay measurements: MSD U-Plex assay platform was utilized to assess the hCG and LH values. The U-Plex plates were coated with mouse anti-human hCG beta monoclonal antibody (5H4-E2, Thermofisher) and mouse anti-human LH beta antibody (L1, Abcam), cross-linked with appropriate cross linkers and then washed and incubated with appropriate calibrators as previously described.(16) Then the plates were washed with wash buffer 4 times and incubated with the 1st detection antibody of rabbit anti-α HCG Antibody (#R-114-C; Thermo Fisher) for hCG and LH at RT on a shaker at 6500 rpm. Plates were then washed and incubated with 2nd detection antibody of SULFO-TAG™ goat anti-mouse antibody for 30 min at RT on a shaker at 6500 rpm. Plates were washed 4 times and blotted. After the addition of 1x Read buffer (MSD), the plates were read on the MSD 6000 sector imager. All DBS were run in duplicate and both values averaged. For all patients, DBS from both timepoints were run on the same assay. To express analytes concentrations per total mg protein of the DBS extract (as other neonatal DBS studies have done)(28), total protein of the DBS extracts were measured using Micro BSA protein assay kit (Thermo Fisher, Catalogue #23235).

Normalization of hormone values: hCG and LH values for DBS samples from an internal control (composed of 28 neonatal DBS, 14 DBS from each time point) were also assessed during different assay runs. Due to variability in DBS internal control values from different assay runs (as commonly occurs in antibody-based multiplex immunoassays, even with good standard curves) (29–31),hCG and LH values for each DBS were normalized to assay-specific internal control hCG and LH values; internal control hCG and LH levels were each normalized to 1 during each assay run. Because of this, the hormone values (initially expressed in picogram/total protein units) were changed to arbitrary units relative to internal control hCG and LH values. In other words, all hormone values in this study represent relative values (without a unit of measure attached to them).

Statistical analyses: SAS 9.4 software (Cary, NC) was utilized for the analysis. Non-parametric tests (i.e., Mann-Whitney U test and Wilcoxon Signed Rank test) were used for statistical tests and a two-sided p-value of 0.05 was used as a significance threshold. Logistic regression models were used to examine the associations between ROP status and hormone levels. Because the DBS had been divided into no-ROP and NP-ROP groups before the hormonal analyses took place, +Low hCG (or +Low LH) was utilized as the outcome variable (with +/−NP-ROP as predictor variable). Since hCG does not significantly differ by sex, we defined +Low hCG as infant hCG below the mean hCG of the study population [Week 1: low hCG <2.03; Week 4: low hCG <2.22]. Since LH significantly differs by sex, we defined +Low LH as infant LH below sex-specific mean LH of the study population. [Week 1: low male LH: <0.16, low female LH <0.33; Week 4: low male LH <0.28, low female LH <1.64. Sex and GA were covariates in all models.

Results:

Descriptive statistics of sex, GA groups and BW groups for the 113 study participants [60 no-ROP 53 NP-ROP] are summarized in Table 1. No-ROPs and NP-ROPs have similar percentages of males (55.0% and 56.6% respectively). The relative mean and median levels of hCG and LH by timepoint, GA category, sex, and ROP status are shown in Table 2. In most sub-groups, infants with ROPs have lower mean hCG levels compared to their no-ROP counterparts.

Graphical representation of relative hCG and LH levels at Week 1 and Week 4 by gender and ROP status are seen in Figure 1 and Figure 2, respectively. hCG levels are not significantly different between the time points or between sexes for either the no-ROP or for the NP-ROP groups. However, LH levels are significantly increased by Week 4 for no-ROP males ($p < 0.05$) and for both no-ROP ($p<0.001$) and NP-ROP females ($p<0.001$); of note, the magnitude of the LH difference between timepoints is considerably more marked for females than for males.

Logistic Regression Analysis

Modeling for +Low hCG: As seen in Table 3, infants with NP-ROP, after adjusting for sex and GA, have (a) 2.14 [95% CI: 0.96–4.73] times the odds of having had low hCG during 1st week of life and (b) 2.42 [95% CI: 1.08–5.40] times the odds of having had low hCG during 4th week of life.

