DISSERTATION

COLOR VISION DURING PREGNANCY

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ABSTRACT

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Color vision deficiencies, both congenital and acquired, are well documented. Acquired color vision deficiencies can arise from a variety of systemic and ocular problems. Previous research has shown that modulation of hormone levels leads to changes in visual perception. Pregnancy involves predictable increases in hormone levels, so this study examined how naturally occurring changes in endogenous levels of steroid hormones during pregnancy may affect color perception and visual acuity. Color vision testing was conducted at regular time intervals over the duration of pregnancy for 6 women as well as a control group of non-pregnant, non-contraceptive using women. As levels of hormones increased over the course of pregnancy, error scores were predicted to increase, indicating increasing losses in color perception. No significant differences were found between pregnant and control participants for any of the color vision tests conducted across any of the time periods tested; however, four of the pregnant participants did show increases in error scores in the shorter wavelengths as time elapsed. The lack of significant differences could indicate that there are compensatory mechanisms for the body to adjust to increasing levels of endogenous hormones from pregnancy. This study has opened up myriad possibilities for future research examining the relationship of hormones and neurosteroids and their effects on color vision.

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Chapter 1: Introduction

Color Vision During Pregnancy

While the average individual may not realize the implications that color vision has on everyday life, there are several occupations where correct color discrimination and identification are crucial. An example of a profession where careful and accurate discrimination of colors is critical is diamond graders, who rely on their visual perception to correctly analyze and appraise the value of diamonds. This takes attention to detail and stringent rules. Early diamond graders graded diamonds in the morning, because of the lighting and because of the toll it took on their eyes over the course of the day. They had to be careful that the clothing they wore did not reflect into their light source and influence their perception of the diamond's color. Even today, diamond graders have to be very aware of any medication that may affect their color perception, because even small mistakes can cost thousands of dollars or more ("Acquiring your Dream Diamond", n.d).

Most daily activities do not require such strict attention to color differences. However, humans use their color vision to function in the world, whether it is analyzing different hues to determine when to stop the car or to know when food is cooked or raw or ripe. Individuals with color vision deficiencies can be seriously impaired in our visually driven world, especially in fields that require perception of colored lights, such as aviation and air traffic control (Yates, Diamantopoulos, & Daumann, 2001).

In order to understand color vision deficiencies one must understand the physiological components of color vision. The normal human retina consists of two types of light sensitive receptors – rods, active under dim illuminations, and three classes of cones, active under brighter illuminations. Each of these classes of cones contains a distinct photopigment that is specialized

to respond to a specific range of wavelengths in the visible spectrum. These ranges correspond to short (S)-, middle (M)-, and long (L)-wavelength lights.

The existence of these three types of cones was proposed by Thomas Young (Young 1802/1970, 1845/1970) and supported by color matching experiments conducted by Helmholtz (1867/1962), which demonstrated that color-normal people needed three wavelengths of light to match any spectral hue. From these experiments, Helmholtz proposed the existence of three sensory processes, comparable to what we now know today as the cone photopigments. He even identified the spectral sensitivities of these processes, which again are quite similar to the actual absorption spectra of the cone photopigments (Dartnall, Bowmaker, & Mollon, 1983).

Individuals with color deficiencies require two or fewer wavelengths of light to match another while an individual with a color anomaly requires three wavelengths of light, similar to a color normal individual but in different proportions. Subsequent studies have revealed that color deficient individuals are missing at least one of the photopigments, while color anomalous individuals have three cone photopigments with one photopigment displaying an absorption spectrum different from a color normal individual (Neitz, Neitz & Kainz, 1996; Deeb, 2004; Nathans, Piantanida, Eddy, Shows, & Hogness, 1986; Nathans, Merbs, Sung, Weitz, & Wang, 1992).

The retina is not the only place where a color vision deficiency or disturbance can occur. Light must pass through pre-receptoral filters, such as the lens; and these filters alter the wavelength of incident light (Melamud, Hagstrom, & Traboulsi, 2004). For example, it is known that with aging, changes in the lens can alter color perception (Shinomori, Schefrin, & Werner, 2001; Sample, Esterson, Weinreb, & Boynton, 1998). As lens density increases, less short-wavelength light reaches the retina. Monet's paintings provide one of the best examples of the

effects of an aging lens on color perception. His later paintings have more reds and yellows and less blues and greens than his earlier paintings. After consenting to cataract surgery, his paintings returned largely to the style that he had demonstrated before his vision failed (Marmor, 2006).

After light passes through the pre-receptoral filters and reaches the retina, it is processed by the photoreceptors. Light is captured by the photopigment in the photoreceptors and transduction occurs wherein the physical signal (light) is translated into an electrical neural signal (Goldstein, 2002). These neural signals are processed by the horizontal, bipolar, and amacrine cells and lastly reach the ganglion cell layer. Conventional color vision models propose three neural pathways--Red/Green (R/G), Yellow/Blue (Y/B), Black/White (Bk/Wh) -- that detect differences in responses from the three cone types. The idea of these opponent pathways originated from Ewald Hering's (1920/1964) observation that there were some color combinations that we do not perceive (i.e., mutually exclusive), such as reddish green and bluish yellow, and that a blue (or red stimulus) produces a yellow (or green) afterimage. In addition, these hue pairs produce simultaneous color contrast, which occurs when an area is surrounded by a color that affects the perception of the surrounded area. Thus, these hue pairings were considered to be opponent to each other.

The ganglion cells have been proposed to mediate these three opponent pathways. Midget ganglion cells process differences in signals from the L and M cones and are assumed to represent the R/G pathway, while the small bistratified ganglion cells process differences between the signal from S cones and a combined signal from the L and M cones to mediate the B/Y pathway (Dacey, 1993; Dacey & Petersen, 1992). The parasol ganglion cells have been proposed to convey achromatic (Black/White) information (Dacey & Petersen, 1992). The neural signals from the ganglion cells are then conducted via the optic nerve to the lateral

geniculate nucleus and then to the visual cortex. Injury or damage anywhere along this pathway may also result in a color vision deficiency. For example, a lesion in the occipital lobe may result in achromatopsia, a complete loss of color perception (Bouvier & Engel, 2006).

Color vision deficiencies

As noted above, congenital color vision deficiencies are most frequently due to either a spectral shift or absence of one or more of the three cone photopigments. For anomalous trichromacy, one of the photopigments has a different (i.e. shifted) absorption spectrum than a color-normal individual (Neitz et al., 1996; Deeb, 2004; Nathans et al., 1986; Nathans et al., 1992). Various models have been proposed to describe the existence of anomalous trichromacy including altered or non-functioning genes for the "correct" photopigment (Melamud et al, 2004), variability in the number of copies of M and L genes, and amino acid differences at spectral tuning sites (e.g. position 180) that shift the peak sensitivity of one pigment relative to the other (Neitz & Neitz, 2011). Impairment or modifications in the long-wavelength-sensitive (L) photopigment results in protanomaly; in the middle-wavelength-sensitive (M) cones, deuteranomaly; and in the short-wavelength sensitive cones (S), tritanomaly.

Dichromacy results when the gene for one of the photopigments is missing or a non-functional hybrid is present for that cone photopigment. This results in a reduction in the number of hues that these individuals are able to perceive. Protanopes are missing L photopigment, deuteranopes are missing M photopigment, and tritanopes are missing S photopigment. The most common congenital color vision dichromacies, protanopia and deuteranopia, are X-linked recessive and therefore occur much more frequently in males than females (Nathans et al., 1986). Roughly 2.5 percent of males are affected by some type of congenital dichromacy while only .03 percent of females are affected (Sharpe, Stockman, Jagle, & Nathans, 1999). Tritanopia,

however, affects male and females approximately equally, since it is not a sex-linked trait, and is much less frequent, affecting less than .005% of individuals (Melamud et al., 2004). It is caused by two amino acid substitutions in chromosome 7 that encode the blue-sensitive opsin (Weitz, Miyake, Shinzato, Montag, Zrenner, Went & Nathans, 1992).

Other deficits arise when the genes for more than one cone are non-functional. Monochromacy results when only one or no cone photopigments are functional. Cone monochromats only have one cone photopigment, but have rod photopigment, resulting in complete loss of hue perception except possibly under mesopic conditions where both rods and cones are activated (Smith, Pokorny, Delleman, Cozijnsen, Houtman, & Went, 1983). Rod monochromats have no functioning cones, resulting in lack of hue perception as well as decreased visual acuity and photophobia (Melamud et al., 2004). Knut Nordby was a vision researcher and a rod monochromat. In his personal account Nordby (1990) details the difficulties associated with this type of visual deficit including the inability to read regular print books and discriminate colors.

Acquired color vision deficits are caused by some type of injury, disease, or compromise to the visual system and can arise from a variety of systemic and ocular problems as well as from cortical damage. For example, aging (e.g. Verriest, Van Laethem, & Uviljls, 1982; Shinomori, Schefrin & Werner, 2001; Werner, Delahunt, & Hardy, 2004; Kraft & Werner, 1999), diabetes (e.g. Lakowski & Morton, 1977; Rockett, Anderle, & Bessman, 1987; Volbrecht, Schenck, Adams, Linfoot, & Ai, 1994), exposure to toxic chemicals (e.g. Iregren, Johnson, & Nylen, 2005; Kishi, Eguchi, Yuasa, Katakura, Arata, Harabuchi, Kawai & Masuchi, 1999), and various medications (e.g., Fraunfelder, Fraunfelder, & Edwards, 2001;Lopez, Thomson, & Rabiniwicz, 1999; Haustein, Oltmanns, Rietbrock, & Alken, 1982) can seriously alter color perception. Oral

contraceptives (Fine & McCord, 1991; Lakowski & Morton, 1977, 1978; Marre, Neubauer, & Nemetz, 1974) have also been shown to alter color perception. Color vision alterations due to side effects of medications can be either permanent or transitory whereas those due to disease tend to be permanent.

Early classification of acquired color vision defects by Köllner (1812) posited that blue-yellow defects were characteristic of retinal disorders and red-green defects were characteristic of optic nerve disorders. Verriest and Caulwaerts (1978) expanded this delineation into four broad categories: Type I Red-Green, Type II Red-Green, Type III Blue-Yellow and a non-specific deficit. Type I Red-Green deficits are associated with retinal diseases and results in poorer red-green chromatic discrimination, with a corresponding loss of visual acuity. These defects eventually result in complete loss of color discrimination. Type II Red-Green deficits are associated with disorders such as optic neuritis, retrobulbar neuritis, or other optic nerve disorders. There is moderate to severe red-green loss as well as mild blue-yellow loss, with eventual loss of all color discrimination. Type III Blue-Yellow defects are the most common. In the early stages, individuals have problems distinguishing blues from violet. Eventually color vision progresses to be similar to tritanopia, with the individual perceiving only blue, red, black and white. These defects can be caused by age-related macular degeneration, glaucoma, cone dystrophies, and retinal vascular disorders (e.g. diabetes).

Congenital and acquired color vision deficiencies differ in significant ways. While the most common genetic color deficiencies affect the R/G opponent pathway, the most common acquired color vision deficiencies tend to affect the B/Y opponent pathway. This pathway is more susceptible to insult from toxic exposure and eye disease than the R/G pathway (Neitz, Carroll, & Neitz, 2001). Unlike congenital color vision deficits, acquired deficits may not have a

clear-cut area of spectral discrimination loss (Yates et al., 2001; Melamud et al., 2004). Also, congenital dichromacy does not affect visual acuity while many acquired color vision deficits may also produce losses in acuity (Pacheco-Cutillas, Sahraie, & Edgar, 1999). Lastly, hue losses with acquired color deficiencies are often progressive, while hue losses with congenital defects are typically stable across time.

Functionally, acquired color vision deficiencies can be asymmetric between the two eyes. Standard color vision tests, which typically test for R/G deficits, often diagnose genetic color vision deficits more readily than acquired deficits; and individuals affected with acquired deficits may have a more difficult time with color naming as they have not learned the compensatory coping mechanisms that many congenitally color deficient individuals have developed. The effects of acquired color vision deficits may also be transient or even reversible unlike congenital impairments (Pacheco-Cutillas et al., 1999). For example, an individual who had suffered carbon monoxide poisoning presented with cerebral achromatopsia (i.e. no hue perception, only achromatic perception) without other visual disturbances five months after the incident. Six months later he recovered spontaneously (Fine & Parker, 1996). Similarly, methotrexate (used for rheumatoid arthritis) can produce transient color deficiencies (Clare, Colley, Kennett, & Elston, 2005). A patient presented with achromatopsia along with serious visual field disturbances. After cessation of the methotrexate, the visual problems were reversed. One of the anecdotal side effects of Viagra has been the reporting of a transient blue haze or halo, with studies showing no long-term effect (Dundar, Topalo, Dundar, & Kocak, 2006).

These transient effects are caused by exogenous substances altering function in the nervous system. However, it is not fully understood if naturally occurring cycles in the body, such as the menstrual cycle or pregnancy, can produce transient color vision deficits. Estrogen and

progesterone fluctuate on a regular basis in humans, especially in females, and receptors for both hormones have been localized in the human retina (Wickham, Gao, Toda, Rocha, Ono & Sullivan, 2000). Research has shown that exogenous modulation of both these hormones may lead to changes in color perception (Eisner & Incognito, 2005; Eisner, Austin, & Samples, 2004a; Eisner, Burke, & Toomey, 2004b; Fine & McCord, 1991; Lakowski & Morton, 1977, 1978; Marre et al., 1974).

Changes in vision related to hormones

Several researchers have examined whether or not changes in hormone levels over the course of the menstrual cycle cause fluctuations in visual functioning. Recent research by Giuffre, DiRosa and Fiorino (2005) examined changes in color vision over the course of the menstrual cycle using the Farnsworth-Munsell (FM) 100-Hue test, a test consisting of four trays of colored caps. They measured performance three times during the cycle: the beginning, at ovulation, and the end. While there were no reports of individual tray scores, they found that overall performance on all four trays (i.e. least number of errors) was higher at ovulation than at the other two points in the cycle.

Flaherty, Cowert-Steckler, & Pollack (1988) examined the magnitude of the fixation effect in 28 menstruating females (14 women who used oral contraceptives and 14 who did not). Fixation effects are perceived changes in the size of a test figure after prolonged viewing of another figure. The researchers found that the magnitude of the fixation effect was greatest with higher levels of estrogen for both groups. The normally menstruating females showed a greater effect than the oral contraceptive users, presumably because the normally menstruating females had a higher level of estrogen than oral contraceptive users, since oral contraceptives keep estrogen levels artificially low.

Yilmaz, Erkin, Mavioğlu, & Sungurtekin (1998) conducted a study of the latency and amplitude of pattern reversal visual evoked potentials (PRVEPs) over the course of the menstrual cycle. They studied 30 healthy females ranging in age from 18-42 years, but their use of oral contraceptives was not documented. The researchers discovered that the latency of the P100, an event related potential associated with sensory memory and early attentional selection, was shortest and the amplitude was highest during the ovulatory phase of the menstrual cycle, when levels of estrogen are highest. They theorized that the shortened time and increased amplitude could be due to estrogen facilitating neural transmission by decreasing transmission time.

Eisner et al. (2004b) evaluated foveal visual sensitivities by measuring visual adaptation changes of women over the course of several menstrual cycles. They examined both women who used oral contraceptives and women who did not, as well as one participant diagnosed with premenstrual syndrome (PMS). The participant with PMS showed alterations in foveal sensitivity prior to taking oral contraceptives with decreases in sensitivity immediately preceding menstruation. Upon starting oral contraceptives, this individual showed fewer changes in sensitivity with cyclical activity. There was slight evidence of cyclical changes in two other participants not using OCs, with one showing isolated sensitivity spikes in a cyclical fashion and one showing small changes in a smooth progression over the cycle.

Guttridge (1994) reviewed research concerning changes in ocular and visual variables over the course of the menstrual cycle. Despite several studies demonstrating anatomical changes in corneal parameters, intraocular pressure, and tear production, most sample sizes were too small and the results too varied to draw definitive conclusions. The results from behavioral tests were similarly inconclusive. For example, there were no reliable changes in color vision over the course of the menstrual cycle in the majority of studies reviewed, and the ones that did show

differences were not replicated in other studies.

Oral contraceptives.

Effects of oral contraceptives (OC) on vision were the topic of much scrutiny in the late 1960's and 1970's. These OCs contained much higher levels of estrogen than the OCs currently on the market. Enovid, the first FDA approved oral contraceptive, had 150 micrograms of mestranol and 9.8 milligrams of norethynodrel. Current versions of "the pill" use mostly 30 to 50 micrograms of ethinyl estradiol (Oesterheld, Cozza & Sandson, 2008). Comparatively speaking, today's versions of "the pill" have approximately a third of the amount of hormones of earlier formulations. Accommodative loss, vascular occlusion, retinal periphlebitis, and headaches have all been attributed to OC use, both in first generation and later versions of OCs (Roever, 1969; Gombos, Moreno, & Bedrossian, 1975; Rock, Dinar, & Romem, 1989).

Another documented side effect of OC use is color vision deficits. Early research (Marre et al., 1974; Lakowski & Morton, 1977, 1978; Fine & McCord, 1991) showed tritanopic deficits in women using certain oral contraceptives. Women with this deficit have difficulty distinguishing among lights in the short-wavelength portion of the visible spectrum. Marre et al. (1974) studied the color vision of two groups of European women using standard diagnostic tests for color deficiencies such as the Nagel anomaloscope, D-15 panel test, and the Roth 28-hue panel test. One group of women was from Dresden and the other from Vienna. A group of control women not using oral contraceptives was tested in each location as well. The researchers found that in the group from Vienna approximately 28% showed an "acquired blue defect" on the D-15 panel and Roth 28-hue panel tests. In addition, women using certain types of pill, especially Anovlar, Ovulen, Eugynon, and Lyndiol, had higher numbers of pathological tritanomalies than those using other contraceptive pills. The Dresden group did not show any

significant differences between groups. The researchers speculated that this might have been due to the type of OC used by these women (Ovoviston) and/or duration of use (Dresden, mean duration=3 years; Vienna, mean duration=5 years).

In 1977 Lakowski and Morton tested diabetic women who were OC users and matched counterparts who were not OC users. Since oral contraceptives can reduce glucose tolerance, the researchers expected more acquired color vision losses in diabetics using OC than those not using OC. These researchers found that diabetic women using contraceptives had higher error scores on the FM-100 hue test than the non-OC diabetics. The women on OC had increased errors in the tritan areas consisting of caps 75-10 (Tray 4 to Tray 1) and 30-50 (Tray 2 to Tray 3). In addition, those using contraceptives had extended red-green match ranges on the anomaloscope, along with higher incidences of blue-yellow and green-blue deficits.
Fine & McCord (1991) reported a similar finding in their study examining the effects of oral contraceptive use and caffeine on color perception. Women on OC had higher error scores on Trays 2 and 3 of the FM-100 test, which are the ones containing the short-wavelength (blue/violet) hues. Duration of OC use was not documented. The researchers found that for women using OCs, caffeine impaired color discrimination (i.e., increase in error scores) while in women not using OCs caffeine facilitated color discrimination (i.e., decrease in error scores).

All of these studies indicate that oral contraceptives may contribute to acquired tritanomaly. While the duration of use and type of OC varied from study to study, all indicated some type of color vision deficiency. To my knowledge, there have not been any longitudinal studies that document if the effects are permanent and if not how long the effects persist.

Tamoxifen.

Other hormone related drugs that may affect color perception are the selective estrogen

receptor modulators (SERMs). SERMs exert both agonistic and antagonistic effects in a tissue- and receptor-specific manner (Riggs & Hartman, 2003). For example, these chemicals may exert antagonistic effects when acting on estrogen receptor beta (ERβ) but as agonists when acting on estrogen receptor alpha (ERα).

Tamoxifen is a SERM that acts as an estrogen antagonist in breast tissue, as a weak agonist in the endometrium, and is used in treating certain types of breast cancer (Riggs & Hartman, 2003). One of the documented side effects of Tamoxifen is color vision deficits (e.g. Eisner et al., 2004a; Eisner & Incognito, 2005).

Eisner et al. (2004a) investigated the changes in short-wavelength (S) sensitivity via short-wavelength automated perimetry (SWAP) studies and traditional color vision tests. In SWAP studies, a blue stimulus is shown against a high luminance yellow background. In theory, the blue stimulus will stimulate the S cones and the yellow background will desensitize the M and L cones (Wild, 2001). The experimenters tested the vision of 47 women aged 42-69 years who were being treated for breast cancer with Tamoxifen.

The researchers found that as retinal eccentricity (distance from the central portion of the retina) increased, sensitivity decreased (Eisner et al., 2004a). Also, as duration of Tamoxifen use increased, the short-wavelength sensitivity decreased. These results showed that Tamoxifen altered the response of the S pathway, although it did not produce an effect on standard diagnostic color vision tests such as the D-15 panel test.

