# An Examination of the Interaction Between Hormonal Contraceptive Use, Behaviour, and Psychology

by

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#### Abstract

The objective of the following studies was to investigate the associations of hormonal contraceptives (HC) with several psychological, behavioural, and hormonal variables. Previous research has suggested that HCs may be linked with a wide variety of changes in women, including increases in borderline-like symptomology, altered partner preferences, and blunted stress responses. In the current studies, I examine differences between HC users, naturally cycling (NC) women with either low or high estrogen depending on their phase in their menstrual cycle, and intrauterine device (IUD) users. I found little support for the hypothesis that HC use increases borderline-like symptoms, and no change in partner preference between HC users, and salivary cortisol levels. I also found several differences in psychological traits between HC users and IUD users. Limitations of the current studies are discussed and directions for future research are considered.

# Preface

This thesis is an original work by Taylor Blake Irvine. The research project, of which this thesis is a part, received research ethics approval from the University of Alberta Research Ethics Board, project name "(REN) Personality and Genetic Polymorphism in Humans", project number Pro00015728, approved on August 27<sup>th</sup>, 2010; project name "Investigating the Relationship Between Hormonal Contraceptive Use and Borderline Personality, Empathy and Oxytocin Level", project number Pro00078461, approved on January 25<sup>th</sup>, 2018; project name "Examining Differences in Personality and Male Attractiveness Ratings Between Hormonal Contraceptive Users and Non-Users", project number Pro00085370, approved on September 26<sup>th</sup>, 2018; and project name "Examining Stress and Cortisol Reactivity in Hormonal Contraceptive Users and Non-Users", project number Pro00085688, approved on January 29<sup>th</sup>, 2019.

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# **Table of Contents**

Abstract	ii
Preface	iii
Acknowledgements	iv
Table of Contents	v
List of Tables	vii
List of Figures	viii
<ul> <li>Chapter 1: Introduction</li> <li>The Menstrual Cycle</li> <li>Hormonal Contraceptives</li> <li>Hormonal Contraceptives and Psychobehavioural Effects</li> <li>Hormones and Partner Preference</li> <li>Oxytocin</li> <li>Cortisol</li> <li>Intrauterine Devices</li> <li>Current Study</li> <li>References</li> </ul>	1 2 3 4 5 6 7 8 9 11
Chapter 2: Investigating the Relationship Between Hormonal Contraceptive Use and Borderline Personality, Empathy and Oxytocin Level Introduction Methods Results Discussion References	19 19 22 28 31 35
Chapter 3: Re-Examining the Influence of Hormonal Contraceptives on Partner Preference Introduction Methods Results Discussion References	48 48 50 53 54 58

Chapter 4: Association Between Stress Reactivity and Hormonal Contraceptive Use	65
Introduction	65
Methods	67
Results	71
Discussion	73
References	77
Chapter 5: A Comparison of Naturally Cycling Women, Combination Pill Users, and	
Intrauterine Device Users	87
Introduction	87
Methods	89
Results	93
Discussion	94
References	96
Chapter 6: Discussion	104
Summary of the Results	104
Conclusions	107
References	111
Bibliography	115

# List of Tables

Table 2–1. T-tests between hormonal contraceptive (HC) users and naturally cycling (NC) women.	40
Table 2–2. T-tests between hormonal contraceptive (HC) users, participants with low estrogen (Low E), and participants with high estrogen (High E) at time of data collection.	43
Table 2–3. Principal components 1, 2, and 3 loadings across each variable.	46
Table 3–1. T-tests between combination pill users and naturally cycling (NC) women.	63
Table 4–1. T-tests between combination pill users and naturally cycling (NC) women.	81
Table 4–2. Principal components 1 and 2 loadings across each variable for hormonal contraceptive (HC) users.	84
Table 4–3. Principal components 1, 2, and 3 loadings across each variable for naturally cycling (NC) women.	84
Table 5–1. United Nations data on the estimates of contraceptive prevalence by method among married or in-union women aged 15 to 49 (percentage) in 2015.	99
Table 5–2. T-tests between contraceptive users (CU; combination pill and intrauterine device users together) and naturally cycling (NC) women	100
Table 5–3. T-tests between combination pill (CP) users and intrauterine device (IUD) users.	102