Modeling for +Low LH: In an overall analysis by adjusting for sex and GA, and in separate analyses of male and female samples with the adjustment of GA, we found no evidence of association of LH with NP-ROP at either time point.

Discussion:

Bioactive hCG is found in at least 9 human fetal tissue types and the CG/LH receptor is found in many different organs.(11,20–22) In addition, small amounts of hCG synthesis by the fetal kidney has been reported.(32) Despite this, the role of hCG in human development has not yet been rigorously studied. Perhaps, this is because the amount of hCG in fetal blood [<0.0025% of maternal hCG levels(21,22)] was assumed too low to have any appreciable effect. Furthermore, most non-primate species do not produce any chorionic gonadotropin; instead LH is the only ligand for most non-primate CG/LH receptors.(11,33) Therefore, there are few (if any) animal models (of lower species) to help distinguish between the potential roles of hCG and LH in human development. To the best of our knowledge, we are the first to describe the presence of measurable blood levels of hCG in preterm infants for at least one month after birth; we found similar levels of hCG at 1 week and 4 weeks of age. Until now, most hCG in fetal cord blood was probably assumed to have originated from the placenta and thus, hCG levels in preterm infants were probably thought to drop below detection soon after birth. Our novel finding that hCG is likely produced by infants after preterm birth greatly tilts the odds in favor of hCG having a firm function in human development.

The knowledge that hCG is present in infant blood postnatally does not change the possibility that preterm infants may be hCG deficient due to the absence of the placental hCG contribution. As hypothesized, we show, in multi-variate regression, that infants with NP-ROP (a proxy for insufficient retinal angiogenesis) have significantly ($p=0.03$) lower levels of hCG at 4 weeks of age (near the time when retinal signs of ROP begin to manifest) than their no-ROP preterm counterparts; this finding is independent of gender and GA. Thus, higher levels of postnatal hCG appears to be protective against NP-ROP. In addition, we found a trend ($p=0.06$) between low hCG and NP-ROP even within the first week of life (before any retinal signs of ROP are manifest).

In our prior CG/LH receptor knockout mouse study, the participation of CG/LH receptor signaling in mouse retinal angiogenesis was unmasked; elimination of CG/LH receptors significantly reduced murine retinal vascular density.(17) This current study supports a similar, potential role of CG/LH receptor signaling in human retinal angiogenesis. However, as previously mentioned, rodents do not produce any chorionic gonadotropin; thus, hCG may possibly function in the developing human eye as LH functions in the developing mouse eye. To the best of our knowledge, this is the first report of a specific function for hCG in human development, namely, its potential association with normal retinal angiogenesis.

Based on prior reports of high LH in preterm infants (34), we were not surprised to find high LH levels in our study population. However, we had expected to find higher LH levels in the no-ROP group compared to the NP-ROP group. The lack of any significant difference in LH

between no-ROP and NP-ROP suggests that increased LH does not help promote normal retinal angiogenesis. Thus, despite their shared receptor, LH and hCG do not appear to have interchangeable roles in promoting retinal vascular growth. Important to note is that the postnatal LH rise (shown by our data) does not reflect normal gestational developmental physiology; in utero, LH levels descend in the fetus during the corresponding time period.(21) Investigating the meaning of this rise of LH after preterm birth, especially prominent in premature girls, might constitute an important research venture.

To avoid the difficulty of prospectively collecting blood from very premature, hospitalized neonates, this proof-of-concept study was conducted via the use of pre-collected DBS; that said, the quantification of picogram (1×10^{-12} gram) amounts of hormone on the neonatal DBS presented challenges. During the initial phase of this study, we evaluated the feasibility of different methods for LH and hCG analyses on neonatal DBS. Considering the miniscule amounts of infant hormone that would be available for analysis and the small sample volume (from 3 mm punches from DBS), we determined that mass spectrometry would not be a viable analytical method for this study.(35,36) Alternatively, we opted to utilize ECL technology on the MSD multi-plex platform since it provides exquisite detection sensitivity with reduced total amount of analyte required;(27,37) moreover, it has been successfully used in prior neonatal DBS analyses.(28) That said, the presence of interassay variability is a known concern in antibody-based multiplex platforms.(29–31) We controlled for this issue by normalizing hormone values from each multiplex assay to internal control values. The marked similarity in odds ratios and confidence intervals (in our models with low hCG as outcome variable) at two different time points [Week 1:OR 2.12 [CI: 0.96–4.69]; Week 4:OR 2.42 (CI: 1.09–5.41)] reduces the likelihood that a measurement error may have influenced the results.