Research by Eisner & Incognito (2005) examined the effects of Tamoxifen on color naming. The researchers projected a 440-nm stimulus (short-wavelength or violet stimulus) on a 580- nm (yellow) background to the fovea. They selected 32 control participants who did not use Tamoxifen and 30 “short-term” and 24 “long-term” users of Tamoxifen. The participants were

given a 3-alternative forced choice procedure in which they had to choose if the 440-nm stimulus appeared white, lavender, or blue. The researchers found that a significantly higher percentage of the Tamoxifen users selected white as opposed to the non-users who tended to choose lavender to describe the 440-nm stimulus. Within the Tamoxifen users, the long-term users tended to choose white more often than the short-term users, indicating compromised short wavelength sensitivity.

Steroid hormones

All of the studies described above involved endogenous modulations of hormones or exogenous substances that altered levels of endogenous hormones. Hormones are chemical substances released by the endocrine system into the circulation and are carried to different target organs and tissues to modify their structure and function (Gupta, Johar, Nagpal, & Vasavada, 2005). Only cells containing receptors for a particular hormone, known as a target tissue, will respond to that hormone. There are two superfamilies of hormones: those with cytoplasmic receptors and those with nuclear receptors. The ones most closely related to the menstrual cycle and pregnancy are sex steroid hormones with nuclear receptors, which means that the receptors for the particular hormone are located in the nucleus of the cell as opposed to the cell surface (Miller & Duckles, 2008).

These hormones are produced by the ovaries in females and are mostly found in the bloodstream bound to proteins. Estrogen, estrogen derivatives, and progesterone enter cells and bind to steroid receptors in the nucleus of the cell. These nuclear receptors have two main functions: first to bind a hormone and second to activate an intracellular signaling cascade that regulates gene transcription upon binding. This process is known as "classic genomic functioning" and involves modulation of genes, requiring hours or days until the effects of the

hormone are observed (Pietras & Szego, 2005).

More recent research has revealed that hormones may also have faster non-genomic effects that occur quickly and do not involve gene regulation (Chaban, Lakhter, & Micevych, 2004; Razandi, Pedram, Merchenthaler, Greene, & Levin, 2004). A pool of estrogen receptors has been identified at the plasma membrane that can cause rapid effects via second messenger systems. These receptors have been isolated in cortical brain areas, the spinal cord, and peripheral nervous system (Melcangi & Panzica, 2006). It has also been demonstrated that hormones are not only synthesized in the adrenals and gonads but also within the central and peripheral nervous systems, with proteins involved in steroid synthesis localized in the brain, spinal cord, and peripheral nervous system (Melcangi & Panzica, 2006). These hormones are dubbed "neurosteroids" (Gupta et al, 2005).

Neurosteroids are synthesized in the nervous system. If the receptors for these hormones are found in the nervous system, this indicates that there are target tissues outside the reproductive system that can be affected by circulating hormones. For example, sex- and age-related differences in rates of ocular pathology, such as age-related cataract, closed-angle glaucoma, and idiopathic macular holes, indicate that differing levels of hormones can affect the visual system with a higher incidence of these ocular pathologies in postmenopausal women than their age-matched male counterparts (Gupta et al., 2005). The hallmark of menopause is a reduction in sex and neurosteroid hormone production (Gupta et al., 2005; Genazzani, Monteleone, Stomati, Bernardi, Cobellis, Casarosa, Luisi, Luisi, & Petraglia, 2001).

Estrogen: Structure and Function.

Estrogens, a class of steroid hormones, are compounds that promote estrus (Johnson, 2003). These hormones are important in the development and maintenance of secondary sex

characteristics, as well as height development, increased metabolism, and endometrial growth (Carr, Blackwell, & Azziz, 2005; Goodman, 2003). Estrogen also has known effects on the cardiovascular system, including altering the tone of blood vessels and reducing peripheral vascular resistance (Gangar, Vyas, Whitehead, Crook, Meire, & Campbell, 1991). New research also explores the role of estrogen in the functioning of the central nervous system, especially the visual system (Gupta et al., 2005). The exact mechanisms have not been elucidated, but studies have shown differences in ocular variables with age and gender. As previously mentioned, certain ocular pathologies have higher incidence in postmenopausal women as opposed to age-matched male counterparts (Gupta et al., 2005). Variables such as ocular pulsatile blood flow have been shown to be higher in premenopausal women compared to age-matched men or postmenopausal women (Tokar, Yenice, & Akpinar, 2003). These studies suggest that hormones, including estrogen, directly affect the functioning of the eye.

There are several types of estrogen in the body. Estradiol is the most powerful, with estrone and estriol being weaker derivatives (Brotherton, 1976). These hormones are synthesized from androstenedione and produced in the ovarian follicles, corpus luteum, and the placenta in pregnant women. Despite being a weaker derivative, estriol is found in large amounts in maternal plasma during pregnancy (Parker, 2005).

Estrogen: Receptors.

Estrogen receptors (ER) function as a normal part of many physiological processes. By binding to heat shock proteins, ERs can bind to DNA and modulate cellular activity both directly and indirectly (Fauser, 2003). There are two distinct types of ERs: estrogen receptor subtype alpha (ERα) and estrogen receptor subtype beta (ERβ). There are also heterodimers of these as well. Estrogen receptors are found in many areas of the body, not all of which are related to typical

reproductive functioning, such as the central nervous system, vasculature, and the eye (Gupta et al., 2005).

Research has localized ERs to the eye in both animal and human tissue. Kobayashi, Kobayashi, Ueda, and Honda (1998) positively identified estrogen receptor protein in bovine and rat retinas. Munaut, Lambert, Noel, Frankenne, Deprez, Foidart & Rakic (2001) found ERβ in the ganglion cell and choroid layers of the retina in both male and female human ocular tissue and isolated mRNA for both ERα and ERβ in the eye.

Ogueta, Schwartz, Yamashita, and Farber (1999) used three methods to determine the prevalence of ERα in human eye tissue and the differences in expression between males and females. They used reverse transcription polymerase chain reaction (RT-PCR), western blot analysis, and immunocytochemistry to find the receptors in a variety of eye tissue from various donors (whole eyes from females 35, 49, 74, and 77 years of age and males 27,45, and 76 years of age; eye sections from females 17, 48, 49, 55, 68, 77, 79, 83, and 93 years of age and males 6, 13, 59, and 65 years of age). In particular, RT-PCR studies showed women who still had estrous cycles showed more mRNA than those who did not. Male retinas had levels of mRNA that fell between women still experiencing estrous cycles and those who were not. Western blot analysis also showed ERα present in the nuclear tissue and retinal pigment epithelium (RPE) and choroid extract of a 17-year old female donor but not in a sample from a 77-year old female donor. Furthermore, immunocytochemistry of the 17-year old donor's eye showed staining for ERα in the inner and outer nuclear layers of the retina, outer plexiform layers of the retina, and in some ganglion cell nuclei (Ogueta et al., 1999). ERα staining was also seen in other ocular tissue such as the lens and ciliary body. Thus, evidence suggests that estrogen receptors are present in the human retina, and the distribution of the receptors varies with sex and age.

Progesterone.

Progesterone is known as the natural anti-androgen (Brotherton, 1976). It is primarily the hormone of pregnancy, and is essential for achieving and maintaining pregnancy (Goodman, 2003). Progesterone is present in lower levels in non-pregnant women, and it works along with estrogen in promoting the menstrual cycle (Carr, 2005).

Progesterone receptors are found not only in reproductive organs and related structures but throughout the central nervous system as well. As with estrogen receptors, there are two types: progesterone receptor alpha (PRα) and progesterone receptor beta (PRβ) (Gupta et al., 2005). Wickham, Gao, Toda, Rocha, Ono, & Sullivan (2000) identified progesterone receptor mRNA in the lacrimal gland, meibomian gland, bulbar conjunctiva, cornea and RPE cells in both female and male human ocular tissue. No differences between the sexes were observed and age of the donors was not listed.

Pregnancy

The typical human pregnancy lasts for approximately 40 weeks. Changes in maternal physiology and fetal development are divided into three separate stages, called trimesters. The first trimester is defined as the 12 weeks following conception. The second trimester lasts from week 12 until week 28, and the third trimester continues until parturition.

As Figure 1 illustrates, during pregnancy, women have increasing levels of both progesterone and estrogen. Plasma progesterone levels and estriol increase throughout the course of pregnancy. In particular, the amount of estriol at term is 1000 times higher than at the normal luteal phase (Carr et al., 2005). Estriol is produced primarily in the fetal adrenal cortices. Besides estriol, estradiol and estrone levels also increase in pregnancy. For example, estradiol

levels at term are 100 times greater than at the normal luteal phase.

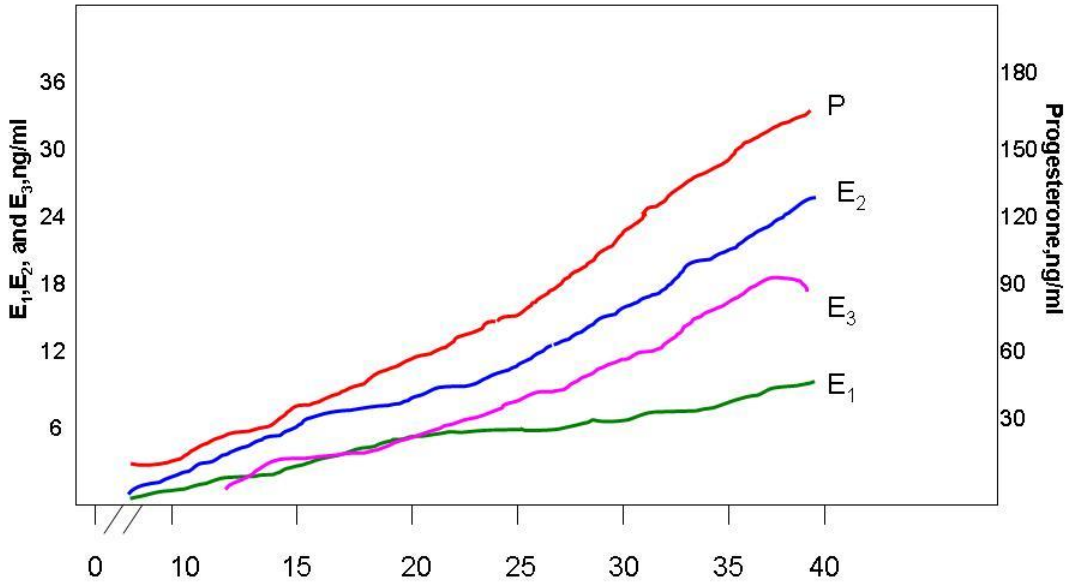

Figure 1: Serum levels of estrone (E1), estradiol (E2), estriol (E3) and progesterone (P) over the course of normal pregnancy (*Adapted from Parker CP Jr*. Endocrinology of Pregnancy, In Carr, Blackwell, & Azziz, *Essential Reproductive Medicine, 2005*).

In addition to the increased hormones produced in the maternal system, the placenta produces hormones as well. The placenta is the primary support system for the developing fetus. It serves several functions, including 1) attachment of the fetus to the uterine wall, 2) a site for exchange of nutrients and gases between mother and fetus, and 3) secretion of hormones and other substances that regulate the mother's homeostasis to benefit the fetus (Goodman, 2003). These hormones, including estradiol, estriol, and progesterone, cause the female physiology to change over the course of pregnancy (Parker, 2005).

Research Direction

Pregnancy produces substantial and predictable increases in hormone levels as compared to the normal menstrual cycle levels. Previous research has shown that modulation of hormone levels may lead to changes in visual perception (e.g. Eisner et al., 2004; Eisner & Incognito, 2005; Eisner et al., 2004; Fine & McCord, 1991; Lakowski & Morton, 1977; Lakowski & Morton, 1977, 1978; & Marre et al., 1974). Therefore, the purpose of this study was to investigate whether modifications of hormone levels associated with pregnancy produce predictable changes in color and visual perception. Specifically, the present research examined how naturally occurring changes in endogenous levels of steroid hormones such as estrogen and progesterone during pregnancy may affect color perception and visual acuity. Color vision testing was conducted at regular time intervals over the duration of pregnancy for six women. A control group of non-pregnant, non-contraceptive using women was also tested for color vision and acuity. Given past research (Marre et al., 1974; Lakowski & Morton, 1977, 1978; Fine & McCord, 1991), it was predicted that the pattern of color vision deficiency would indicate a loss of color processing in the Y/B opponent pathway. Furthermore, it was hypothesized that the color vision deficits would be dynamic and correlate with increasing levels of steroidal hormones in pregnant women.

Chapter 2: Methods

Participants

Six pregnant (P) and six non-pregnant (C) women participated in this study. All were non-smokers between the ages of 18 and 35 and were screened via a questionnaire for use of medications and any medical conditions [see Appendix A (Pregnant participants) and Appendix B (Control participants)]. Participants were eliminated from the experiment if the color vision tests indicated a color vision deficiency. Since C2's anomaloscopic match range was relatively large for a color-normal and suggested the presence of a potential color vision deficiency, she was eliminated from the study. All control participants were nulliparous (i.e. had never given birth to a child) and none of the control participants used any oral contraceptive or steroidal birth control. Regarding the pregnant participants, P1, P3, P4, and P6 were having their first child, P2 was having her second child, and P5 was having her third child. During the second post-test, P4 was pregnant with her second child.

Participants were solicited by flyers posted on campus and in the local community and by word-of-mouth from other participants in the study. At the conclusion of the study, each participant received $10 for each experimental session they completed. This study was approved by the Institutional Review Board at Colorado State University.

Color Vision Tests

A battery of standard color-vision diagnostics was employed in this study. This included three panel tests [D-15 panel test, L'anthony desaturated D-15 panel test, Farnsworth-Munsell (FM) 100-Hue panel test], the pseudo-isochromatic tritan plate test, and anomaloscopic color matches (Neitz OT-II).

Pseudo-isochromatic tritan plate test.

This test is a single plate test that assesses tritan deficiencies. The test consists of pseudo-isochromatic circles creating a triangle and a square. The hues used in the plate create a tritan confusion such that an individual with a tritan defect is unable to identify and locate the two embedded figures. The task of the individual is to identify the shapes and locations of the figures.

D-15 and Adams L'anthony desaturated D-15 Panel test.

Both the D-15 and the L'anthony desaturated D-15 panel tests consist of 15 colored caps in a hinged wooden case, representing a range of colors across the visible spectrum. At one end there is a fixed cap used as a reference for the placement of the remaining caps. The objective of the test is to arrange the colored chips in successive order by placing the cap closest in hue to the reference cap and continuing by placing the remaining caps in the same fashion. All caps are numbered on the underside for scoring.

The D-15 panel test is a broad diagnostic test for extreme color vision deficiencies (e.g. dichromacies). The L'anthony desaturated D-15 uses Munsell colors with lower saturation and higher lightness and is more sensitive in diagnosing subtle color vision losses (Good, Schepler, & Nichols, 2005).

Farnsworth-Munsell (FM) 100-Hue Panel test.

The FM 100-hue test was first developed by Farnsworth in 1943, and later revised in 1957 to the current version, and consists of 85 colored caps. The test represents a full range of colors spanning the visible spectrum and requires finer hue discriminations than the two D-15 panel tests described above. The caps are arranged in four separate trays, each containing a fixed anchor cap at each end. There are 22 caps in one of the trays and 21 caps in each of remaining

three trays. The trays progress from Tray 1 containing hues from red to yellow, Tray 2 containing hues from yellow to green, Tray 3 containing hues from green to blue, and Tray 4 containing hues from blue to red. The test is performed much like the D-15 test with the exception that the participant can start by placing the caps at either reference (anchor) point, but they always place caps most similar in hue next to each other, resulting in a gradual transition in hue across the tray.

Anomaloscope (Neitz OT-II).

The anomaloscope is an optical instrument which produces a bipartite field comprised of monochromatic lights and requires the observer to make a color match, referred to as a "Rayleigh" match. The Rayleigh color match is extremely sensitive and diagnoses and screens for protan and deutan deficiencies (i.e., red/green deficiencies). Using the anomaloscope, participants view a bipartite field measuring 2° 10' in diameter. The top half of the field is composed of a mixture of two monochromatic lights, red (671 nm) and green (546 nm); the bottom half is a monochromatic yellow (589 nm) light. The ratio of red to green in the top half can be adjusted as can the intensity of the yellow light on the bottom half. Participants are asked to first create a perceptual match by adjusting both half fields (i.e., the ratio of red to green on the top half and the intensity of yellow on the bottom half). From several of these matches, the experimenter determines the range of the potentially acceptable matches. The experimenter then sets the red-green ratio and asks the observer to determine if a complete color match can be made by adjusting the intensity of the yellow field. Various red-green ratios from the predetermined range are presented to the observer, and each time the observer sets the yellow field to match the red-green field in brightness and indicates whether a complete color match has been obtained (i.e., the two half fields are indistinguishable). Between settings, the observer views a white

adapting field. The experimenter is able to determine the range of red-green ratios that the observer can match to the yellow field and compares them to values obtained from individuals with normal color vision (Linksz, 1964).

Bailey-Lovie Eye Chart.

The Bailey-Lovie eye chart is a simple, standard diagnostic eye chart that assesses visual acuity and consists of thirteen rows of five uppercase letters. From the top to the bottom of the chart, the size of the letters decreases in log units, with each line being 1.2589 times (or 0.1 log unit) smaller than the line above it. The chart has two sides: one with high contrast (black letters on a white background) and one with lower contrast (dark gray letters on a light gray background). By measuring where the participant makes an error, the logMAR (logarithm of the minimum angle of resolution) or Snellen acuity can be determined (Bailey & Lovie, 1976). The smaller the line of text the observer can correctly identify, the higher the visual acuity.

Procedure

Upon their initial visit, all participants read and filled out an informed consent form. They then completed a questionnaire in which they listed any medications being taken and any family history of color vision deficiencies (see Appendices A and B). All participants were asked to notify the experimenter if, over the course of the study, their responses to any of these initial questions changed (e.g. they started smoking and/or started taking any drugs or medications). No significant changes were self-reported by any of the participants. The participants were allowed to wear their corrective lenses if they chose, and none reported changes in their refraction over the course of the entire experiment. Any changes in habits after pregnancy were not recorded.

After completion of the forms, the participants were seated at a table illuminated by a MacBeth illuminant C lamp. This lamp approximates lighting conditions at high noon on a

sunny day and is the appropriate lighting in which to conduct color vision testing. All panel and plate tests were conducted in darkness except for the MacBeth lamp illumination. All participants were permitted to choose which eye they wanted to use for the color and acuity tests, and they used that eye for all experimental sessions. The other eye was covered with an eye patch. They were allowed to wear corrective lenses if they chose to minimize discomfort. If they wore corrective lenses for the first session they were required to wear them for all sessions.

During a test session participants completed the following color vision tests: the tritan plate, D-15 panel test, L'anthony desaturated D-15 panel test, and the FM 100-hue test. The Macbeth lamp was then turned off for the anomaloscope test. The procedure as outlined above for anomaloscopic matches was followed. Before setting the hue/brightness for each trial the participant viewed the white light at the bottom of the anomaloscope to obviate any adaptation effects

Lastly, the participants entered the hallway outside of the testing room to complete the Bailey-Lovie exam. The chart was posted twenty feet from the participant. The high contrast side was displayed first. Participants started by reading the first line that was clearly visible and continued down the chart until they made a mistake. The same procedure was repeated for the low contrast side of the chart.

The same tests were administered in the same order for all participants. Each session lasted approximately 30-40 minutes. The number of sessions for each participant are presented in Tables 1 and 2, and varied due to availability of individual participants. All of the pregnant participants were also tested within three months of giving birth. Three of the six pregnant participants (P3, P4 and P5) also completed a follow up test, up to a year later.

Table 1: Number of sessions completed by pregnant participants before and after the births of their children.

Participant	Sessions Before Birth	Sessions After Birth
P1	9	1
P2	9	1
P3	11	2
P4	9	2
P5	12	2
P6	8	1

Table 2: Number of sessions completed by control participants.

Participant	No. Sessions Completed
C1	4
C3	6
C4	5
C5	7
C6	4
C7	5

Analyses

Non-parametric statistics were used due to the small group sizes. Friedman's test was used to detect differences across multiple sessions within groups. Mann-Whitney/Wilcoxon signed ranks tests were used to compare differences between groups. Wilcoxon dependent test were used to compare within groups.

Chapter 3: Results

Farnsworth-Munsell 100 hue Test (FM 100-hue)

The FM100-hue test was scored using a computer program that automatically generated Kinnear error scores (Kinnear, 1970). As noted previously (see Chapter 2), each cap in the FM 100-hue test is a different color; and each color is assigned its own number from 1 to 85. As Figure 2 demonstrates, the cap numbers form a circle from 1 to 85. When an observer has completed the test, the experimenter notes the order of the cap numbers. Error scores for each cap are then computed by taking the sum of the absolute differences between the number of the cap and the number of the caps immediately adjacent to it. For example, if the observer arranged caps 9-17 in the following order: 9 10 11 14 12 13 15 16 17, the error score for each of the middle seven caps would be 2 4 5 3 3 3 2, with a total error score of 22 for the seven caps. The distance a point deviates from the inner circle indicates the magnitude of error. An error score of 2 is expected for each cap if it has been placed in proper order since the scores for a cap are the sum of the differences between the number of that cap and the caps adjacent to it (Farnsworth, 1957). If the line connecting the error scores appears to form a circle within the error score of 2, the person is color normal. However, if the error pattern is oblong and falls along one of the axes in Figure 2, a color deficiency exists. The dotted line indicates the axis that tritan errors would fall along, and the other two lines indicate the protan (solid line) and the deutan (dashed line) axes.