# List of Figures

Figure 2–1. Example of Reading the Mind in the Eyes Test (RMET).	39
Figure 2–2. Log transformed salivary oxytocin levels (pg/mL) in naturally cycling (NC) women and hormonal contraceptive (HC) users.	41
Figure 2–3. Representation of MANOVA where empathy (EQ), happiness (OHQ), neuroticism (NEO-PI N3), borderline symptoms (BSL-23 and BSL-Supplement), and salivary oxytocin levels (log transformed) are dependent variables and HC use (HC and NC) is the independent variable in the model	42
Figure 2–4. Log transformed salivary oxytocin levels (pg/mL) in hormonal contraceptive (HC) users, participants with high estrogen, and participants with low estrogen at time of data collection.	44
Figure 2–5. Representation of MANOVA where empathy (EQ), happiness (OHQ), neuroticism (NEO-PI N3), borderline symptoms (BSL-23 and BSL-Supplement), and salivary oxytocin levels (log transformed) are dependent variables and estrogen level (High E, Low E, and HC) is the independent variable in the model.	45
Figure 2–6. Visualization of a correlation matrix between principal components (PC) and variables in the model.	47
Figure 3–1. Example of the Facial Preference Task.	62
Figure 3–2. Differences between hormonal (HC) users and naturally cycling (NC) women on the Trait Preference Survey (TPS).	64
Figure 4–1. Salivary cortisol levels in hormonal contraceptive (HC) users and naturally cycling (NC) women at baseline and after the mental arithmetic task.	82
Figure 4–2. Hormonal contraceptive (HC) use and salivary cortisol level interact and influence subjective stress levels ( $\Delta VAS$ ).	83
Figure 4–3. Visualization of a correlation matrix between principal components (PC) and variables in the model in hormonal contraceptive (HC) users only.	85
Figure 4–4. Visualization of a correlation matrix between principal components (PC) and variables in the model in naturally cycling (NC) women only.	86

#### **Chapter 1: Introduction**

Hormonal contraceptive (HC) usage has increased steadily since its first introduction in the early 1960's (Chiappori, & Oreffice, 2008). HCs are the most commonly used method of birth control in the United States (Mosher & Jones, 2010), and more than 100 million women use HCs worldwide (Christin-Maitre, 2013). Between the years 2006 to 2008, the number of women in the US who had used oral contraceptive pills at some point in their lives was 82% (Mosher & Jones, 2010). HC use is not limited solely to developed nations, as high proportions of HC users can be found in emerging and developing nations (United Nations, 2015). Given the popularity of HCs, any potentially relevant consequences that HC use carries – such as effects on mate choice, behaviour, and psychology – need to be considered, as the widespread use of HCs affect millions of women globally and over several generations.

Since hormonal fluctuations occur continually across the menstrual cycle, it is imperative for researchers to take menstrual cycle phase into account when collecting data from female participants. Previously, the hormonal fluctuations that accompany the menstrual cycle had led many researchers to exclude females from studies, and the need for females to be included in research has only recently been highlighted (Taylor et al., 2000). Furthermore, similarly to how an increased understanding on hormonal fluctuations would help advance research, an understanding that a large proportion of women currently do not have cyclical variations in sex hormones – due to HC, menopause, or other possible reasons – is vital. Along with recognition of how cyclic variations, and a lack thereof, influence researchers' understanding of females, we should also seek to understand how HCs influence women in their day-to-day lives in terms of psychology and behaviour.

# The Menstrual Cycle

The menstrual cycle consists of two phases: the follicular phase and the luteal phase. Both phases can be further divided into early, mid, and late periods. The early follicular phase begins on the first day of the cycle and it is also the first day of menstrual bleeding. During the early follicular phase (days 1 to 5 of a typical 28-day menstrual cycle), when estradiol and progesterone levels are low, the anterior pituitary gland begins to release the follicle-stimulating hormone (FSH), which triggers the growth of ovarian follicles (Reilly, 2000). In the mid follicular phase (days 6 to 10 of cycle), the ovarian follicles begin to mature and release increasing amounts of estradiol. Estradiol peaks by the late follicular phase (days 11 to 13 of cycle) as the dominant ovarian follicle reaches maturity (Reilly, 2000).

Ovulation occurs in the late follicular phase when serum estradiol concentrations are at their peak and when luteinizing hormone (LH) is released from the anterior pituitary. The release of LH from the anterior pituitary stimulates the release of the secondary oocyte (i.e., an immature ovum) from the follicle, which is the process known as ovulation. Ovulation persists for 16 to 32 hours, and occurs approximately 13 to 14 days into the cycle. The time surrounding ovulation is associated with maximal likelihood of conception (Wilcox, Dunson, & Baird, 2000).

The luteal phase begins once ovulation has ceased. During the early luteal phase (the first five days after ovulation; days 15 to 19 of cycle), the ruptured follicle closes and forms a body known as the corpus luteum, which will then produce increasing amounts of progesterone. The mid luteal phase begins approximately five to six days following ovulation (days 20 to 24 of cycle) and is characterized by elevated levels of progesterone, and low levels of LH and FSH (Soules et al., 1984). At this time, estradiol levels are intermediate between the high levels at ovulation and the low levels at menses. During the late luteal phase (day 25 to 28 of cycle),

progesterone levels begin to decline and the drop in progesterone levels trigger the re-release of FSH and the beginning of a new menstrual cycle. Without fertilization, the corpus luteum will degenerate, leading to a decrease in progesterone secreted. Then, the blood-enriched uterine lining sloughs off, initiating menstruation, and the menstrual cycle begins again (Reilly, 2000).