Another limitation of the study was the limited information available for each patient due to use of precollected, de-identified samples. In a future, prospective study, we plan to confirm the hormonal/ROP relationship, with adjustment for variables such as oxygen exposure, breastfeeding, antenatal steroid use and transfusion protocols. Moreover, the use of ICD codes for distinguishing between cases and non-cases might have led to some misclassification between groups for a variety of reasons including coding errors and inter-observer variability. However, if this had significantly limited our study, we would have expected a shift towards the null hypothesis. That said, our study remained powered to demonstrate the significant association between low hCG and NP-ROP.

We also acknowledge that some proliferative ROP cases may have been included in the NP-ROP group. At the time that we requested that the State of Michigan (a) match birth data with hospital records, (b) query their available DBS and (c) locate DBS that would qualify as de-identifiable (meaning that the state had 5 or more infants with identical characteristics), we thought that perhaps only a small number of NP-ROP DBS would be available. We, therefore, cast a wide net to include as many infants with NP-ROP as possible. Thus, we included “unspecified ROP” since the vast majority of these infants would likely have NP-ROP. We reasoned that infants with proliferative ROP would more likely have diagnostic codes specific for ROP Stages 3–5 (codes which were excluded from this particular investigation), especially since these infants are closely monitored to assess

the potential need for treatment. However, for a number of reasons (including coding errors and inter-observer variability), the possibility remains that a few infants with proliferative ROP were included in the NP-ROP group.

Our novel finding that hCG is present in preterm infant blood for at least 4 weeks after birth will likely open new investigations into potential roles for hCG in human development. Given the many roles that hCG serves during pregnancy, it is probable that it serves more than one role during human development as well. Our key conclusion--that infants with NP-ROP have 2.4 times the odds of having had low hCG at 4 weeks of age--provides new insight into a potential relationship between hCG and angiogenesis during eye development. Confirmation of these results with a prospective study may help to inform new strategies for ROP prevention.

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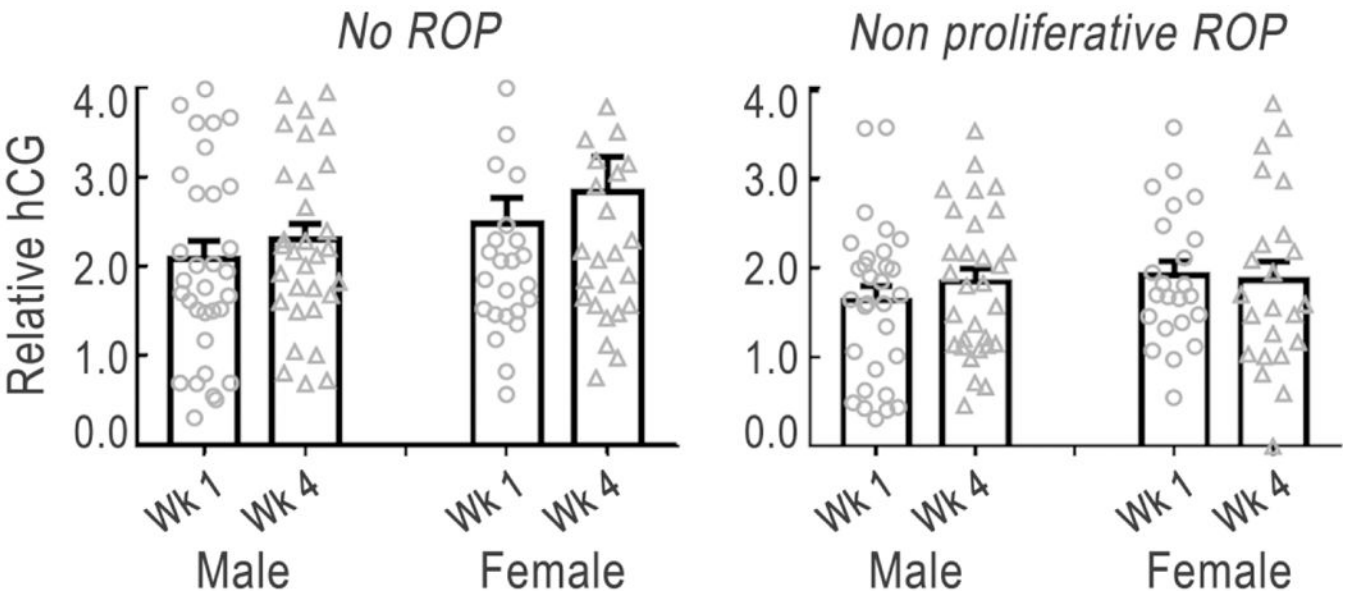