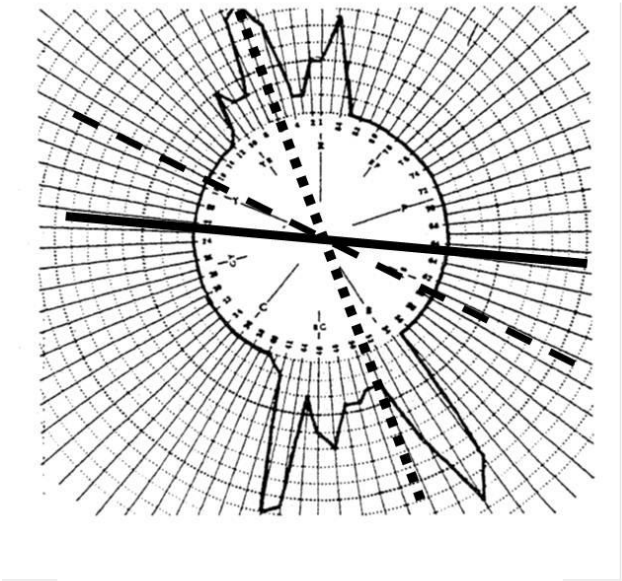

Figure 2: Error scores plotted for an individual with a tritan deficiency. The solid line indicates the protan axis, the broken line shows the deutan axis, and the dotted line indicates the tritan axis.

Previous research with oral contraceptive users showed acquired tritanomaly on the FM-100- Hue test (Marre, Neubauer, & Nemetz, 1973; Lakowski & Morton, 1977/1978, Fine & McCord, 1991), so this test was used to detect if similar patterns of color loss were present in the pregnant participants of this study. Tray 3 was of most interest because it contains the short-wavelength hues ranging from blue to violet. If acquired tritanomaly does develop, the error scores from this tray should be the greatest compared to the other trays.

Tray error scores across groups.

Figure 3 shows mean error scores across sessions for each tray in the FM 100-hue test for pregnant (before giving birth; black bars) and control (checkered bars) groups. Error bars indicate ± 1 standard error of the mean (SEM) across test sessions during pregnancy for the pregnant participants and all sessions for control participants. Although the largest errors were found in Tray 3, the Mann Whitney nonparametric test confirmed there were no significant differences between the groups (See Table 3) for any of the four trays.

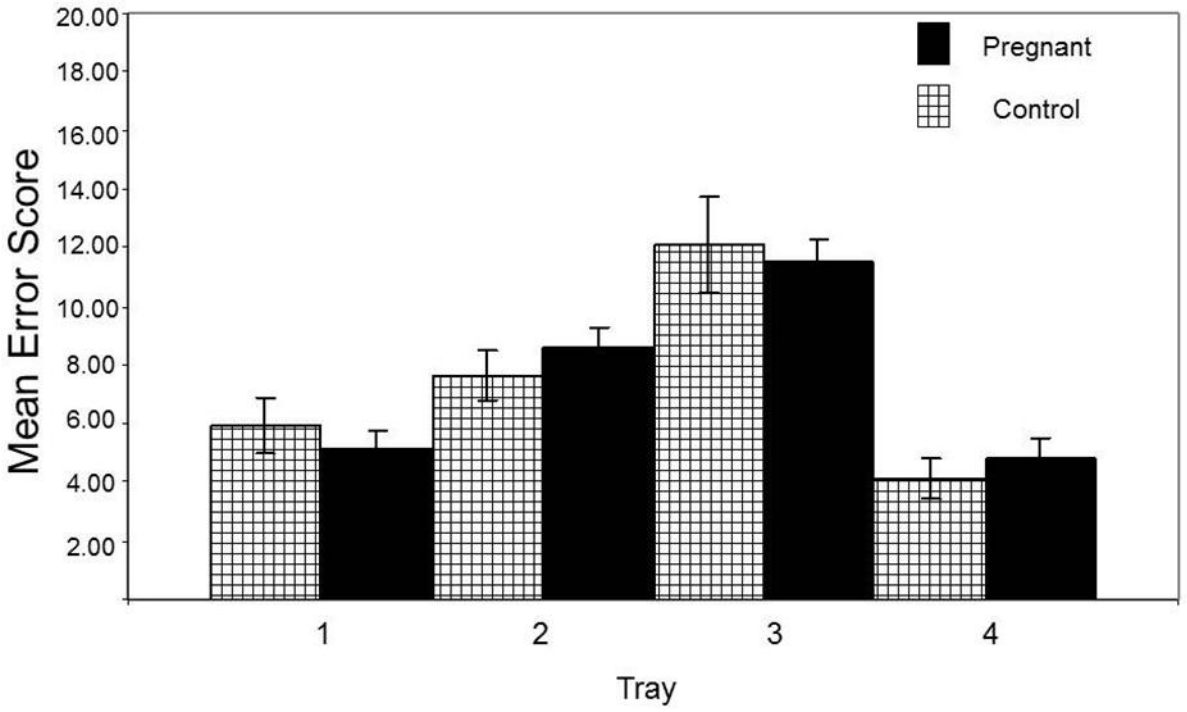

Figure 3: Mean error scores for pregnant participants versus control participants

Table 3: Mann-Whitney statistics comparing pregnant and control participants

Tray	U	n_1	n_2	P value (one-tailed)
1	859.5	59	31	0.32
2	814.5	59	31	0.198
3	911.5	59	31	0.49
4	914.5	59	31	0.393

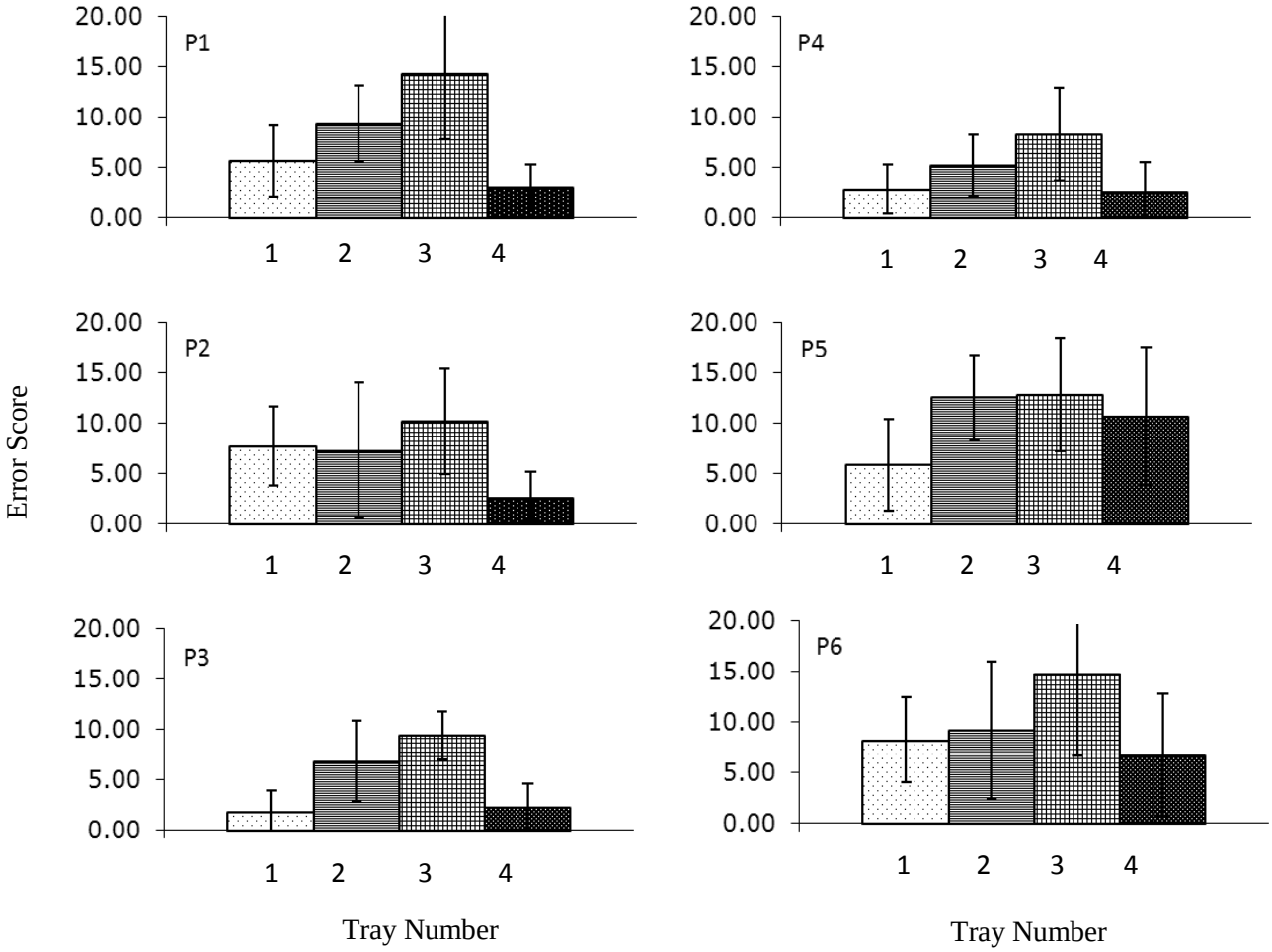

Figure 4: Individual mean error scores for pregnant participants during pregnancy

Figure 4 presents the mean error scores for each pregnant participant before giving birth on each tray of the FM100-Hue test. Error bars indicate ± 1 standard deviation (SD) across test sessions. As expected, for five of the six pregnant women, errors were greater for Tray 3, which is associated with a tritan loss. The next highest error score was on Tray 2 with the exception of Participant P2, who had the second highest score on Tray 1. Tray 1 and Tray 4 error scores varied by participant, but were always less than Tray 3 scores. Friedman's tests confirmed that there were statistically significant differences among the tray scores for five of the individual participants.

For the five showing a significant difference among error scores, further analyses were conducted on their error scores using Wilcoxon dependent tests. These tests focused on the error scores for Tray 3 to see if they were significantly greater than the error scores on the other three trays. The analyses revealed that Tray 3 error scores for P1 and P3 were greater than the error scores on the other three trays. The error score on Tray 3 were greater than Trays 1 and 4 for P4 while the Tray 3 error score was only greater on Trays 1or 4 for P5 and P2, respectively. It was expected that the Tray 3 error scores would be larger than error scores from Trays 1 and 4 since these trays do not test a tritan loss. It was not necessarily the case that differences between Trays 2 and 3 would be predicted since Tray 2 is also involved in determining a tritan deficiency.

Table 4: Friedman's test results for tray differences for each pregnant participant

	X_2	DF	P
P1	13.3	3	0.004
P2	10.367	3	0.016
P3	18.45	3	0
P4	8.367	3	0.039
P5	8.875	3	0.031
P6	4.988	3	0.173

Table 5: Wilcoxon dependent test results for tray differences for pregnant participants

		Wilcoxon	Z score	P (one-tailed)
P1	Tray 2-3	3.5	-2.03	0.021
	Tray 3-4	44	2.547	0.005
	Tray 1-3	2.5	-2.369	0.009
P2	Tray 3-4	36	2.521	0.006
P3	Tray 2-3	14	-1.961	0.05
	Tray 3-4	78	3.059	0.002
	Tray 1-3	0	-2.934	0.003
P4	Tray 3-4	32	1.96	0.05
	Tray 1-3	32	1.96	0.05
P5	Tray 1-3	8.5	-2.393	0.017

Figure 5 shows the mean error scores for each control participant on the FM 100 - Hue test across all sessions. Each tray is represented by a separate bar in the figure. Error bars indicate ± 1 SD across test sessions.

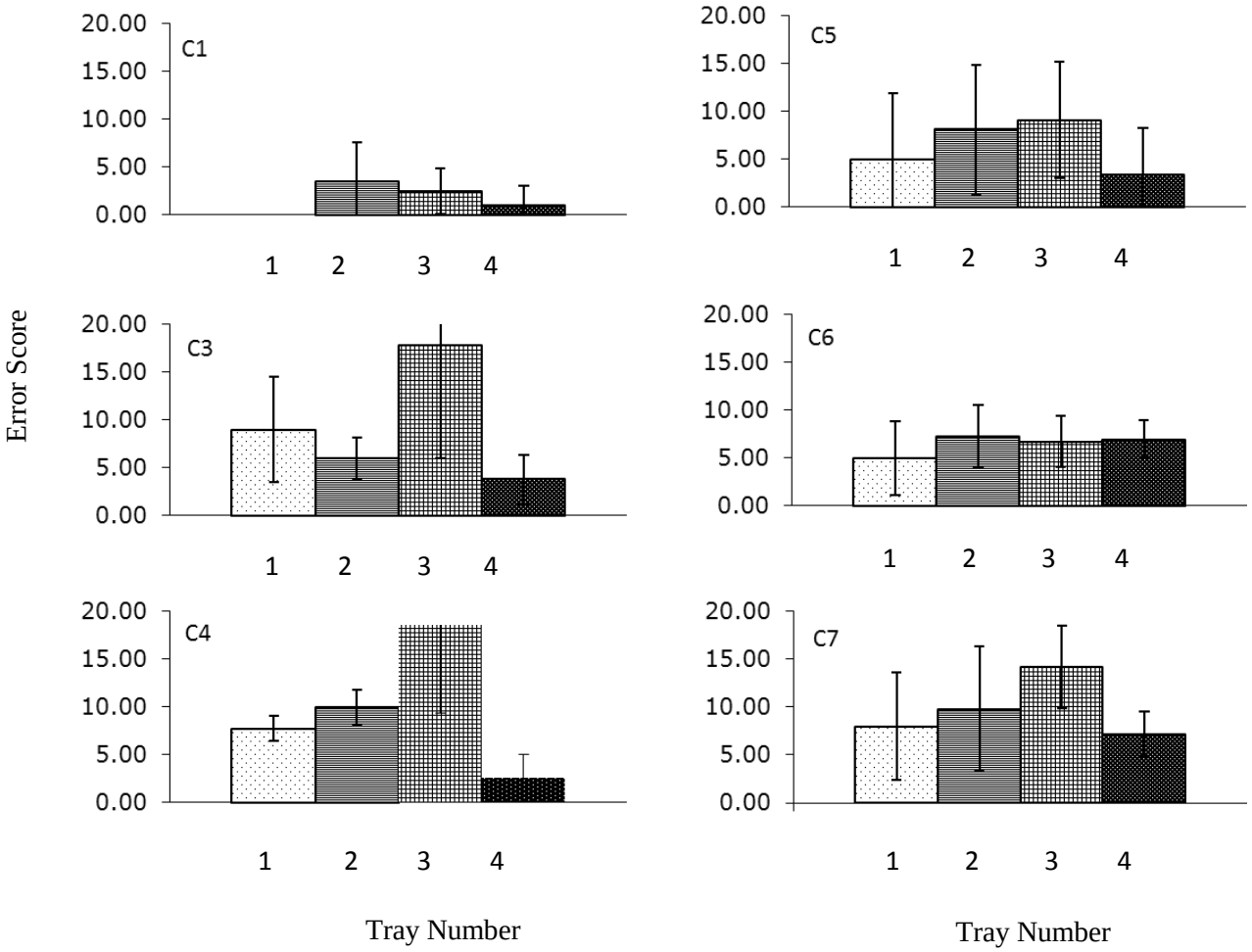

Figure 5: Individual mean error scores for control participants

The patterns for these participants showed greater variability than pregnant participants. Tray 3 elicited the highest error scores for all but one observer (C6). The error scores for C4 and C5 appear to be higher than those of the pregnant participants. This could have been due to the fact that the control participants participated in fewer sessions than the pregnant participants. Friedman's tests were used to ascertain if these differences among error scores was statistically significant. As shown in Table 6, error scores from two of the control participants were significantly different. Further analyses of the data from these two participants, using Wilcoxon dependent tests, showed two differences. For one control participant, the Tray 3 error score was significantly greater than those for Trays 2 and 4. For the other participant, the Tray 2 and 3 error scores were significantly larger than the Tray 4 error score.

Table 6: Friedman's test results for tray differences for each control participant

	X_2	DF	P
C1	2.7	3	0.44
C3	10	3	0.019
C4	8.7	3	0.034
C5	5.271	3	0.153
C6	0.9	3	0.825
C7	2.52	3	0.472

Table 7: Wilcoxon dependent test results for tray differences for control participants

		Wilcoxon	Z score	P (one-tailed)
C3	Tray 2-3	0	-2.201	0.028
	Tray 3-4	21	2.201	0.028
C4	Tray 3-4	21	2.201	0.028
	Tray 2-4	21	2.201	0.028

Tray error scores across time.

As Figure 6 shows, over the course of pregnancy, endogenous hormone levels increase. If changes in hormone levels influence color perception, we would expect to see a concurrent rise in error scores. This was examined in Figure 7 by plotting mean error scores for each tray as a function of time (weeks) before delivery.

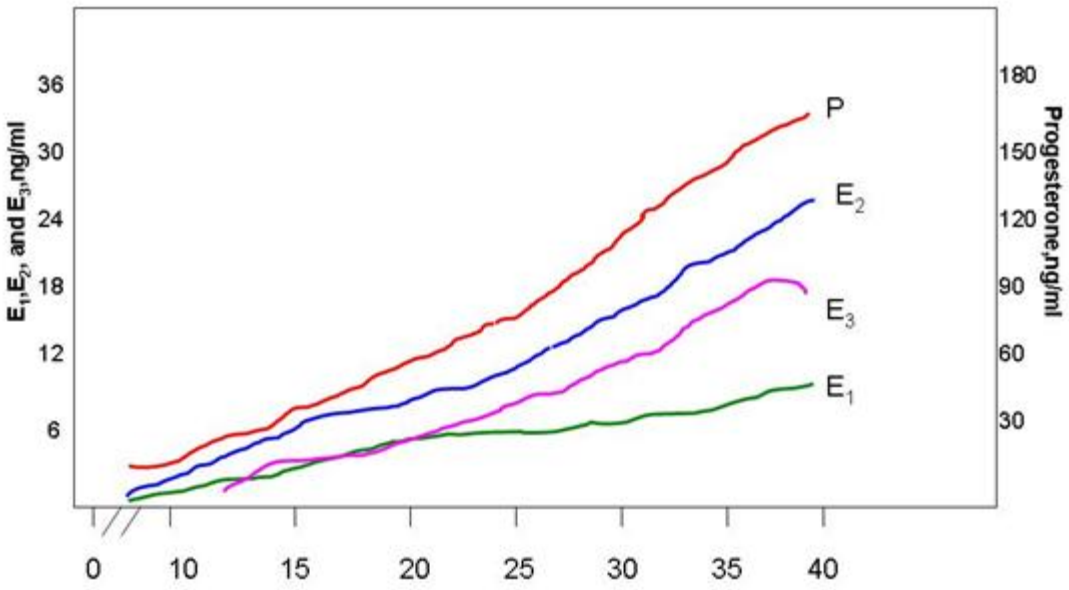

Figure 6: Serum levels of estrone (E1), estradiol (E2), estriol (E3) and progesterone (P) over the course of normal pregnancy (*Adapted from Parker CP Jr*. Endocrinology of Pregnancy, In Carr, Blackwell, & Azziz, *Essential Reproductive Medicine, 2005*).