### **Hormonal Contraceptives**

Estrogen and progestin are active components of combination pills. Their primary mechanism of action is believed to be the prevention of ovulation. The estrogen in HCs prevents the release of gonadotropin-releasing hormone (GnRH) from the hypothalamus, thereby blocking the signal to the pituitary gland to produce FSH and LH (van Heusdan & Fauser, 1999). This inhibits follicles from maturing and releasing a secondary oocyte. Thus, the ovaries are relatively dormant with HC use. Progestin has several effects, including thickening of cervical mucus, decreased tubal mobility, changes in the endometrium, and prevention of ovulation (Frye, 2006). Thickening of cervical mucus impedes sperm penetration. Reduced tubal mobility prevents ovum transportation and fertilization, and may decrease sperm migration and activity. Lastly, changes in the endometrium may reduce survival of a blastocyst within the uterus or prevent implantation into the uterine lining (Chi, 1993). Since HC users do not experience the progesterone-induced thickening of the uterine wall, they do not experience true menstruation. Instead, HC users experience "withdrawal bleeding" in response to withdrawal from the hormones in HC pills in the last week of their cycle (van Heusdan & Fauser, 1999). In summary, HC use mimics the hormonal state of pregnancy by increasing and flattening a woman's levels of both progesterone and estrogen, resulting in the prevention of ovulation and a loss of normal fertility (Alvergne & Lummaa, 2010).

In addition to having exogenous estrogen and progestin circulating in their bloodstream, HC users also demonstrate a different endogenous hormonal pattern across the menstrual cycle than naturally cycling (NC) women. HC users exhibit lower endogenous sex steroid levels and decreased hormonal fluctuations across the cycle than NC women (van Heusdan & Fauser, 1999). In a study measuring estradiol and progesterone levels in healthy women before and after initiation of HC usage, Follesa et al. (2002) found that the rise in estradiol and progesterone levels that previously occurred in the luteal phase was completely eliminated after three months of HC usage. Additionally, estradiol and progesterone levels measured during the follicular phase after three months of HC usage were lower than the estradiol and progesterone levels measured in the follicular phase prior to HC use. HCs have even been shown to lower serum levels of estradiol and progesterone even after discontinuation of use (Balogh, Ditroi, & Lampe, 1981). Therefore, HC use directly alters hormonal levels across the menstrual cycle.

# Hormonal Contraceptives and Psychobehavioural Effects

There is a growing body of literature to support that not only can HCs impact women's physical well-being but HCs can also influence women's psychological well-being. Negative effects on mood and psychological well-being as a result of HC usage have been documented (Kahn & Halbreich, 2001; Kurshan & Epperson, 2006). HC users demonstrated more symptoms of borderline personality disorder (BPD; DeSoto, Geary, Hoard, Sheldon, & Cooper, 2003), which is a disorder characterized by a pattern of instability in affect regulation, impulse control, interpersonal relationships, and self-image (DSM 5, 2013). Furthermore, women with already high pre-existing levels of BPD symptomology became significantly worse after HC initiation (DeSoto et al., 2003). HC use has also been linked to other negative psychological changes such as increased jealousy (Cobey, Pollet, Roberts, & Buunk, 2011) and emotional reactivity

(Armbuster et al., 2017). Some research has found that HC users have higher rates of depression than NC women (Kulkarni, 2007). Negative changes in emotional and sexual well-being are significant predictors of HC discontinuation (Sanders, Graham, Bass, & Bancroft, 2001); however, some studies suggest that HCs provide a stabilizing effect on mood (Oinonen & Mazmanian, 2002). HC users reported experiencing decreased day-to-day variability in their affect and decreased negative affect throughout "menstruation" (i.e., withdrawal bleeding) compared to NC women (Oinonen & Mazmanian, 2002). Other studies have demonstrated that a low-dose oral contraceptive improved women's subjective perceptions of their quality of life (Ernst, Baumgartner, Bauer, & Janssen, 2002), emphasizing the need for continued research.