Figure 1: Relative blood hCG levels within 1ST and 4Th week of postnatal life in infants who were born at ≤30 weeks of gestation.

Data are presented as relative mean ± SEM. By non-parametric paired t-testing (Wilcoxan signed rank test) , there are no significant differences in mean rank hCGlevels between time points in the groups without ROP or in the groups with nonproliferative ROP group

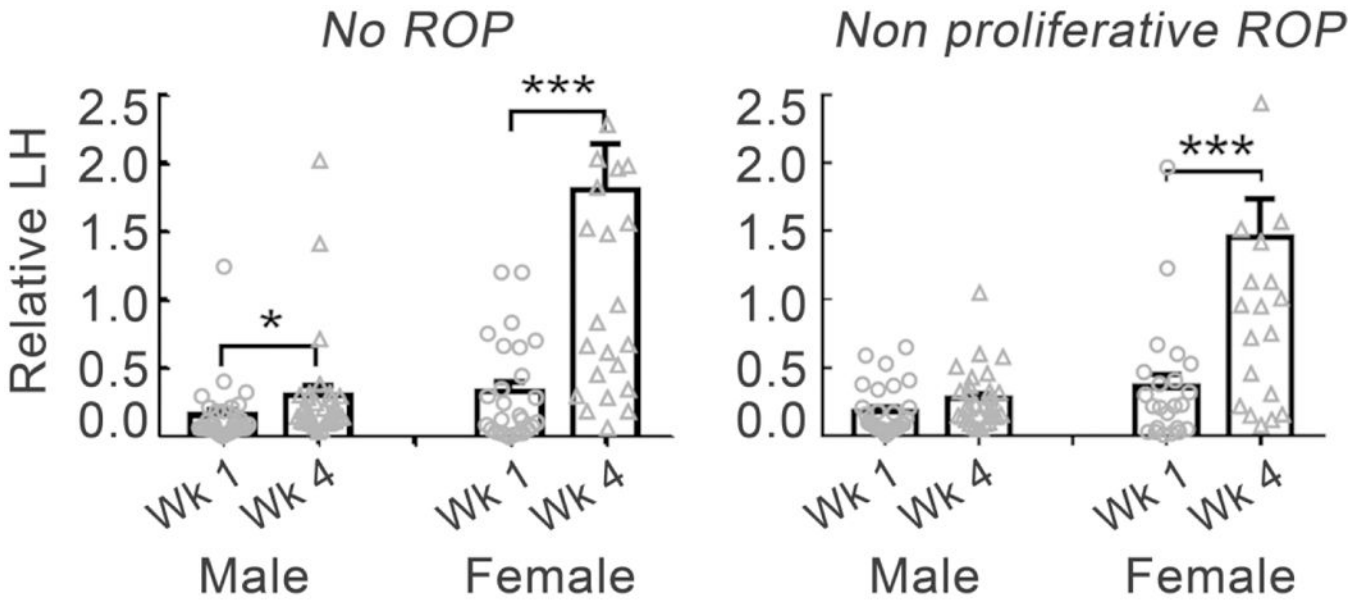

Figure 2: Relative blood LH levels within 1ST and 4Th week of postnatal life in infants who were born at ≤30 weeks of gestation.