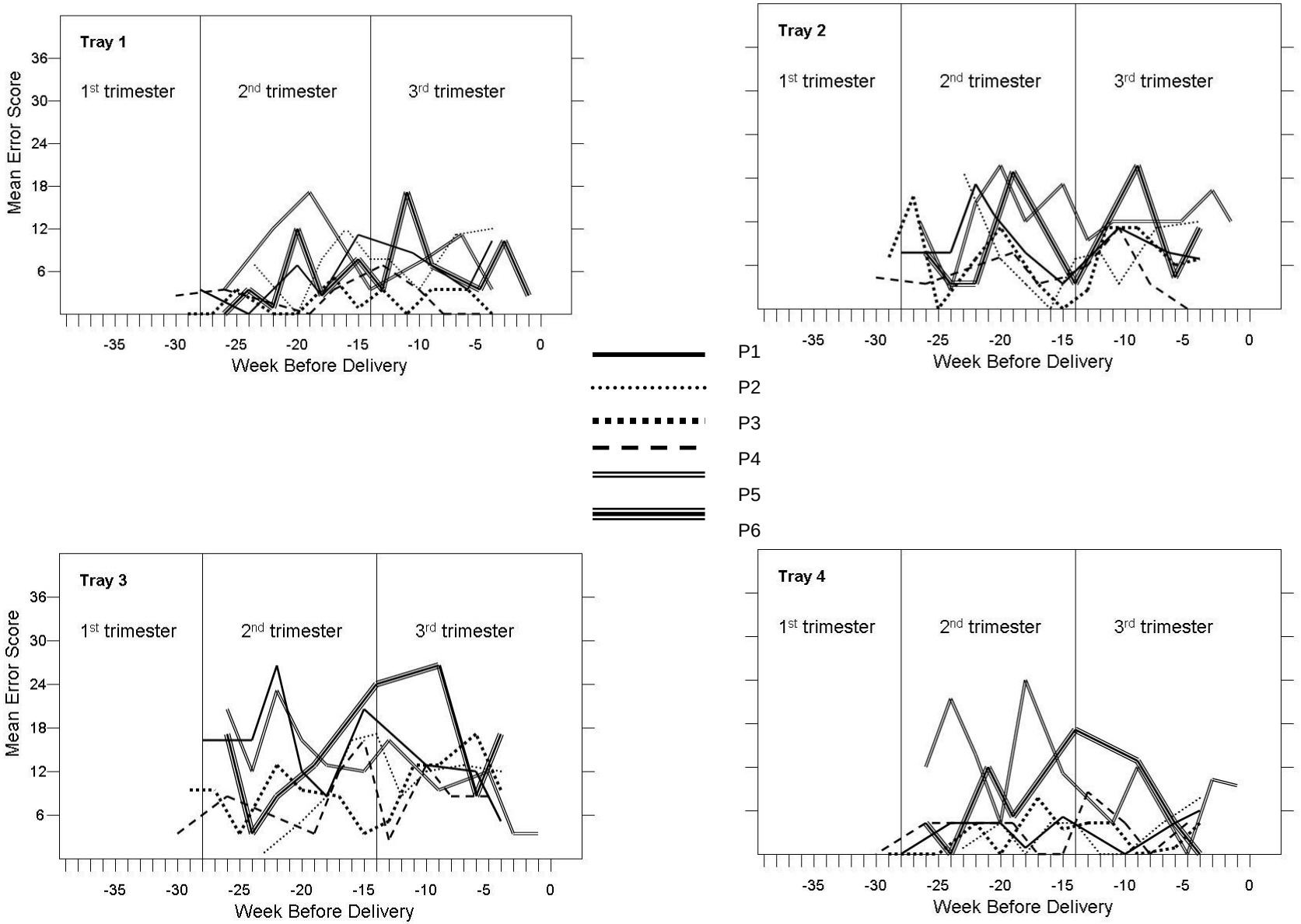

Figure 7: Error scores for each tray as a function of time (weeks) before delivery

Since the plots of the raw data made it difficult to see any relation between color perception and weeks before delivery, a linear regression analysis was performed. Figures 8-13 show the results of this analysis for each pregnant participant. Each participant's error score was plotted as a function of time using date of delivery as a fixed point set to 0. From this, weeks of pregnancy before delivery could be determined. The slope of the line for each data set is shown in each panel, with a positive slope indicating a decrease in performance (i.e., larger error score) with weeks of pregnancy and a negative slope indicating an increase in performance (i.e., smaller error scores) with weeks of pregnancy. Again the expectation is that if hormone levels are increasing in a linear manner with weeks of pregnancy performance on the FM 100-hue test should also systematically changing with weeks of pregnancy. In particular, it was expected if there is a tritan loss with pregnancy, error scores on Tray 3 should increase with pregnancy while the other trays should show very little change. In general there was little change in performance on Trays 2 and 4 during pregnancy. Four of the pregnant women (P2, P3, and P6) showed an increase in errors with pregnancy for Tray 3 while P1 and P5 became better at discriminating the color chips in Tray 3 from each other, i.e. fewer errors. For Tray 1, some showed an increase in error scores with weeks of pregnancy while others showed no change in error scores.

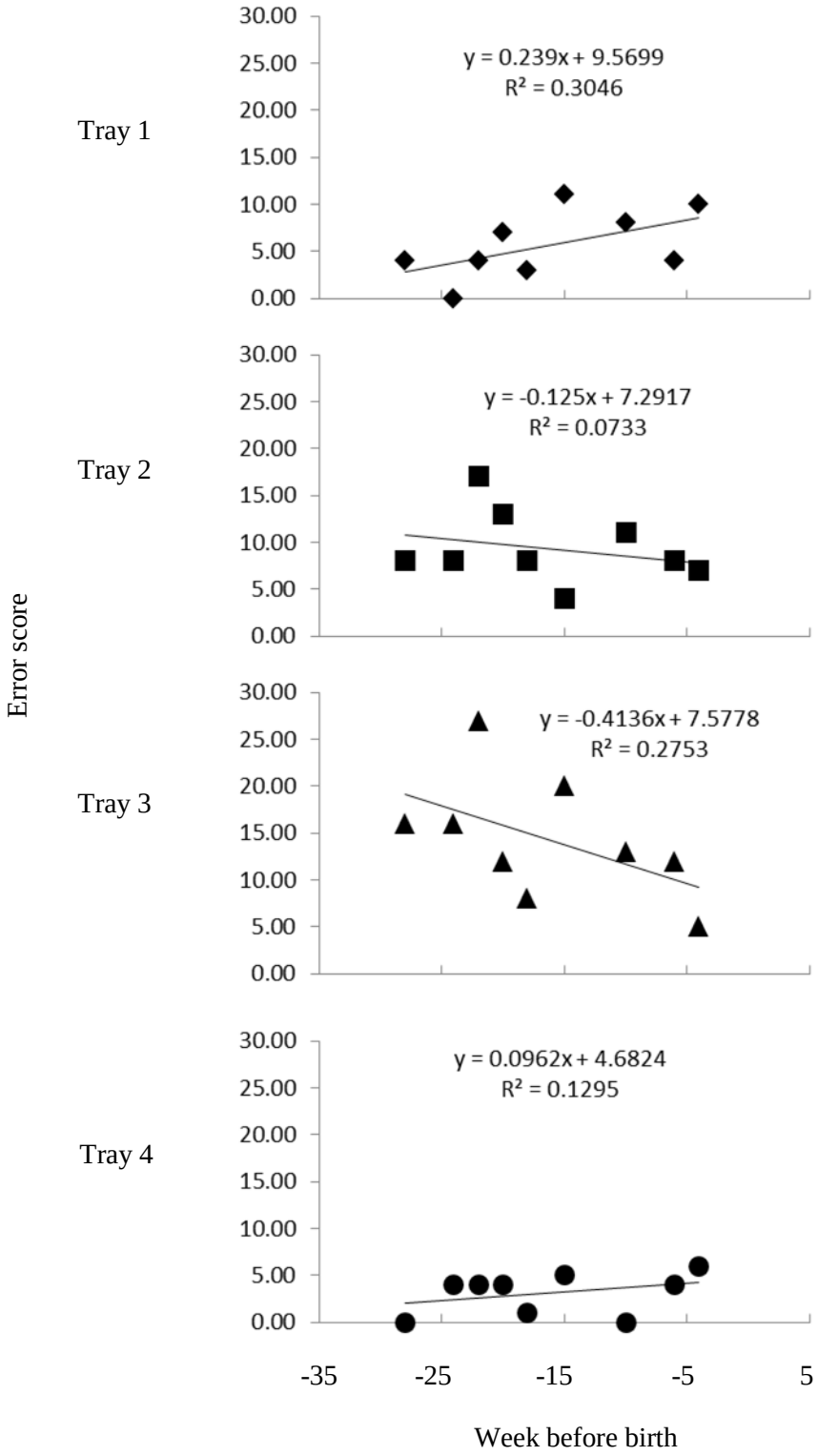

Figure 8: Error scores for P1 over time

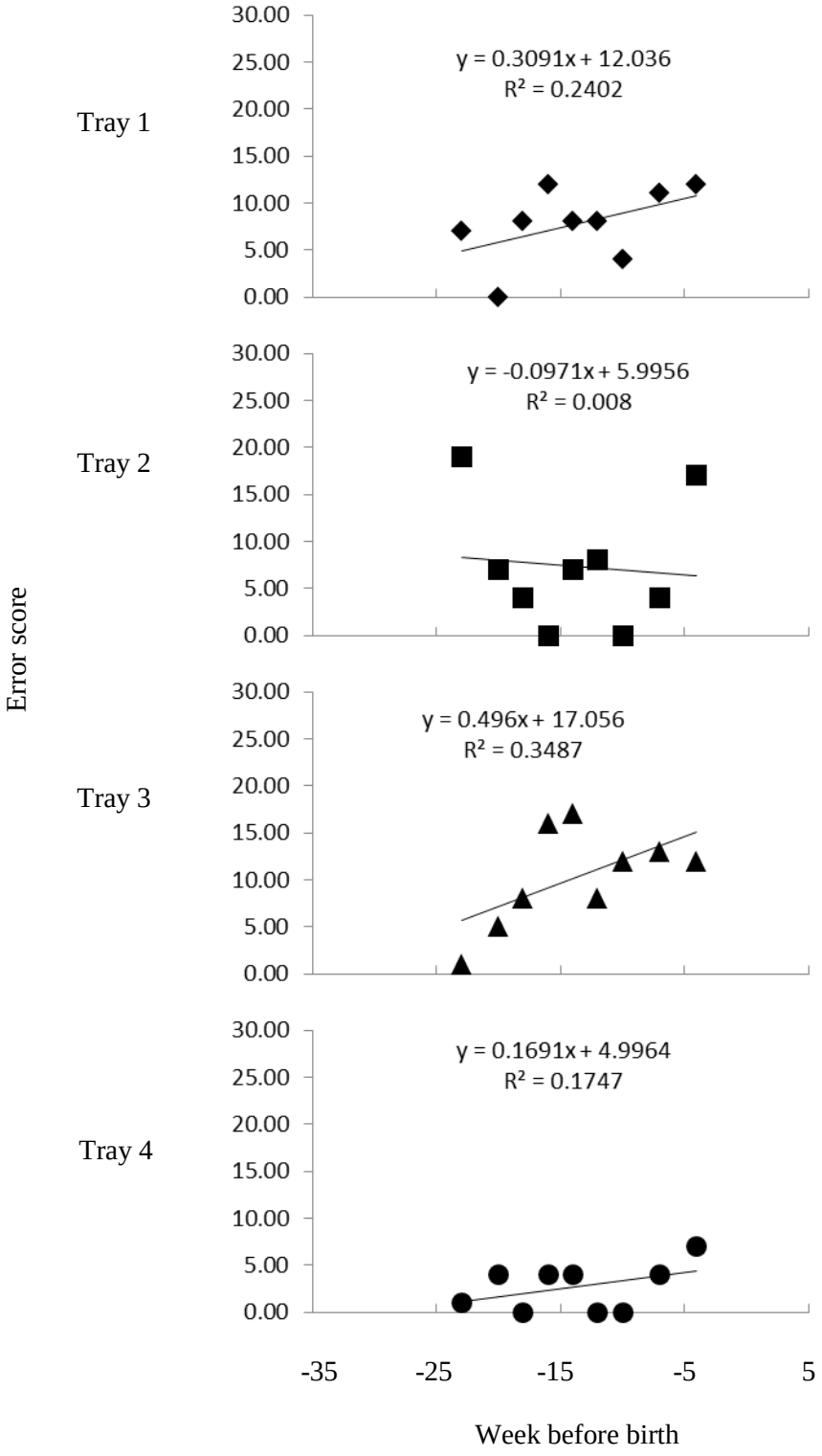

Figure 9: Error scores for P2 over time

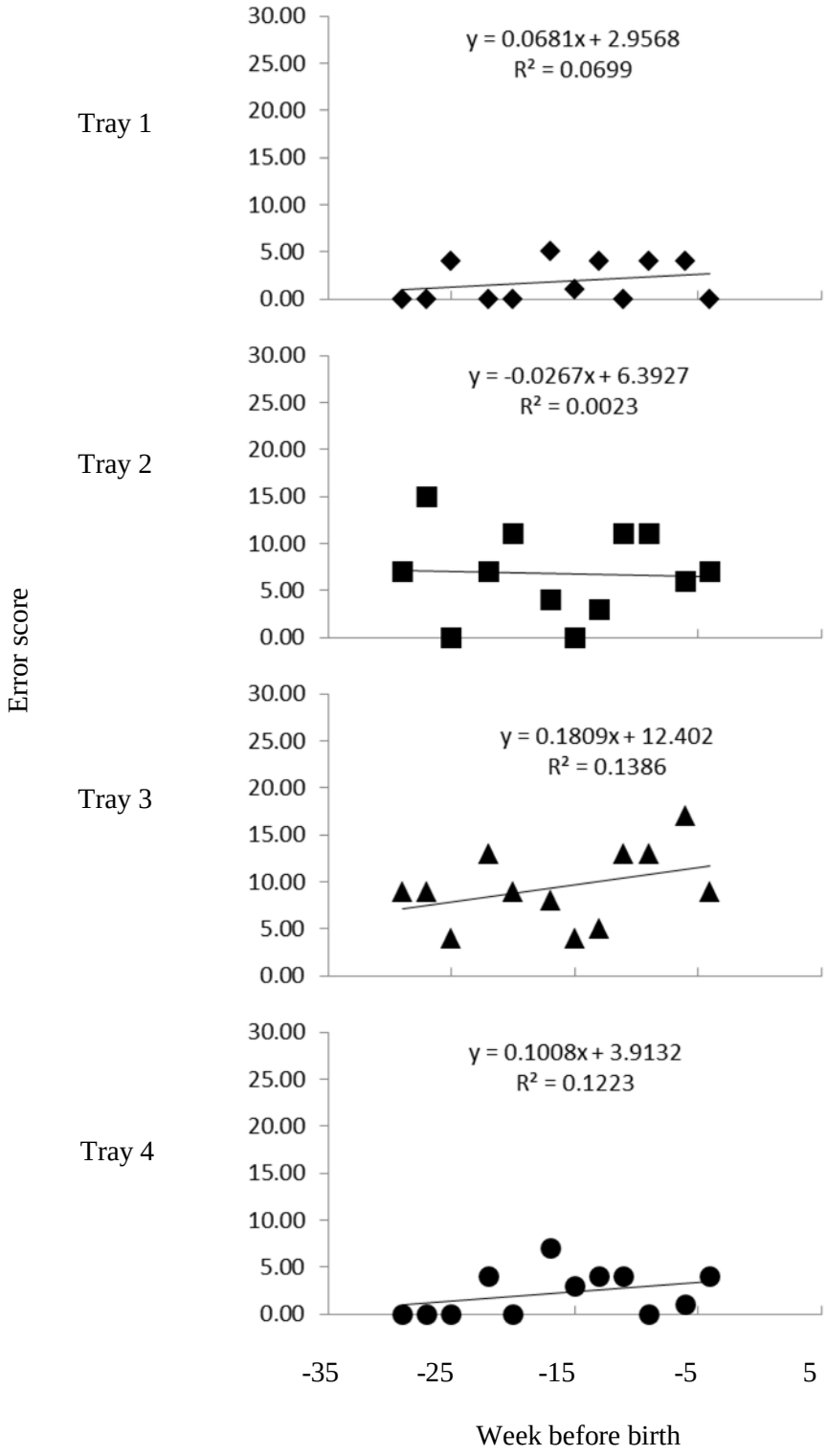

Figure 10: Error scores for P3 over time

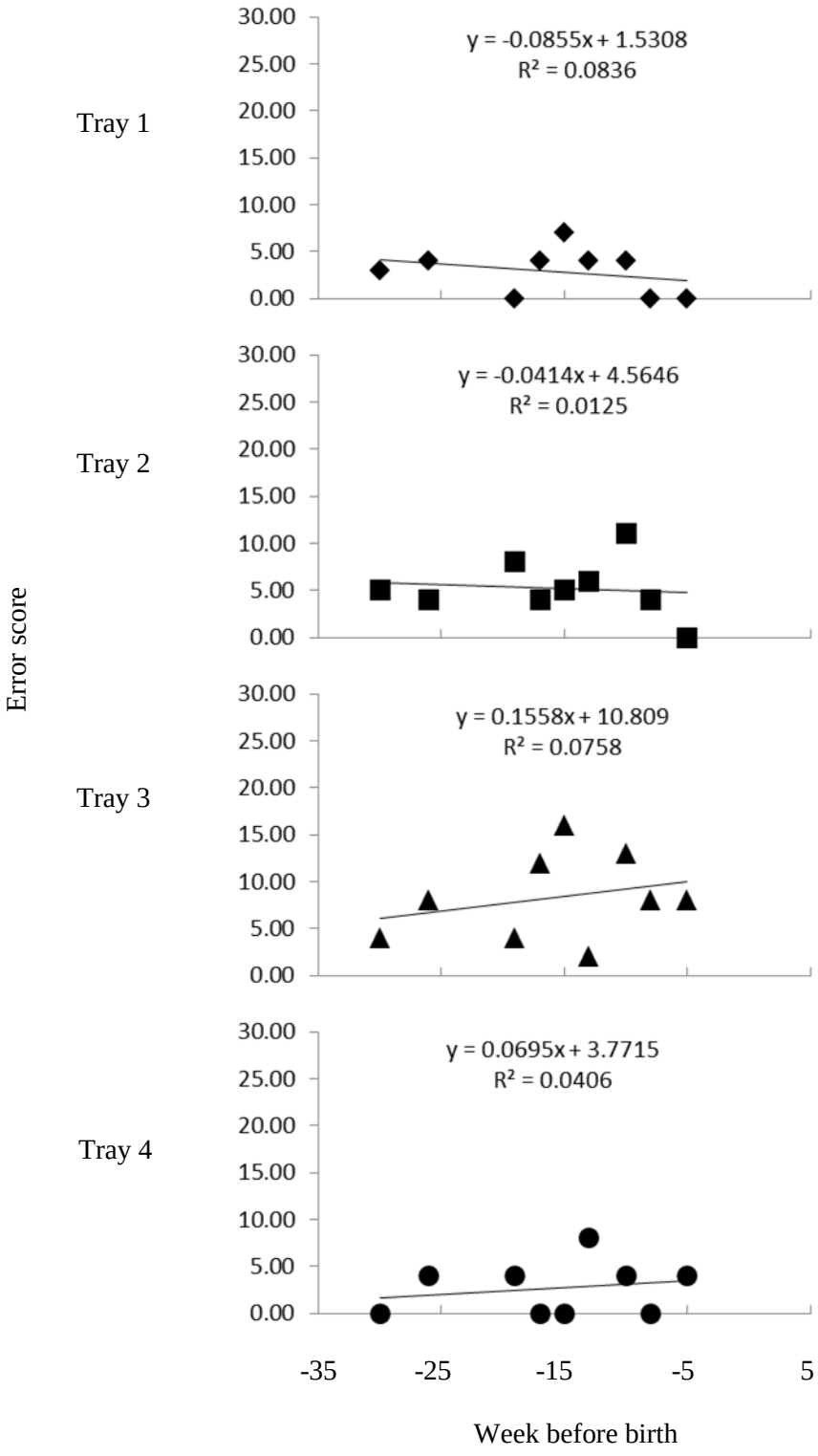

Figure 11: Error scores for P4 over time

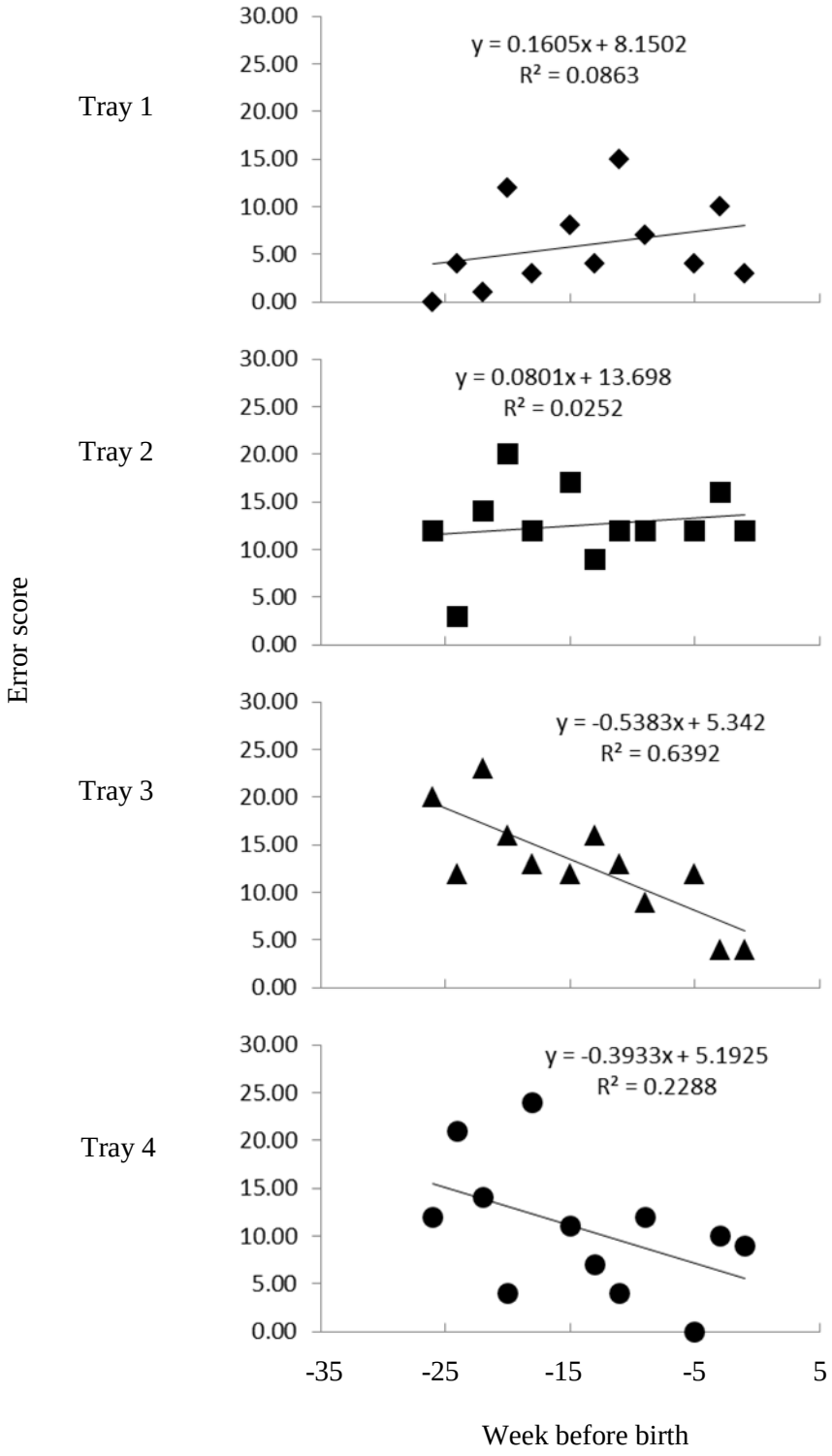

Figure 12: Error scores for P5 over time

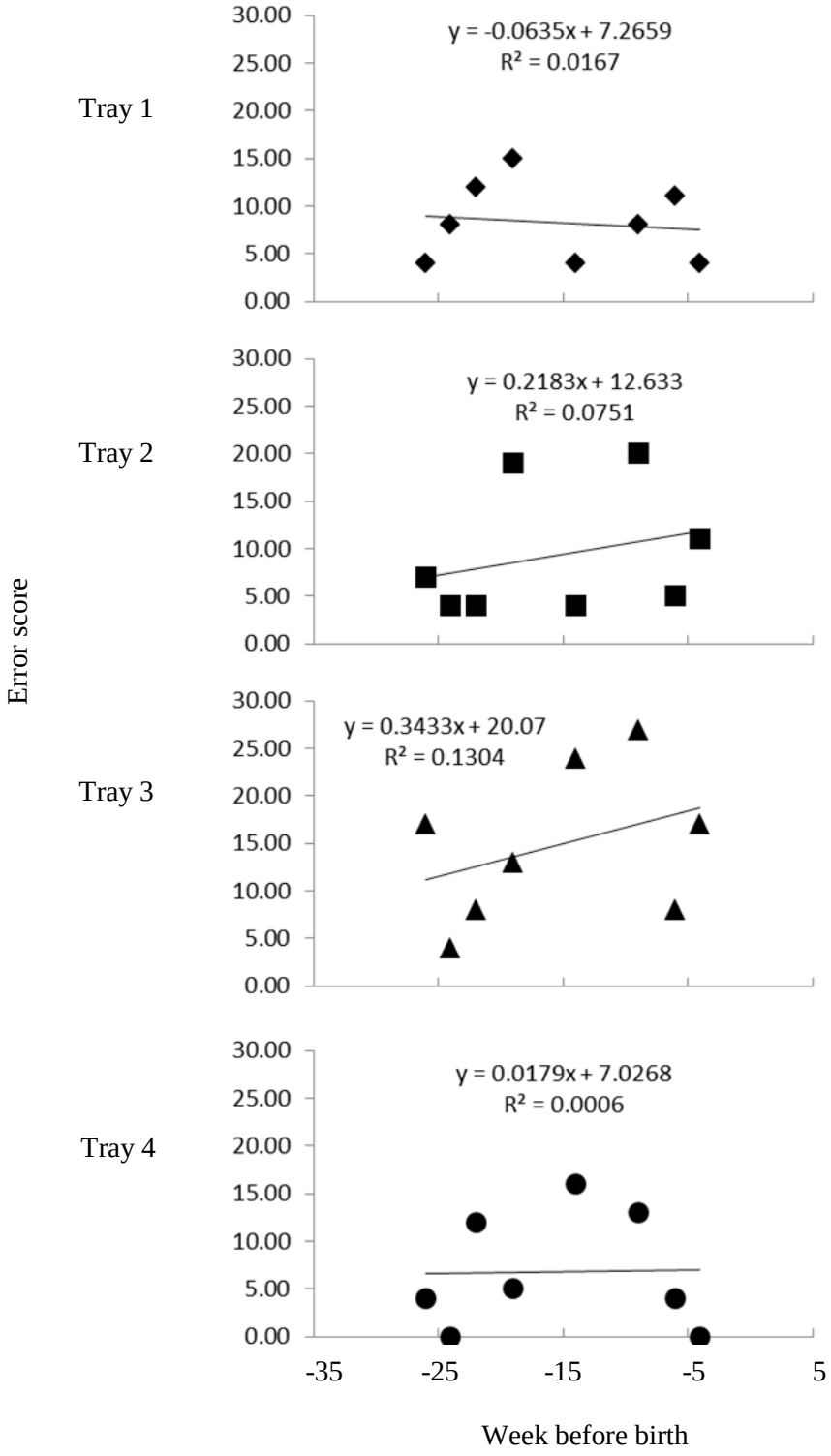

Figure 13: Error scores for P6 over time

A similar analysis was done with the control participants to see if these individuals showed an increase or decrease in performance with time. If these individuals show a similar pattern as the pregnant participants, then it is unlikely that changes in hormones are responsible for their changes in color discrimination. In general, the control participants showed little change in error scores with time. The only exception was C5 who showed increases in error scores with time for all four trays. If there was a change in error scores, the control participants were more likely to show a decrease in error scores such as for Tray 3 for C3, C4 and C6, perhaps indicating a practice effect. It is possible, then, that the three pregnant participants who showed an increase with error scores on Tray 3 with pregnancy were affected by increases in their hormone levels.