Data are presented as relative mean ± SEM. By non-parametric paired t-testing (Wilcoxan signed rank test), there are significant differences in mean rank LH levels between time points for both male and female groups without ROP as well as for females with non-proliferative ROP; *p< 0.05, ***p< 0.001

Table 1:

Summary statistics of key characteristics in the study

	No ROP N=60(%)	**Non-proliferative ROP N=53 (%)**
Sex	Male: 33 (55.0) Female: 27 (45.0)	Male: 30 (56.6) Female: 23 (43.3)
Gestational Age Categories (weeks)	≤24: 7 (11.6) >24–26: 20 (33.3) >26–28: 19 (31.6) >28–30: 14 (23.3)	≤24: 13 (24.5) >24–26: 19 (35.8) >26–28: 11 (20.7) >28–30: 10 (18.8)
Birthweight Categories (grams)	<500: 2 (3.3) 500–749: 17 (28.3) 750–999: 24 (40.0) 1000–1249: 11 (18.3) 1250–1500: 6 (10.0)	<500: 1 (1.8) 500–749: 22 (41.5) 750–999: 20 (37.7) 1000–1249: 4 (7.5) 1250–1500: 6 (11.3)

Table 2:

Relative levels* of LH and hCG at 1 Week and 4 Weeks after preterm birth in infants without ROP (No ROP) and in infants with non-proliferative ROP (+NP-ROP)

		ROP Status	N	Week 1 LH	Week 4 LH	Week 1 hCG	Week 4 hCG
				Mean/ Median	Mean/ Median	Mean/ Median	Mean/ Median
GA ≤24 weeks	**MALES**	No ROP	4	0.41/0.18	0.44/0.12	1.70/1.32	2.13/2.31
		+ NP-ROP	5	0.25/0.17	0.32/0.15	1.65/1.66	2.11/2.51
	FEMALES	No ROP	3	0.48/0.45	1.47/1.49	4.14/4.83	2.38/2.90
		+ NP-ROP	8	0.59/0.35	2.32/2.21	2.21/2.31	1.96/1.65
GA >24–26 weeks	**MALES**	No ROP	9	0.09/0.07	0.27/0.29	2.71/2.81	2.88/2.66
		+ NP-ROP	11	0.15/0.09	0.26/0.20	1.55/1.92	2.00/2.04
	FEMALES	No ROP	11	0.41/0.25	2.10/0.67	2.30/2.12	3.26/2.11
		+ NP-ROP	8	0.25/0.22	0.64/0.59	1.68/1.68	1.65/1.03
GA >26–28 weeks	**MALES**	No ROP	11	0.12/0.09	0.18/0.18	2.02/1.10	2.13/1.84
		+ NP-ROP	7	0.09/0.01	0.23/0.16	1.3S/1.01	1.42/1.16
	FEMALES	No ROP	8	0.16/0.05	1.53/1.12	2.25/1.92	2.04/1.95
		+ NP-ROP	4	0.18/0.12	0.93/0.58	1.31/1.36	1.92/2.34
GA >28–30 weeks	**MALES**	No ROP	9	0.11/0.05	0.31/0.11	1.11/1.61	2.01/2.01
		+ NP-ROP	7	0.14/0.06	0.28/0.32	1.98/2.03	1.13/1.96
	FEMALES	No ROP	5	0.26/0.03	1.51/1.53	1.93/1.52	3.45/3.15
		+ NP-ROP	3	0.22/0.22	2.03/1.51	2.43/2.12	2.04/1.48

* LH and hCG values were normalized to assay-specific internal controls; thus, all hormone values in this table represent relative LH and hCG values

Table 3:

The association results between non-proliferative ROP (+NP-ROP) and low mean hCG at Week 1 and Week 4 by using regression models (N=113)

	p-value	Odds Ratio	95% Confidence interval
+Low hCG at Week 1			
+NP-ROP	0.062	2.12	0.96–4.69
Gender	0.494	1.30	0.61–2.77
Gestational Age (per 2 weeks decrease)	0.281	1.11	0.92–1.35
+Low hCG at Week 4			
+NP-ROP	0.030	2.42	1.09–5.41
Gender	0.998	1.00	0.46–2.14
Gestational Age (per 2 weeks decrease)	0.278	1.12	0.92–1.36