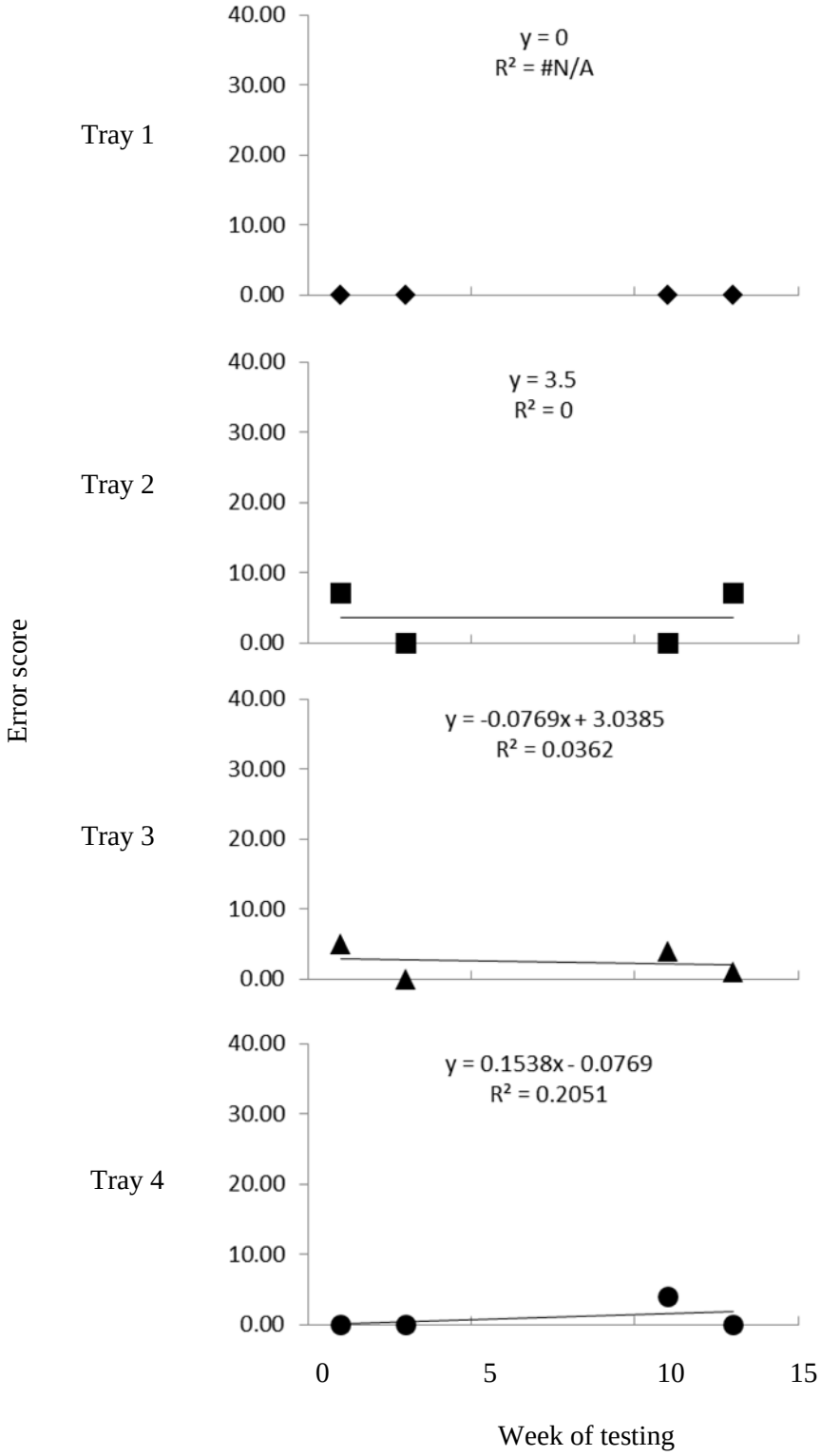

Figure 14: Error scores for C1 over time

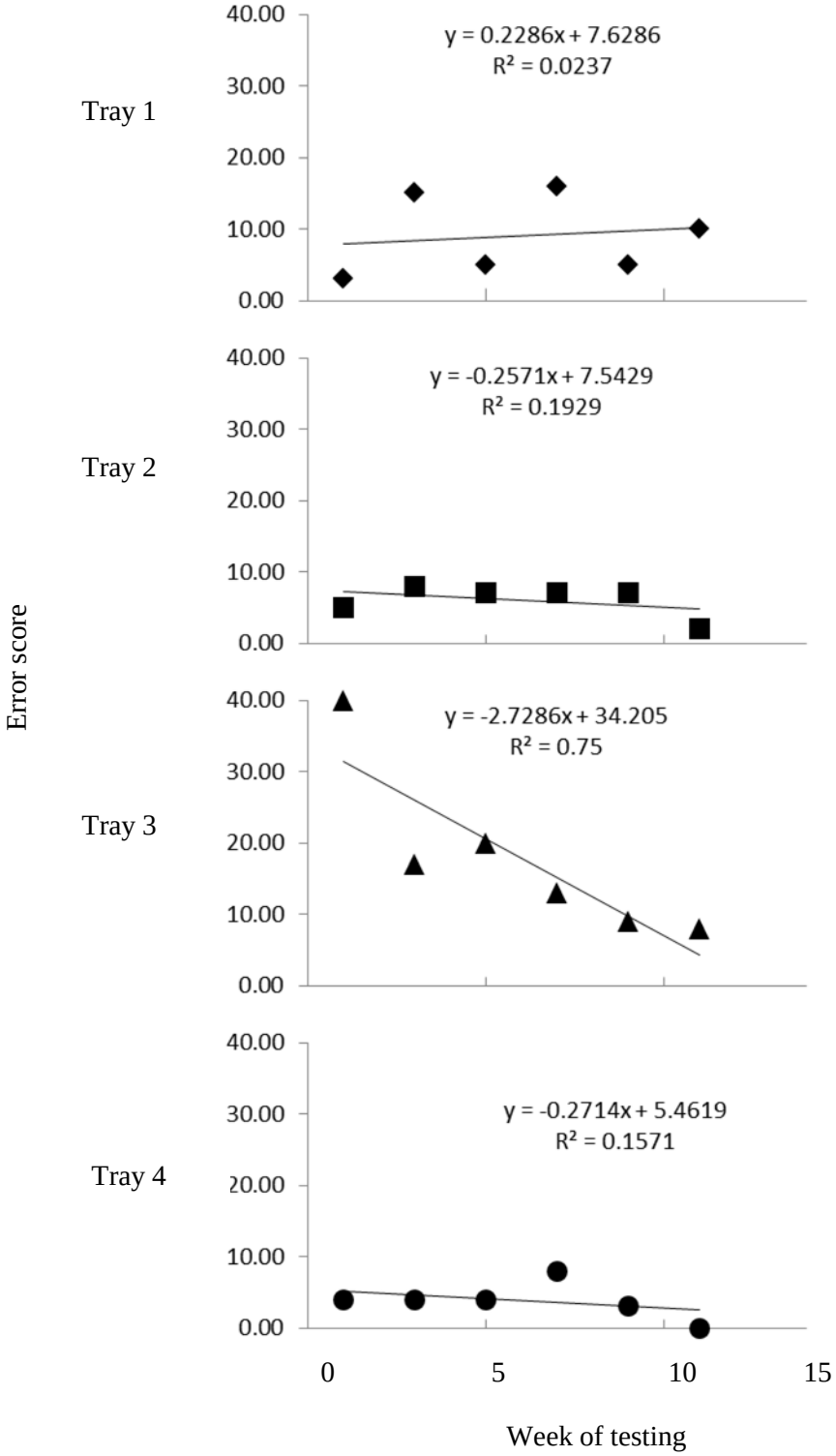

Figure 15: Error scores for C3 over time

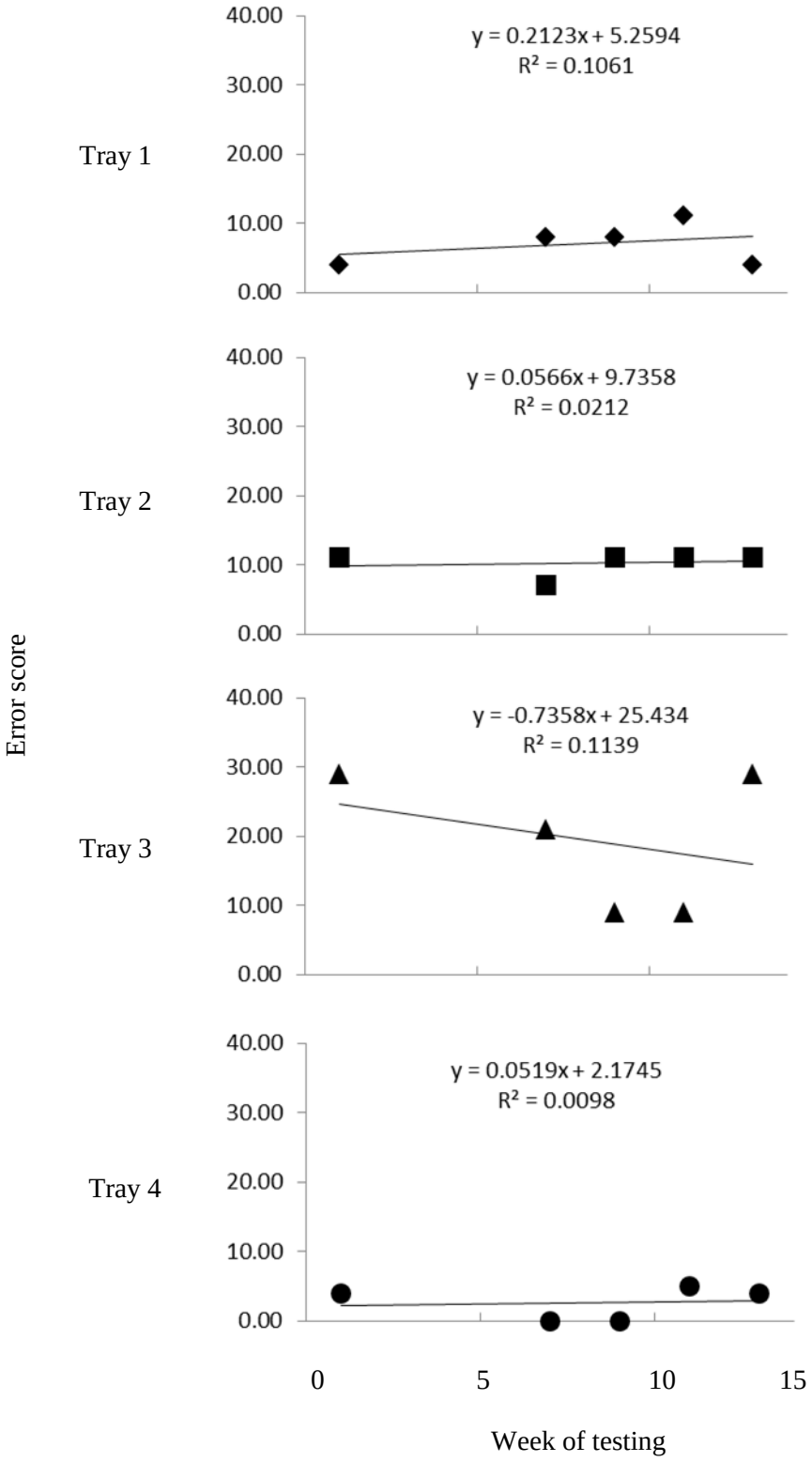

Figure 16: Error scores for C4 over time

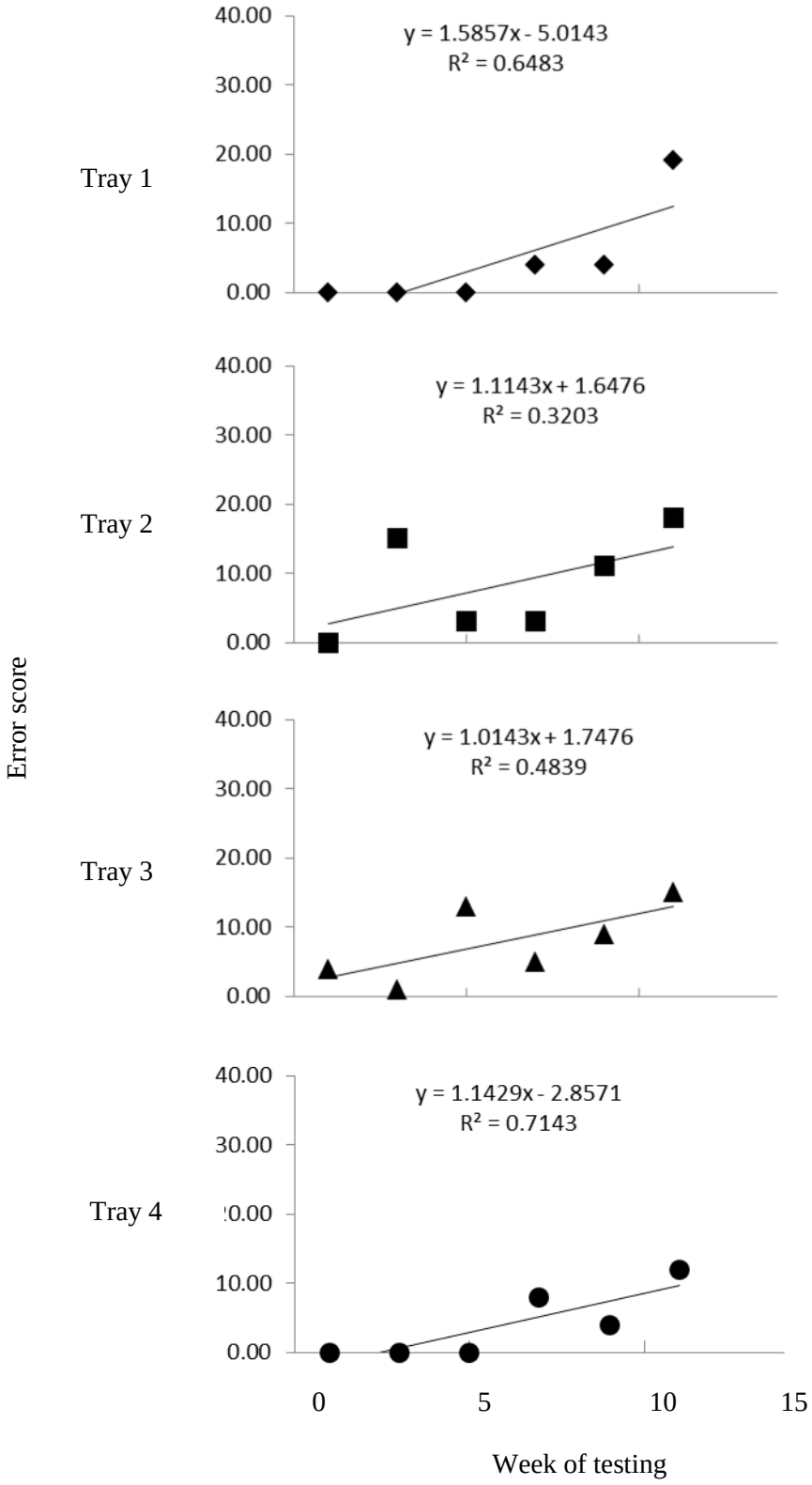

Figure 17: Error scores for C5 over time

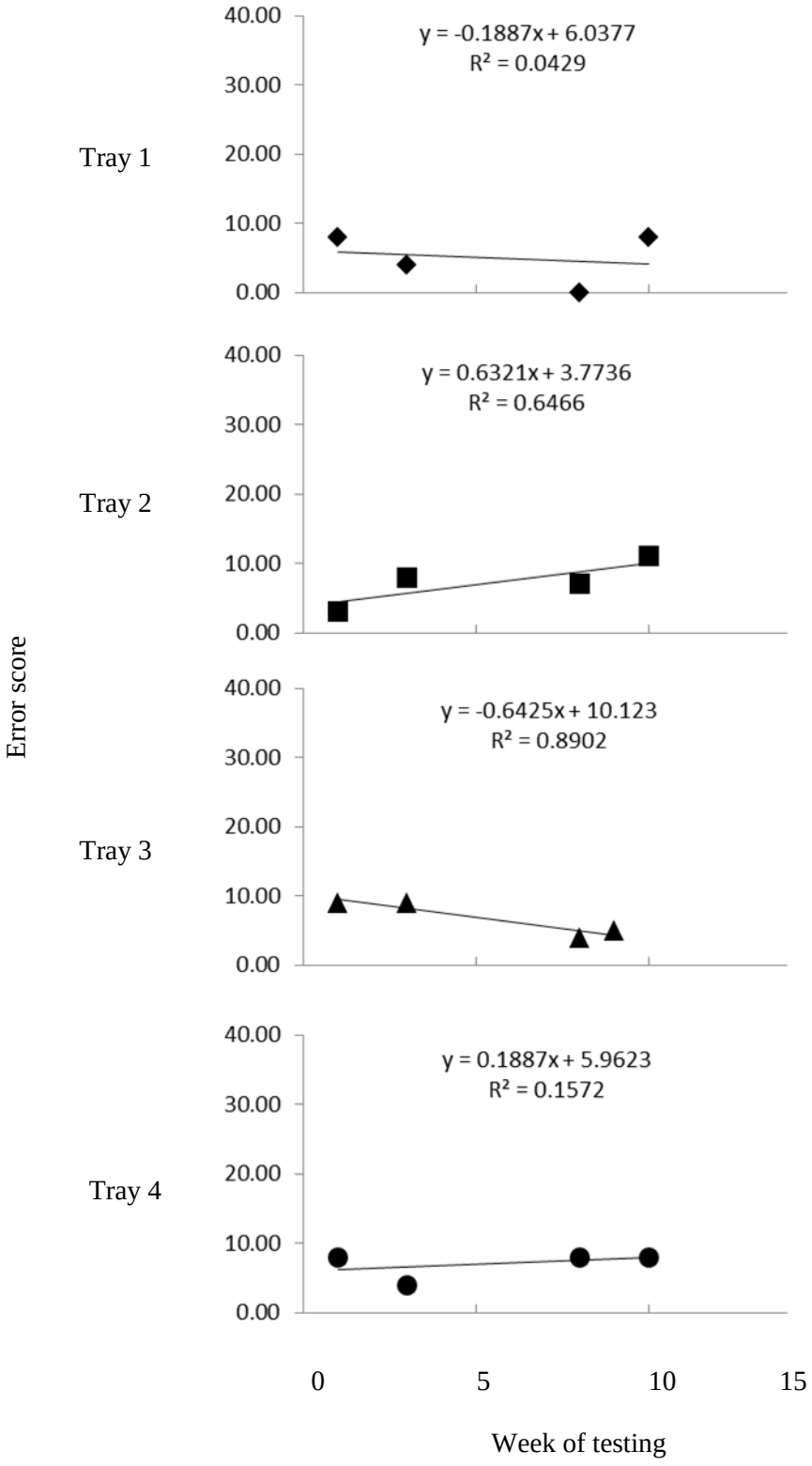

Figure 18: Error scores for C6 over time

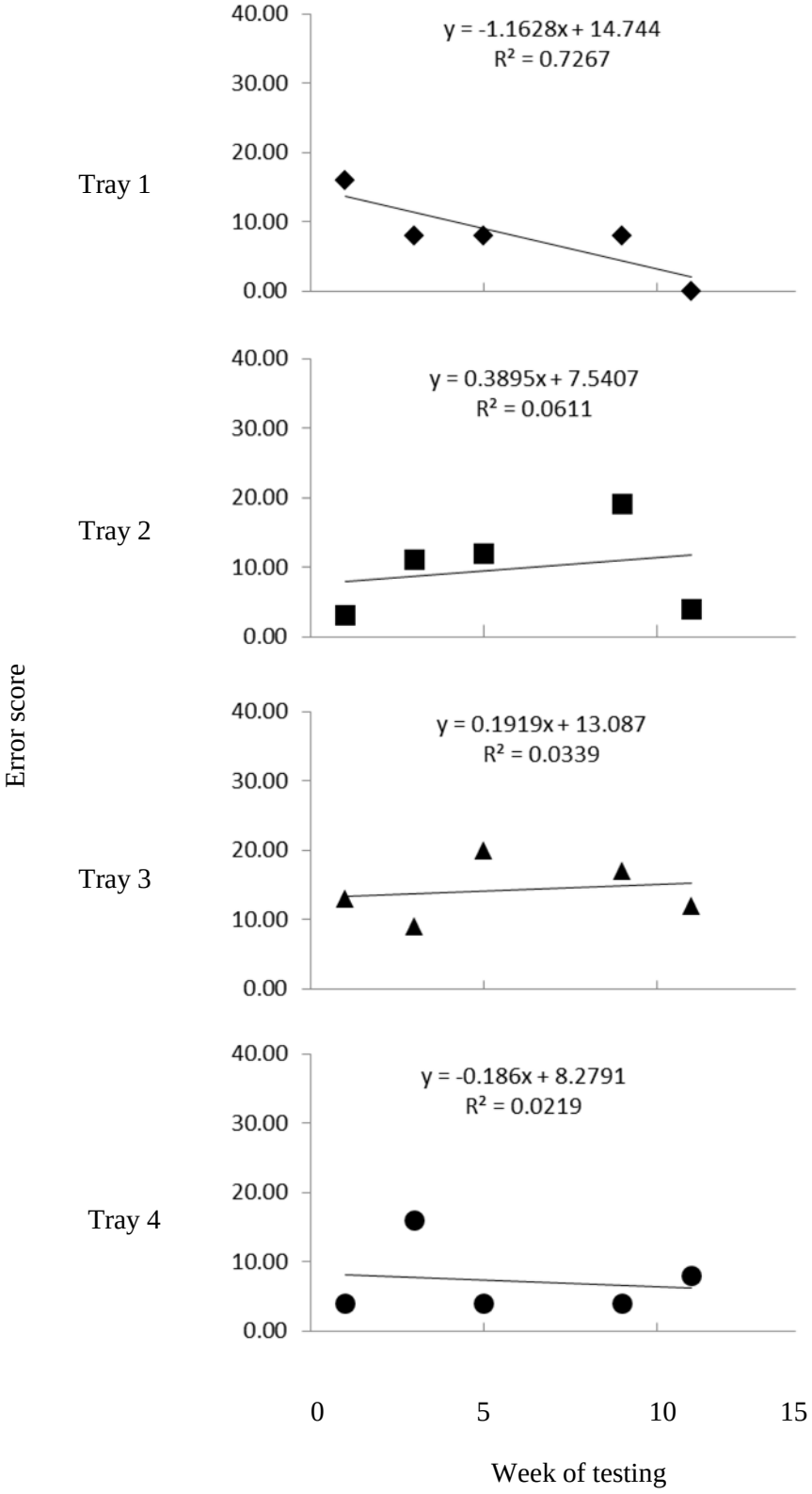

Figure 19: Error scores for C7 over time

The pregnant participants also returned after giving birth to determine if there were changes in performance on the FM 100-hue test from when they were pregnant. Figure 20 replots data from Figure 3 to compare to the post-pregnancy measures. In this figure the mean error scores across time and participants are shown for each tray from the FM 100-hue test. As Figure 20 illustrates, error scores did not vary reliably between when the participants were pregnant and after the babies were born in relation to control participants. Statistical tests confirmed no significant differences between the groups.

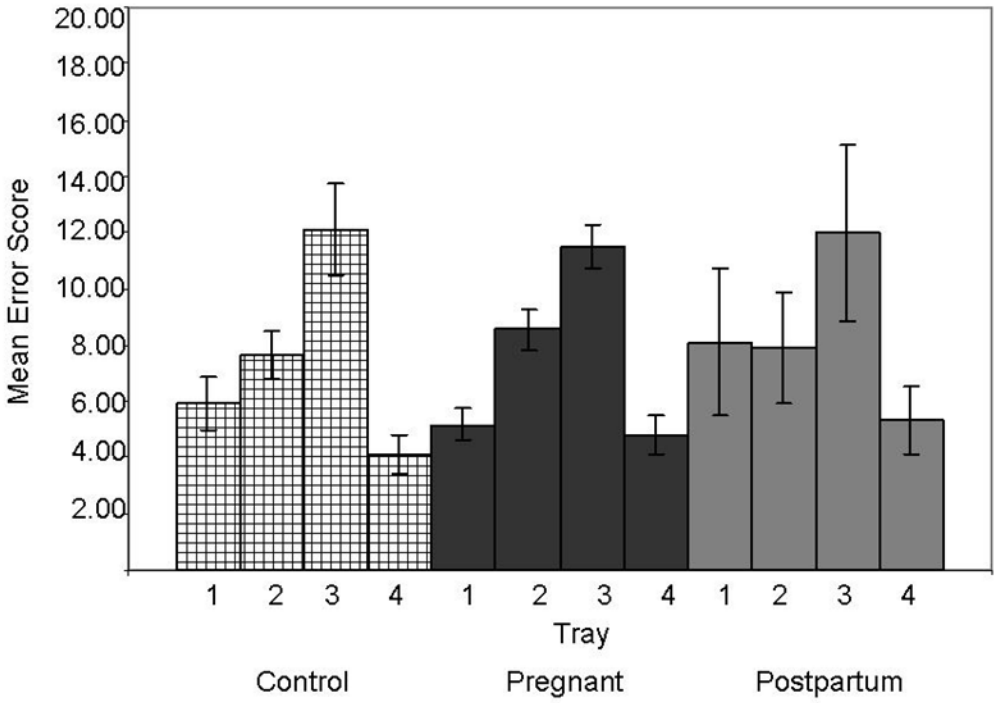

Figure 20: Mean error scores for pregnant participants versus control participants before and after delivery

Figure 21 shows the mean error scores for each tray and for each pregnant participant during pregnancy and after pregnancy. For posttests, all of the participants had different patterns. In the initial posttest, P2, P3, and P5 showed decreases in error scores on at least three trays. P4

showed a decrease in Tray 3 error scores and increases in all other scores. P1 and P6 showed substantially higher scores postpartum than when pregnant, probably because they were distracted by extenuating circumstances at the time (see discussion chapter). Also, at the time of her second post-test P4 was pregnant again. However, statistical tests did not show a significant difference between pre- and post-tests overall.

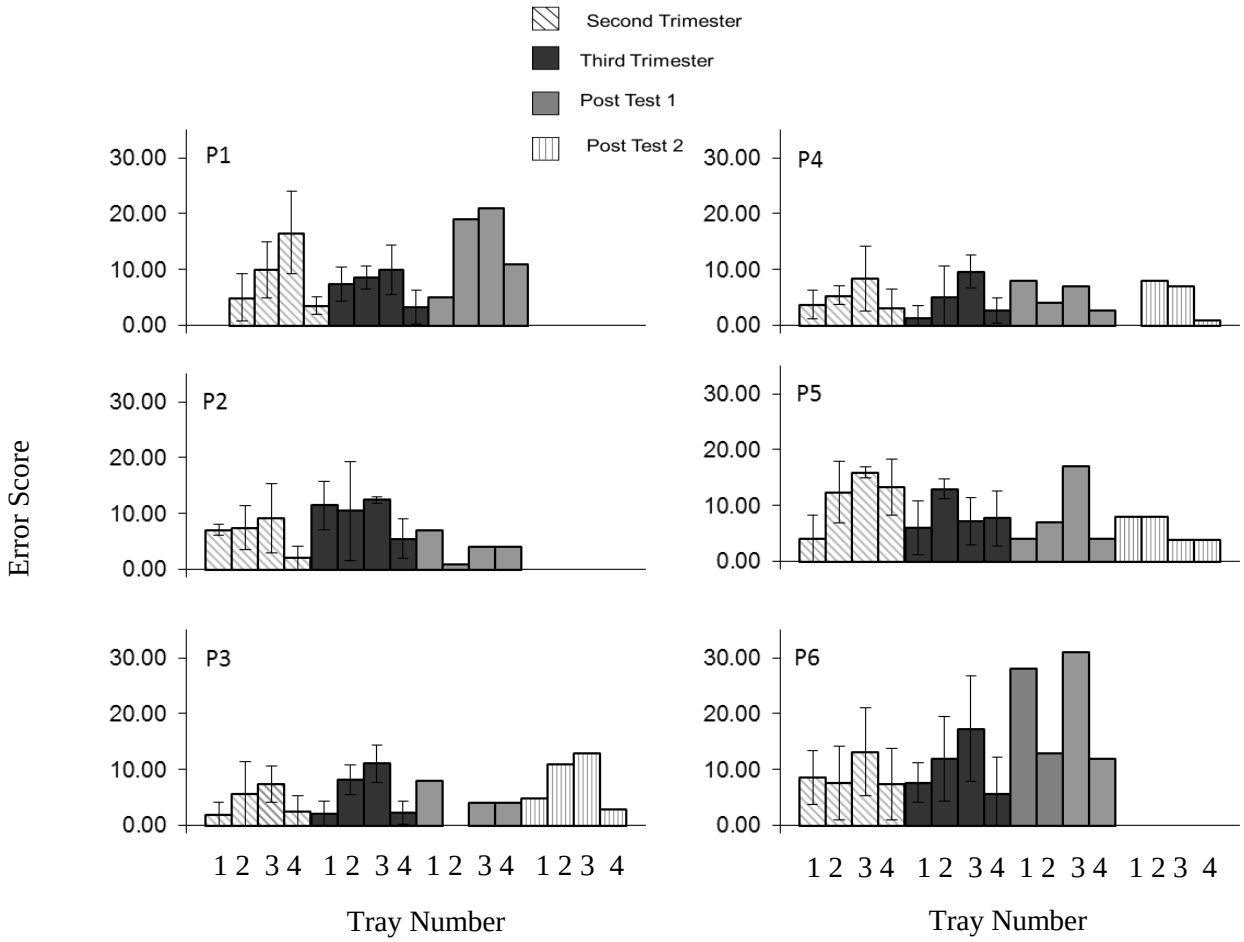

Figure 21: Mean error scores for trimesters and post-tests

Mean error scores across parity.

Figure 22 compares mean error scores across time and participants for those having their first child (P1, P3, P4, P6) and those having their second (P2) or third (P5) child. In general, those having their first child showed lower error scores on Trays 1, 2, and 4 when compared to those having their second or third child. Statistical analysis of this data showed differences between the groups on Trays 1, 2, and 4 (See Table 8) but not on Tray 3.

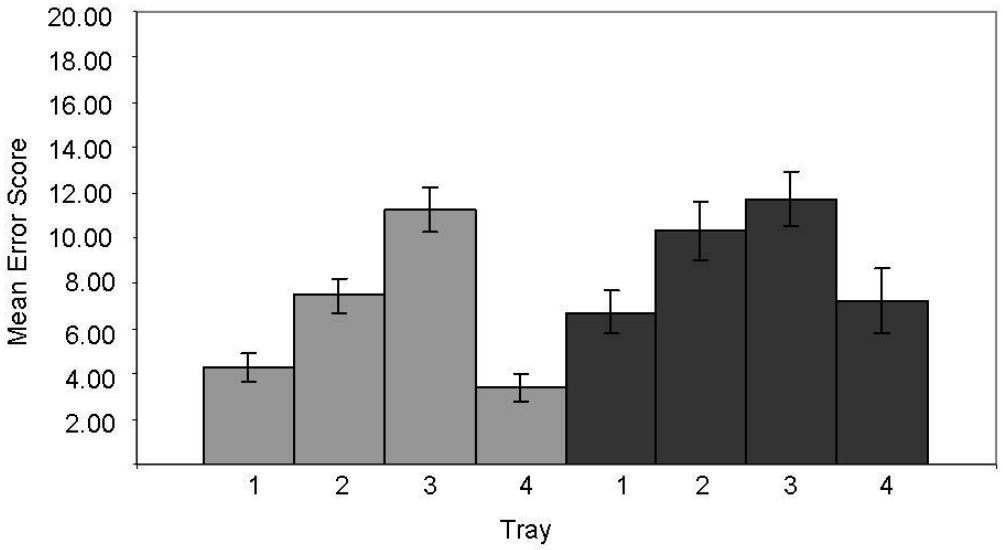

Figure 22: Mean error scores for pregnant participants having their first child vs. multiple births

Table 8: Wilcoxon Signed Ranks statistics comparing pregnant participants by parity

	Z score	P (two-tailed)
Tray 1	1.979	.048
Tray 2	2.406	.016
Tray 4	2.137	.033

Tritan Plate

Tritan plates are used to access the presence of a tritan deficiency. While the FM 100-hue test indicated that some of the participants did more poorly on the tray associated with a tritan loss, all participants, both pregnant and control, correctly identified the two shapes embedded in the color plate on every trial.

D-15 and L'Anthony D-15

The participant's cap order for the D-15 and L'anthony test is graphically illustrated by "connecting the dots" based on the order of the cap numbers. As presented in the upper left panel of Figure 23, if no errors are made in the cap placement, a smooth line is obtained between the "dots". If there are errors, the line may show positional switches (i.e., two adjacent caps are switched) as highlighted in the upper right panel of Figure 23 or crossovers (i.e., caps are placed more than one position away from their correct location) as seen in the lower panels of Figure 23. As with the FM 100-hue test, there are color deficient axes noted on the figure. The pattern of crossovers is compared to these axes to assess whether a color deficiency is present.

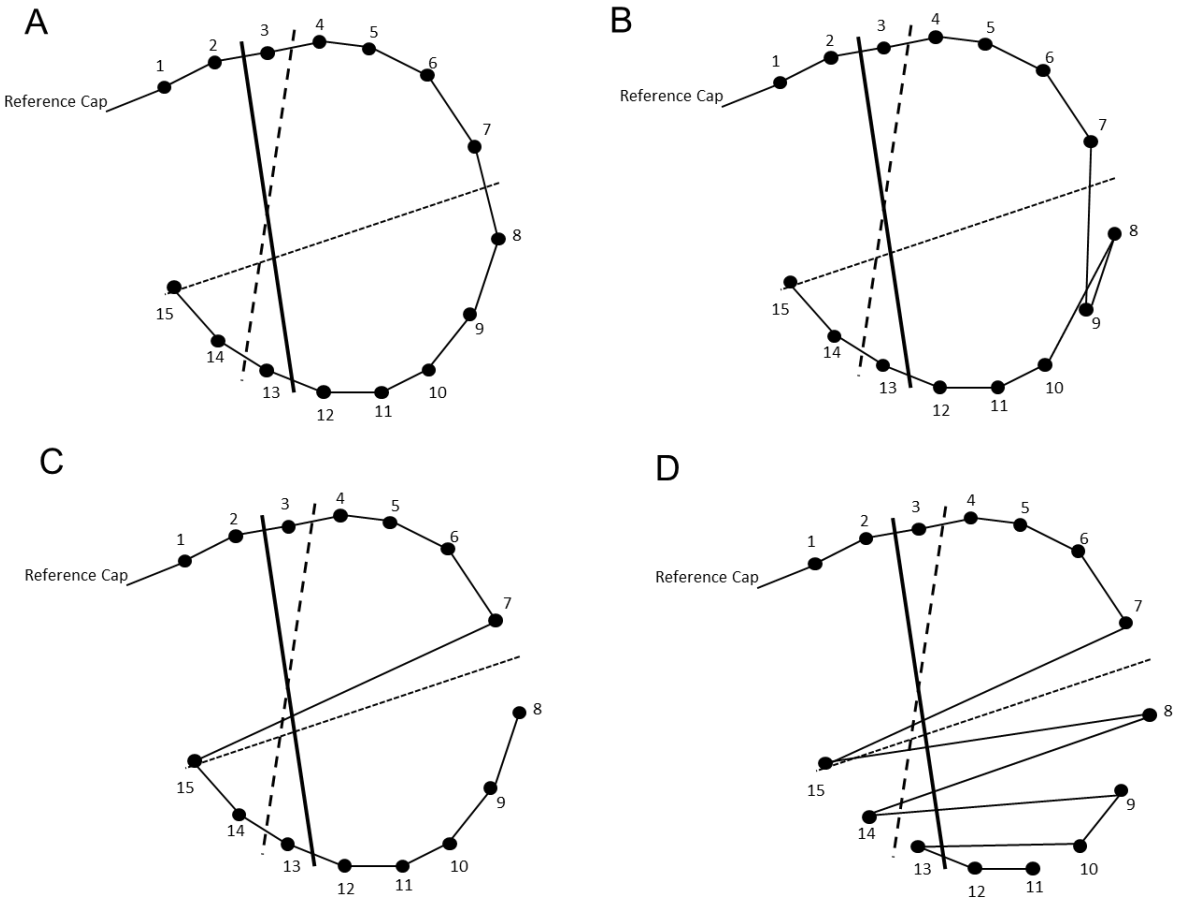

Figure 23: Examples of crossover and transpositional errors for the D-15 Panel test. The solid line indicates the protan axis; the dashed line the deutan axis; and the dotted line the tritan axis. (Modified from Vingrys & King-Smith, 1988). Figure 23 shows results from a perfect arrangement (A), minor crossover error (B), normal tritan crossing (C), and the predictable patterns of individuals with a tritan color vision deficiency (D).

None of the pregnant or control participants committed any errors on the D-15 panel test. With the L'anthony desaturated D-15 panel test, the- colored caps are less saturated, i.e., the caps have more white in them and less hue than the D-15 and FM 100-hue panel tests. Thus, this panel test is more difficult and sensitive to smaller changes in color perception.

Pregnant Participants.

Both transpositional (1 position switch) and crossover (2 position switch) errors were committed by the participants (See Table 9). P6 had the most errors, committing at least one

error in four out of nine sessions. The mean number of total errors for pregnant participants was M=0.35 (SD= 0.86). Scores ranged from three (3) errors in one session to no errors. All pregnant participants made at least one error during the course of their testing. The magnitude of the errors committed on the D-15 desaturated panel test basically indicated little, if any, color vision loss with pregnancy.

Control Participants.

The mean number of total errors for control participants was M=0.26 (SD= 0.58). Scores ranged from two (2) errors in one session to no errors. C1 and C4 had no errors at all during the course of their testing. Only one crossover error was committed by C5 in her last session.

Table 9: Mean errors over sessions for all participants on the L'anthony Desaturated D-15 panel test

	Transpositional	Crossover
P1	.10	0
P2	0	.20
P3	.36	.14
P4	.18	0
P5	.14	0
P6	.67	.11
C1	0	0
C3	.5	0
C4	0	0
C5	.14	.14
C6	.25	0
C7	.4	0

Anomaloscope

The anomaloscope is used to assess the presence of very minor to very major red/green (R/G) color deficiencies (i.e., protan or deutan losses in color perception). Although the

prediction was that the color loss observed with pregnant women would be a yellow/blue (Y/B) deficiency (i.e., a tritan deficiency), the anomaloscope was used to ascertain if there were any modest changes in R/G discrimination that would not be detected by the various panel tests (e.g., FM 100-Hue, D-15, and Desat D-15). In figures 24 and 25, the midpoint range of the match is plotted as a function of time. The midpoint range represents the ratio of red to green light needed to match a yellow field. This midpoint range is then compared to that of known color normal. The error bars represent the range of red/green ratios used to match the yellow light. Most individuals can match a subset of red/green ratios to a yellow field; and for a color normal, this range of ratio values is very small. Red/green ratio values can range from 0 (completely green field) to 70 (completely red field). The midpoint range for the color normal on the instrument used in this study was approximately 50±3. The midpoint range values for the pregnant women all fell within the color normal range. Thus, neither the control participants nor the pregnant participants showed any protan or deutan losses with pregnancy. As linear regression reveals in Figures 24 & 25, there was also no systematic change in midpoints with weeks of pregnancy (Figure 24) and across test sessions for control participants (Figure 25).

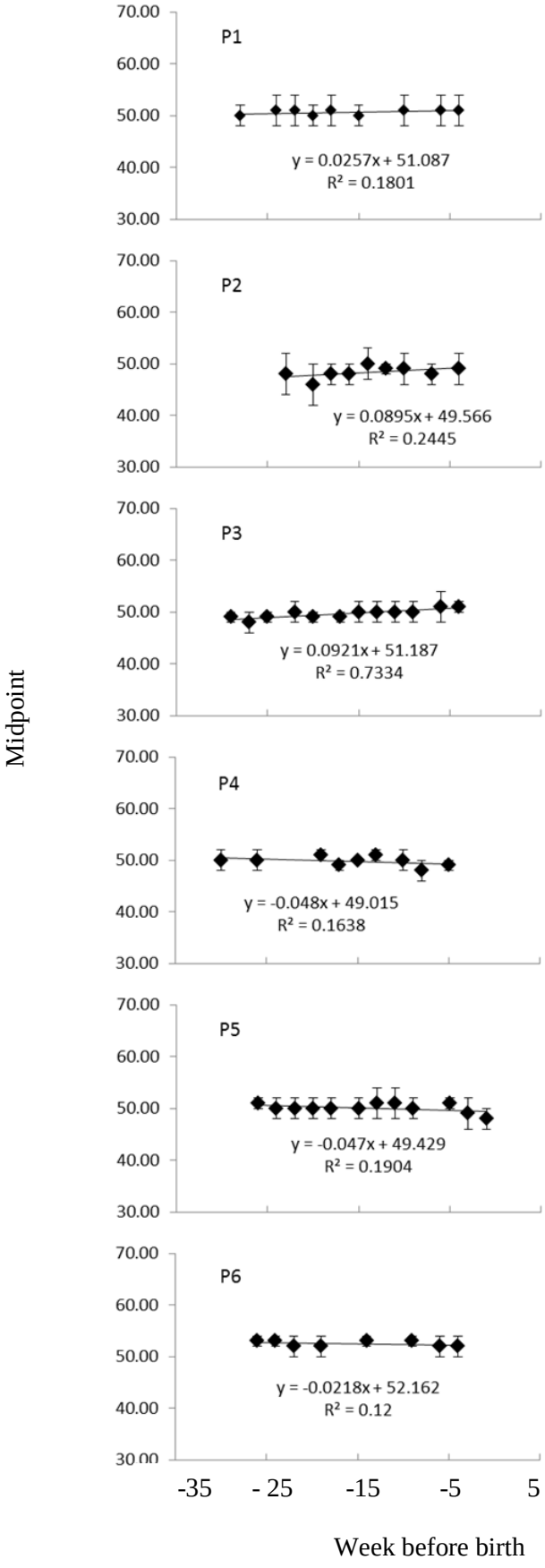

Figure 24: Pregnant participants anomaloscope scores over time

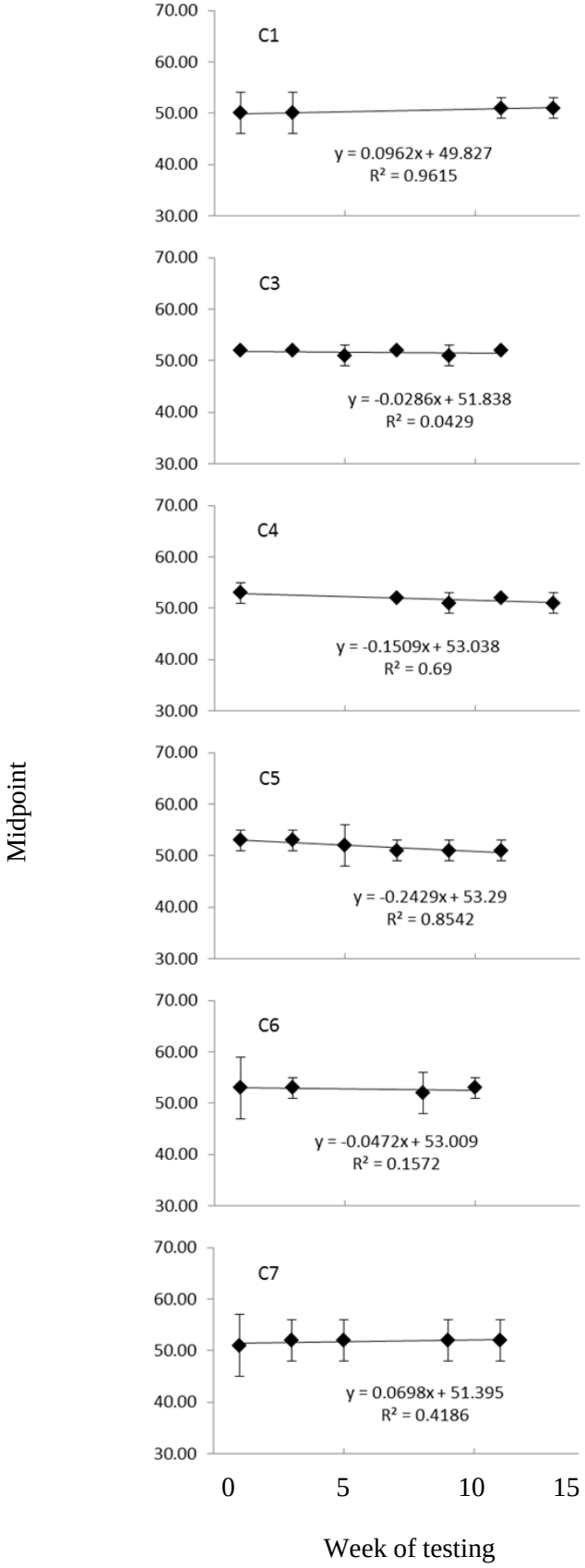

Figure 25: Control participant anomaloscope scores over time

Bailey-Lovie Acuity Chart

Snellen acuity was obtained from the Bailey-Lovie eye charts. Acuity is judged in relation to what an emmetrope, a person requiring no refractive correction, perceives. If acuity is 20/50, it means the person being tested sees at 20 feet what an emmetrope sees at 50 feet. If a person has vision comparable to an emmetrope then the acuity measure is 20/20; and if a person has vision better than an emmetrope then the second value in the measure will be less than 20.

Both high (black letters on a white background) and low contrast (dark gray letters on a light gray background) charts were used in order to determine if there were changes in acuity with pregnancy. As Tables 10 & 12 show, pregnant participants showed slightly higher scores than control participants, with a mean acuity of 20/31 on the high contrast and 20/44 on the low contrast, versus a mean acuity of 20/27 on the high contrast and 20/37 on the low contrast for control participants. Measures obtained on the Bailey-Lovie with the pregnant women after delivery (Table 11) revealed some improvement in acuity, with a mean acuity of 20/29 with the high contrast chart and 20/43 on the low contrast chart .

Table 10: Mean Acuity Scores for Pregnant Participants During Pregnancy

Participant	High Contrast	Low Contrast
P1	23	38
P2	47	67
P3	30	42
P4	51	65
P5	20	27
P6	18	29

Table 11: Mean Acuity Scores for Pregnant Participants After Pregnancy

Participant	High Contrast	Low Contrast
P1	25	40
P2	50	63
P3	30	41
P4	48	65
P5	20	27
P6	16	32

Table 12: Mean Acuity Scores for Control Participants

Participant	High Contrast	Low Contrast
C1	20	25
C3	29	46
C4	24	32
C5	20	29
C6	29	28
C7	42	51

Figures 26 and 27 present Snellen acuity measures for each pregnant and control participant over time, respectively. Tables 13 & 14 specify the slope, intercept, and R^2 values for the linear regression fitted to the data. P2 complained of acuity problems in her previous pregnancy, and as shown in Figure 26 her acuity decreased over the course of pregnancy. The other pregnant participants showed minimal change in acuity over weeks of pregnancy. Some of the control participants showed a decrease in acuity with time (e.g. C3, C4, & C5).

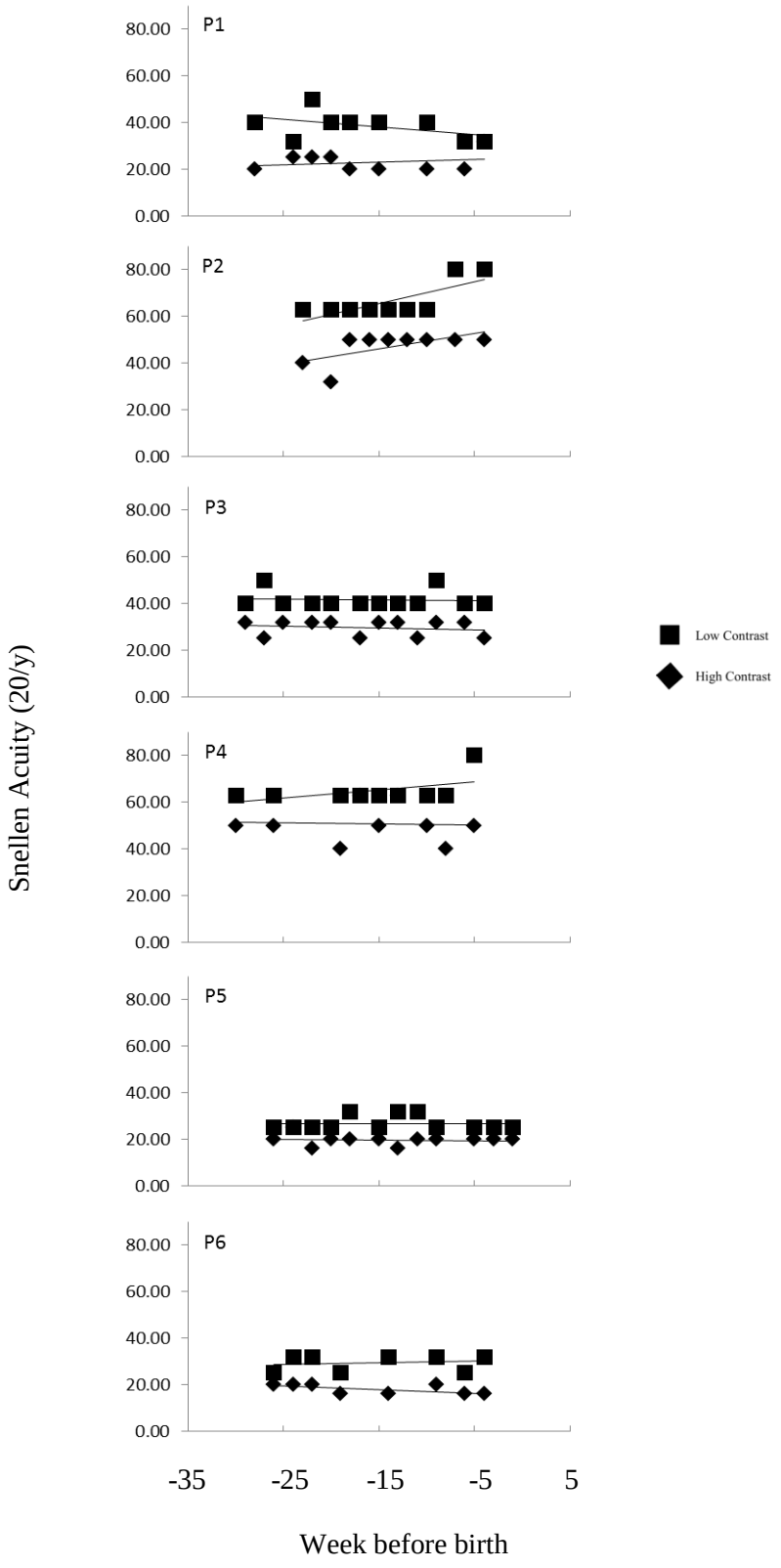

Figure 26: Pregnant participant Bailey-Lovie scores over time

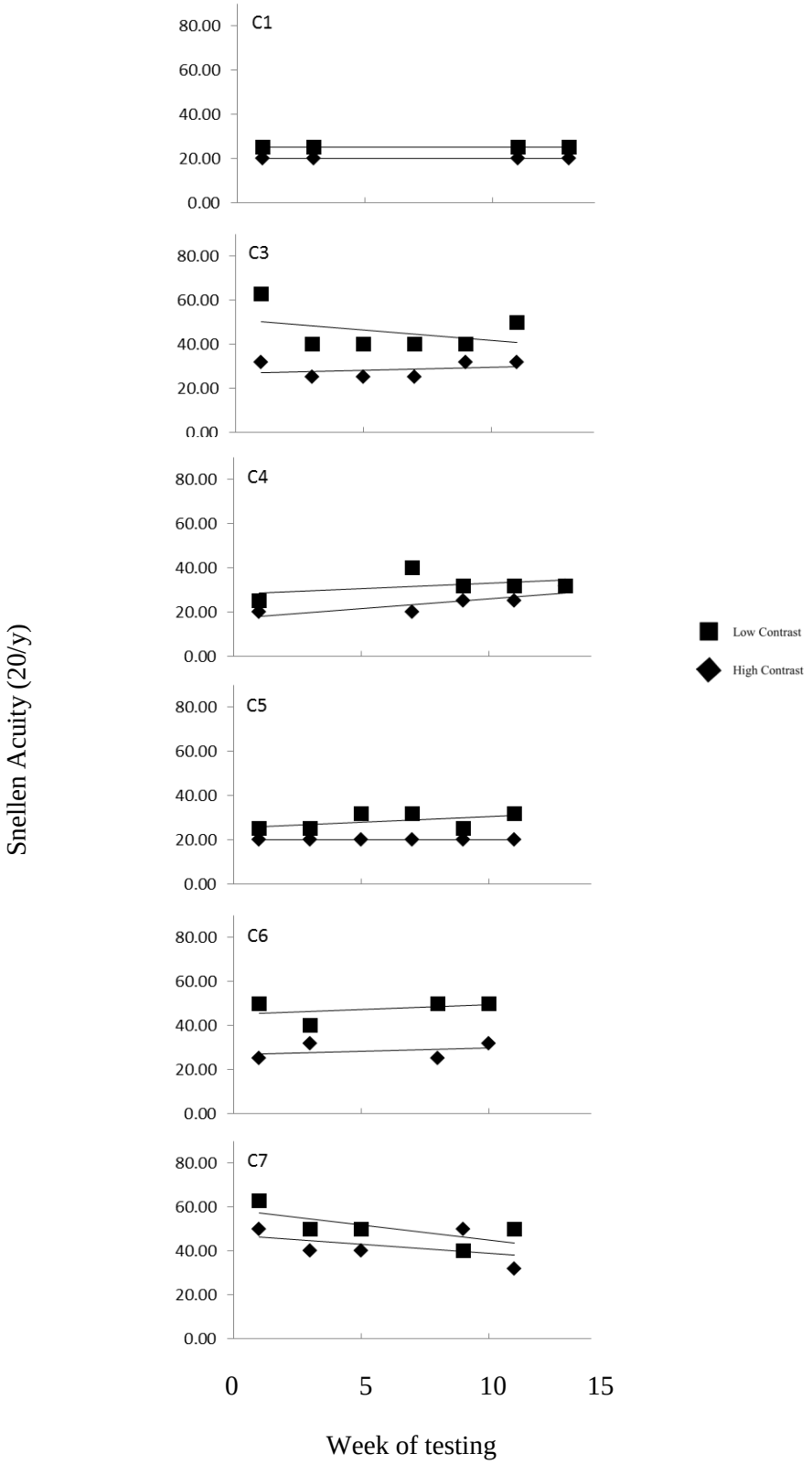

Figure 27: Control participant Bailey-Lovie scores over time

Table 13: Regression values for acuity across time for pregnant participants

	Slope	Intercept	R^2
P1 High Contrast	0.325	33.140	0.212
P1 Low Contrast	0.116	24.892	0.053
P2 High Contrast	0.668	56.097	0.405
P2 Low Contrast	0.921	79.468	0.577
P3 High Contrast	-0.065	28.587	0.025
P3 Low Contrast	-0.040	41.006	0.007
P4 High Contrast	-0.046	49.937	0.002
P4 Low Contrast	0.345	70.367	0.248
P5 High Contrast	-0.028	19.355	0.011
P5 Low Contrast	0.002	26.718	0.000
P6 High Contrast	-0.151	15.663	0.358
P6 Low Contrast	0.063	30.344	0.021

Table 14: Regression values for acuity across time for control participants

	Slope	Intercept	R^2
C1 High Contrast	0.000	20.000	N/A
C1 Low Contrast	0.000	25.000	N/A
C3 High Contrast	0.300	26.700	0.086
C3 Low Contrast	0.929	51.071	0.135
C4 High Contrast	0.892	17.090	0.693
C4 Low Contrast	0.481	28.255	0.174
C5 High Contrast	0.000	20.000	N/A
C5 Low Contrast	0.500	25.500	0.238
C6 High Contrast	0.264	27.047	0.076
C6 Low Contrast	0.472	44.906	0.157
C7 High Contrast	-0.837	47.256	0.205
C7 Low Contrast	-1.372	58.558	0.485

Wilcoxon Signed Rank Sum tests did not show any significant differences between control and pregnant participants on the either the low contrast ($Z = .529$, $p = .597$) or the high contrast ($Z = 1.258$, $p = .208$) charts.

Chapter 4: Discussion

The present research examined whether known changes in endogenous levels of steroid hormones (e.g., estrogen and progesterone) with pregnancy affect hue perception. Past studies (Marre et al., 1974; Lakowski & Morton, 1977, 1978; Fine & McCord, 1991; Eisner et al. 2004 a,b; Eisner & Incognito, 2005) have reported changes in color perception in women who have experienced exogenous changes in hormone levels with oral contraceptives and Tamoxifen. These losses in color perception with exogenous hormones are specific to shorter wavelengths, suggesting that the yellow/blue (Y/B) opponent process is affected more by hormone changes than the red/green (R/G). Based on these previous results, it was predicted that endogenous changes in hormone levels with pregnancy would produce similar changes in color perception, in particular tritan deficits would increase as hormone levels increased with pregnancy (Melamud et al, 2004).

Summary of Results

Overall there were no statistically significant differences observed between the pregnant and control participants on any of the tests. Upon examining the error patterns of the FM 100-hue test over time, four of the pregnant participants did show an increases in error scores on Tray 3, indicative of a tritan loss (see Figures 9-11 and 13). The control participants did not show this pattern across sessions (see Figures 14-19). In general, the errors on Tray 3 were higher than errors on the other trays of the FM 100-hue test for the pregnant women (see Figure 4). This was not necessarily observed for the control group (see Figure 5). These increases in error scores were observed over weeks of pregnancy. The pregnant women performed slightly worse on the desaturated D-15 panel test and the Bailey-Acuity tests than the control participants, but the differences was not statistically significant. As expected, neither the pregnant nor the control

participants showed a red/green loss with their anomaloscopic matches (see Figures 24 & 25). Although the tests were given on repeated days, in general, the pregnant participants did not show evidence of a practice effect. Since participants in the control group made few errors thereby creating a floor effect, practice effects were practically non-existent across the diagnostic tests administered (see Figures 14-19, 25, and 27).

Neuroprotective effects of steroid hormones

Since the inception of this experiment there has been a growing body of research into the function of hormones. It has become clear that hormones may perform several functions. For example, hormones have traditionally been shown to engage in classical genomic functioning, which involves receptors entering the nucleus of the cell and regulating gene transcription through an intracellular signaling cascade (Yamamoto, 1985). This is a fairly slow process, and the effects are not seen immediately. More recent research, however, has revealed that hormones may produce faster non-genomic effects that do not involve gene regulation (Chaban et al., 2004; Razandi et al., 2004). A pool of estrogen receptors has been identified at the plasma membrane that can cause rapid effects via second messenger systems. These receptors are not isolated only to cortical brain areas but are also found in the spinal cord and peripheral nervous system (Melcangi et al., 2006). If the receptors for these hormones are located in the nervous system, then presumably they play a part in the functioning of the nervous system.

It has also been demonstrated that hormones are not only synthesized in the adrenals and gonads but also within the central and peripheral nervous systems as well (Melcangi et al., 2006). Proteins involved in steroid synthesis have been localized in the brain, spinal cord, and peripheral nervous system (Melcangi et al., 2006). If steroids are synthesized in the nervous system as well as the endocrine system, then there could be different derivatives of typical

steroid hormones that may produce different physiological effects than the "traditional" steroid hormones.

In addition, several studies have shown pleiotropic, or neuroprotective, effects of progesterone and estrogen in the nervous system (Gonzalez, Labombarda, Gonzalez Deniselle, Guennoun, Schumacher, & De Nicola, 2004). In particular, progesterone increases neurological function after contusion in rats (Thomas, Nockels, Pan, Shaffrey, & Chopp, 1999) and increases survival of ventral motoneurons in Wobbler mice (a mutant strain of mice that mimic human hereditary motor system disease) (Gonzalez Deniselle, Lopez-Costa, Saavedra, Piatrenera, Gonzalez, Garay, Guennon, Schumacher & Denicola, 2002). Furthermore, it attenuates neural loss of rat facial motoneurons after axotomy (Yu, 1989) and acts as a neuroprotective agent in male rats after ischemia (Jiang, Chopp, Stein, & Feldblum, 1996).

In comparison, estradiol protects oligodendrocytes from cytotoxicity in vitro (Takao, Flint, Lee, Ying, Merrill, & Chandross, 2004) and is beneficial in the function and viability of neurons, possibly influencing learning and memory (Garcia-Segura, Azcoita, & Doncarlos, 2001; McEwen, 2001). Estradiol also promotes neurogenesis in the adult hippocampal form (Gould, Tanapat, Rydel, & Hastings, 2000) and reduces edema following stroke (Stein et al., 2008).

The study of neuroprotective effects has been extended to the study of multiple sclerosis (MS) in women, since women are more susceptible to inflammatory autoimmune diseases. Often females with MS show improvement of symptoms during pregnancy, especially during the third trimester, with a temporary increase of severity of symptoms postpartum (Confavreaux, Hutchinson, Hours, Cortinovis-Tourniaire, & Moreau, 1998). Estriol is a derivative of estrogen that is present in much higher levels during pregnancy than at any other time during a normal menstrual cycle. A study by Gold and Voskuhl (2009) examined the effects of estriol use in

women with both relapsing/remitting (RRMS) and secondary progressive MS (SPMS). Improvements were shown in the RRMS group with the number of lesions returning to pretreatment levels when the estriol treatment was terminated.

These findings challenge the idea that increased levels of estrogen and progesterone impair functioning in the nervous system, including the visual system. If these hormones are neuroprotective, the increased levels during pregnancy may not be deleterious to nervous system functioning (including the visual system) and arguably may be beneficial.

The idea that steroid hormones may provide neuroprotective effects is supported by behavioral research in non-pregnant women. Giuffre et al. (2005) found that performance on the FM 100-Hue test actually improved for 10 out of 15 participants at the time of ovulation when estrogen levels are highest, as opposed to during menstruation or at the beginning of the cycle. Flaherty et al. (1988) reported an increase in the magnitude of the fixation effect (i.e., changes in size perception of an object after viewing another object) with higher estrogen levels, indicating increased performance on the test. Yilmaz et al. (1998) noted an increase in latency and a decrease in amplitude of the pattern reversal evoked potentials (PRVEPs) during the menstrual phase and decreased latency and increased amplitude during the ovulatory phase. These studies suggest that increased levels of estrogen lead to increases in the sensitivity of the visual system, and, thus suggest that perhaps color vision is unaffected by fluctuating hormones during pregnancy. This may explain the lack of a significant difference in the performance of pregnant women and control women in the current study.

Physiological Issues

Endogenous vs. exogenous hormones.

The hypothesis of this study was based on experimental results showing color vision deficits in women with changes in hormones (Marre et al., 1974; Lakowski & Morton, 1977, 1978; Fine & McCord, 1991; Eisner et al, 2004a; Eisner & Incognito, 2005). Unlike my study, these studies altered endogenous hormone levels using exogenous sources such as oral contraceptives and SERMs (Marre et al., 1974; Lakowski & Morton 1977, 1978; Eisner et al, 2004a; Eisner & Incognito, 2005, & Fine & McCord, 1991). The question arises whether synthetic steroid derivatives used to modify hormone levels affect the nervous system in the same manner as the endogenous increase in estrogen and progesterone associated with pregnancy. It is possible that synthetic hormones affect the nervous system differently than endogenous hormones and could account for the results found in this study between pregnant and non-pregnant women.

Additionally, hormonal changes with pregnancy are gradual while initial use of an oral contraceptive or SERM can produce an acute change in hormonal profiles. It is possible that the vision defects reported in earlier studies (Marre et al., 1974; Lakowski & Morton, 1977, 1978; Fine & McCord, 1991) with oral contraceptives could have been produced by massive amounts of hormones abruptly entering the nervous system. In contrast, with pregnancy, the visual system may adapt to the gradual changes in hormonal levels associated with pregnancy; thereby, allowing the visual system to adjust and compensate for changes in hormonal levels and reset its normal acceptable baseline level. If this rationale completely explains our findings, then there should not have been a change in error scores with the FM 100-hue test during the entire

pregnancy. This was not necessarily the case; some of the pregnant participants of this study showed an increase in error scores with duration of pregnancy (see Figures 8-13 and 26).

Other side effects of oral contraceptives.

Oral contraceptives affect not only the endocrine system but the vascular and nervous systems as well. For example, the side effects of thrombosis and stroke are well documented, especially in aging women and smokers (Farley, Collins, & Schlesselman, 1998). The color vision deficits reported in earlier studies could be a result of vascular problems in oral contraceptive users.

SERMS.

SERMs are selective estrogen receptor modulators, and whether they exhibit agonistic or antagonistic reactions in the retina is yet to be determined. At this time the effect on SERMs is not known. Also, in the vision studies (Eisner et al., 2004a; Eisner & Incognito, 2005) with Tamoxifen the participants have or have had breast cancer. While breast cancer may not directly affect color perception, it is possible that past treatments for cancer, such as radiation or chemotherapy, may have affected the visual pathway or retina Thus, changes in color vision may not be due to hormonal changes associated with SERMs (Hazin, Abuzetun, Daoud, & Abu-Khalaf, 2009; Eisner et al., 2004a; Eisner & Incognito, 2005), but to damage in the visual pathway from the radiation and chemotherapy.

Age.

Ogueta and colleagues (1999) found differences in gene expression of ERα in individuals of different sex and age. In particular, RT-PCR studies showed differing levels of ERα between women still experiencing estrous cycles and those who were not (Ogueta et al., 1999). Those who still had estrous cycles showed more mRNA than those who did not. Male retinas had

levels of mRNA that fell between old and young women, with no change in male mRNA levels with age.

Differences in rates of ocular pathology, such as age-related cataract, closed-angle glaucoma, and idiopathic macular holes have been seen in individuals of different sex and age (Gupta et al., 2005). Women who have gone through menopause (resulting in lower levels of endogenous hormones) tend to show higher levels of these disorders.

Age may have also been a factor in a second body of research by Eisner and colleagues (Eisner et al., 2004a, & Eisner & Incognito, 2005) that examined peri- to post-menopausal women using SERMs, where the levels of endogenous progesterone and estrogen are significantly lower in this group than in the pregnant participants of this study. The studies from this research group (Eisner et al., 2004a; Eisner & Incognito, 2005) showed deleterious SERM effects on color vision, such as decreased sensitivity in the short-wavelength-sensitive (S) pathway and problems with correctly identifying short-wavelength stimuli with color naming.

Methodological issues in past and current studies

Time in Menstrual Cycle.

There were several problems with previous studies (Marre et al., 1974; Lakowski & Morton, 1977 , 1978; Fine & McCord, 1991) investigating the relationship between estrogen dosage in oral contraceptives and color vision. The first is that none of these studies mentioned at which point in the menstrual cycle the color vision testing was conducted. It is possible that the acquired deficits are more pronounced at a particular time in the administration of the pill. If so, that could explain why there was a wide range of color vision deficits among women taking the same pill. Our study suffered from the same problem in that we did not track the menstrual cycle of the control participants or the pregnant participants after they had given birth.

Problems with color vision panel tests.

Another problem is that some of the color vision tests are not very sensitive. For example, the D-15, L'anthony Desaturated D-15, and pseudoisochromatic plate tests are for the most part "quick tests" that are used to quickly determine if a person is color normal or color deficient. Thus, these tests provide a clear delineation of color-normal individuals from severely color deficient observers but often miss those individuals with minor color vision losses (Kaiser & Boynton, 1996).

Standardization of OCs.

The types of oral contraceptives used were not always standardized within a study. For example, in the Fine & McCord (1991) study, the brand of pill was not even mentioned; so it is possible that the women were taking different types of pills, which could mean not only different levels of estrogens and progesterone, but also completely different chemical structures. Another factor is that the pills used in earlier studies such as Marre, et al. (1974) may have contained up to 150 micrograms of estrogen, while current formulations of the pill use much less (30-50 micrograms) (Oesterheld et al., 2008).

Disease and Substance Interactions.

The use of special populations (e.g., diabetics) in oral contraceptive studies also presents problems (Lakowski & Morton, 1977). Diabetes is known to negatively impact vision (Klein & Klein, 1995), in particular creating tritan losses (Lakowski & Morton,1977). These experiments are most likely measuring an interaction of diabetes with oral contraceptive use.

Other Considerations

Several other factors may have contributed to the results that were observed. For example, top-down influences such as attention and motivation may have affected the

participants' performance. The tests that were used, especially the FM 100-hue test, took a considerable amount of time and concentration. The participants' individual attention span and intrinsic motivation may have varied from session to session and even within sessions. Also, the physical testing environment may have proved to be more challenging and/or uncomfortable for the pregnant participants over time as their bodies changed due to the pregnancy. This could possibly have led to them to try to complete the tasks faster (which could increase numbers of errors), to alleviate the discomfort. Similarly, the control participants may have rushed their performance with increased sessions in order to complete the experimental sessions sooner. Future studies could incorporate extrinsic motivators, such as payments associated with accuracy, to address this concern.

Future Direction

It is doubtful that a future study using the exact same methodology would show large-scale differences between pregnant and non-pregnant women. The possibility of pregnancy actually improving color vision performance due to increased levels of pleiotropic hormones could be explored by testing many pregnant women towards the end of their pregnancy and then several times over the course of the next few years to detect if there were decreases in performance after the babies were born. Different color vision tests, such as an anomaloscope that can detect Y/B discrimination, could be utilized. In order to accurately determine increases in hormone levels, blood tests should be administered at the start of each experimental session.

Based on the literature discussed above and in Chapter 1 and the results of this study, there are several alternative approaches to investigating hormonal effects on color vision. The first would be to examine different participant pools with different hormone levels. The approach could be three-fold: to examine the contributions of increased exogenous estrogen on younger

participants, which could produce similar results to previous research in OC users; to examine the contributions of increased exogenous estrogen with hormone replacement therapy (HRT) in older participants, which could either be a benefit if the hormones act in a neuroprotective manner or be deleterious if HRT negatively affects the cardiovascular system; and to study the effects of increased endogenous estrogen (namely estriol) in populations with autoimmune disorders, such as MS to detect if increases in endogenous estrogen have effects on color perception.

The effects of abruptly increased exogenous estrogen on color perception could be examined in women who are using hormone therapy to aid in fertility. An ideal population to study would be women who choose to be egg donors. They are carefully screened for many health problems and cannot use oral contraceptives. They are given high doses of hormones to facilitate release of multiple eggs. Some may use Clomid, which is a SERM. It is possible that results from previous studies (Marre et al., 1974; Lakowski & Morton, 1977, 1978; Fine & McCord, 1991) could be replicated if indeed losses in color perception were influenced by exogenous synthetic estrogenic effects. If differences were seen between starting hormone therapy and after treatment, it would lend credence to the idea that massive fluctuations in hormonal levels from exogenous sources affect color discrimination. It could also differentiate between the effects of endogenous and exogenous estrogen on the visual system. If the results from egg donors are similar to those of oral contraceptive users, then a case could be made that exogenous and/or synthetic hormones can contribute to impaired visual function.

Another population of interest would be postmenopausal women. Even after normal ovulatory cycles end, differences exist between women who use hormone replacement therapy and those who do not. Studies have shown differences in contrast sensitivity between these two

populations: Guaschino & colleagues (2003) found improvements in contrast sensitivity in postmenopausal women using hormone replacement therapy (HRT) and impairments in women not using hormone replacement therapy. Contrast sensitivity refers to the ability to discern between different levels of contrast between two objects. For example, studies often present bars of light of two different luminances. The observer notes when they just perceive the presence of two bars of light based on a difference in luminance level. Women with hormone replacement therapy do not require as large of a difference in luminance in order to perceive two bars. Other research has shown that estrogen appears to facilitate neural transmission by decreasing neural transmission time (Yilmaz et al., 1998).

Studying this population could present challenges, since age does affect the physiology of the eye and performance on color diagnostic tests. Increased age in and of itself produces many ocular and perceptual changes, such as increased intraocular pressure (Qureshi, 1997), increased lens density which creates a reduction in short-wavelength sensitivity (Pokorny, Smith & Lutze, 1987; Shinomori, Schefrin, & Werner, 2001), senile miosis (reduction in pupil size), (Winn et al, 1994), and reduction of contrast sensitivity (Scialfa et al., 1988; Siesky et al., 2008; Owsley, Sekular, & Siemsen, 1983). These age-related changes are more prevalent in women as opposed to age-matched males (Gupta et al., 2005). Color vision changes may not be overtly prevalent until after age 60 but tend to be in the Y/B spectrum (Haegerstrom-Portnoy, Schneck & Brabyn, 1999). However, if increases in performance on color vision tests were seen (and not attributed to practice effects) in women using HRT then it may give some support to the idea that estrogen and progesterone may have neuroprotective effects.

Another population that could be studied is women with MS. Similar to the pilot study by Gold and Voskuhl (2009), it would be interesting to see if estriol may actually improve color

discrimination in women with MS. Most MS patients suffer some optic neuritis (inflammation of the ocular nerve), which causes dyschromatopsia in most patients. Y/B, R/G, and non-selective deficits have been shown, with Y/B deficits more prominent early and R/G deficits developing later (Katz, 1995; Schneck & Haegerstrom-Portnoy, 1997). If the patients' performance on color vision tests improved with estriol therapy, it would be a strong support for the neuroprotective effects of estriol. Studying the color vision of women with MS who were pregnant during and after pregnancy would also be a great opportunity to investigate the effects of endogenous estriol.

In addition to different populations, different methodologies could also be used to elucidate more subtle changes in color discrimination performance. A portable apparatus permitting measurement of the spectral sensitivities of the different cone mechanisms with chromatic adaptation, (Volbrecht, Schneck, Adams, Linfoot & Ai, 1994) could help to elucidate the underlying retinal mechanisms affected by changes in endogenous and/or exogenous hormonal levels. The Nagel anomaloscope could be used to test the severity of deutan deficiencies and the Pickford-Nicholson anomaloscope to examine for the severity of tritan deficiencies. In addition, several computer based tests have been developed that can diagnose color vision deficiencies and the severity of the deficiency. The Color Assessment and Diagnosis (CAD) test (Barbur, Harlow & Plant, 1994). and the Cone Contrast test (CCT) (Rabin, Gooch & Ivan, 2011) could be used separately or in conjunction to specify the differences within and between the target populations.

Conclusion

The current study did not find any significant or systematic differences in color vision performance between pregnant women and a control group of women not using oral

contraceptives. Despite the lack of statistically significant differences, this study has opened up a myriad of possibilities for future research examining the relationship of hormones and neurosteroids on color vision. Different methodologies offer the possibility of detecting more subtle variations in color perception while studying distinct populations with different hormonal profiles could bring to light a small part of the puzzle of how both exogenous and endogenous hormones affect visual perception.

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Acquiring Your Dream Diamond

http://www.vandiamond.com/Articles/DreamDiamond.html

A new web-based colour vision test

http://www.city.ac.uk/health/research/research-areas/optometry/a-new-web-based-colour-vision-test

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Appendix A1

Participant Number: _____

Pregnancy and color vision questionnaire

Please note that all answers to these questions will be kept confidential with the rest of your data.

1. Birthdate (mm/dd/yy) ___/____/____
2. How many weeks along are you? _____
3. When is your due date? _____
4. Is this your first pregnancy? _____
5. Please list any medications you are currently taking, including prenatal vitamins.

_____

_____

_____

_____

6. Do you have any pre-pregnancy medical conditions such as diabetes or hypertension?

_____

_____

_____

7. Do you have any medical conditions that have arisen since your pregnancy (e.g. diabetes, anemia, hypertension)?

_____

_____

_____

8. Were you a smoker prior to your pregnancy? _____
9. Are you a smoker currently? _____

10. Do you regularly consume caffeine? If yes, please estimate how much you drink per day (example: 2 cups of tea, 1 diet soda, etc.)

11. Do you have any history of past neurological or psychological conditions? If yes, please explain.

12. Do you wear contacts or glasses (or neither)? _____

13. Does anyone in your family have a history of colorblindness or color vision defects? If yes, what relation are they to you and what is the nature of the deficit?

14. Have you noticed any changes in your vision since you have become pregnant? If so, please list them below.

All of the information provided above will be kept confidential with the rest of your data. We will be using a linked list until your testing sessions are complete. After the tests are complete, there will be no information on this page linking you with the data obtained.

Appendix B

Participant Number: _____

Color vision questionnaire

Please note that all answers to these questions will be kept confidential with the rest of your data.

1. Birthdate (mm/dd/yy) ___/____/____

2. Please list any medications you are currently taking, including vitamins.

_____

_____

_____

_____

3. Do you have any medical conditions such as diabetes or hypertension?

_____

_____

_____

_____

4. Have you ever been a smoker? _____

5. Are you a smoker currently? _____

6. Do you regularly consume caffeine? If yes, please estimate how much you drink per day (example: 2 cups of tea, 1 diet soda, etc.)

_____

_____

_____

7. Do you have any history of past neurological or psychological conditions? If yes, please explain.

8. Do you wear contacts or glasses (or neither)? _____

9. Does anyone in your family have a history of colorblindness or color vision defects? If yes, what relation are they to you and what is the nature of the deficit?

All of the information provided above will be kept confidential with the rest of your data. We will be using a linked list until your testing sessions are complete. After the tests are complete, there will be no information on this page linking you with the data obtained.