# **Hormones and Partner Preference**

Heterosexual women's preferences for male traits vary over the ovulatory cycle in a possibly adaptive manner. Traits that signify masculinity, symmetry, and dominance are hypothesized to signal genetic quality in males (Gangestad & Thornhill, 2008). Therefore, an increase in preferences for such traits during fertile periods is a potentially adaptive mechanism. Women's preferences for facial masculinity (Penton-Voak et al., 1999), the odor of masculine men (Havlíček, Roberts, & Flegr, 2005), masculine body shape (Little, Jones, & Burriss, 2007), and masculine voices (Feinberg et al., 2006) are greatest near peak fertility. These cyclic changes in preferences could increase the probability that women would mate with men of "higher genetic quality", which would maximize the likelihood of offspring survival (Gangestad & Thornhill, 2008) or provide offspring with advantages of heritable sexual attractiveness across generations according to the sexy son hypothesis (Weatherhead & Robertson, 1979). Adaptive mechanisms at other points in the menstrual cycles may also exist. During the luteal phase when progesterone levels are high and likelihood of conception is low, women show increased

preferences for facial self-resemblance in pictures of females and young males (DeBruine, Jones, & Perrett, 2005), and apparent health in faces (Jones et al., 2005). These preference changes may function to promote women's associations with kin and healthy individuals during pregnancy when progesterone levels are also high. Associations with such traits during pregnancy would be adaptive to both the mother and the fetus by increasing affiliation with individuals who are more likely to offer support.

Changes in male preferences over the ovulatory cycle are likely under the influence of cyclical hormone levels (Jones et al., 2008). Consequently, these possibly adaptive preferences are reduced in HC users (Jones et al., 2005; Penton-Voak et al., 1999). HC users show either a weak or a lack of preference for facial masculinity compared to non-users (Little, Jones, Penton-Voak, Burt, & Perrett, 2002). Unlike NC women, HC users do not demonstrate a change in preference for masculinity in male faces across the cycle (Penton-Voak et al., 1999). Since ovulatory cycle shifts in preferences are potentially adaptive (Gangestad & Thornhill, 2008) and HCs alter cycle shifts (van Heusdan & Fauser, 1999), HC use may negatively alter mate preferences, and thus, mate choice (Alvergne & Lummaa, 2010).

## Oxytocin

Oxytocin is a small nonapeptide hormone and neurotransmitter that consists of nine amino acids. It is predominantly produced in the supraoptic (SON) and paraventricular nuclei (PVN) of the hypothalamus. Oxytocin is released into the central nervous system (CNS), periphery, brain stem, and spinal cord by the posterior pituitary gland in a pulsatile manner (Neave, 2007, p. 189). Oxytocin has numerous central and peripheral physiological effects in women. The effects of oxytocin range from modulating neuroendocrine reflexes to the formation of complex social and bonding behaviours associated with reproduction and care of offspring

(Carter, Grippo, Pournajafi-Nazarloo, Ruscio, & Porges, 2008). Oxytocin is a crucial component for uterine contractions during pregnancy (Fuchs et al., 1982), and the initiation and maintenance of lactation (McNeilly et al., 1983). Oxytocin levels increase during sexual stimulation and arousal, and oxytocin levels peak during orgasm in both men and women (Carmichael et al., 1987). Oxytocin also plays a critical role in social and emotional processing as well as empathy (Rodrigues et al., 2009).

Plasma oxytocin levels fluctuate throughout the menstrual cycle in NC fertile women. Research found that oxytocin levels were lower during the luteal phase than during the follicular and ovulatory phases in NC women (Salonia et al., 2005). On the other hand, mean oxytocin values did not significantly fluctuate throughout the menstrual cycle in HC users (Salonia et al., 2005). Moreover, HC use has been linked to a disruption in oxytocin signaling (Scheele, Plota, Stoffel-Wagner, Maier, & Hurlemann, 2016).

# Cortisol

Cortisol is a steroid hormone and is produced mainly in the adrenal cortex within the adrenal gland (Neave, 2007, p. 28). Cortisol binds to glucocorticoidreceptors that are present in almost every tissue of the body. Cortisol is secreted in an unbound state and after entering the circulation system, ~83% is bound to corticosteroid-binding globulin (CBG), ~12% is bound to albumin, and the remaining ~5% circulates in free form (Siiteri et al., 1982). Cortisol is involved in many metabolic processes including mobilization of energy, local glucose utilization, enhancing cardiovascular output and respiration, redistributing blood flow, and energy delivery to the brain and muscles (McEwen & Seeman, 1999). Cortisol also plays an important role in the stress response. Studies demonstrate significant positive correlations between anxiety, subjective autonomic reactivity, and basal cortisol levels (Takahashi et al., 2005). Cortisol synthesis and

release follows a diurnal pattern: cortisol levels rise sharply before waking, and levels show a steady decline throughout the day. This increase after awakening is a phenomenon termed the Cortisol Awakening Response (CAR; Fries, Dettenborn, & Kirschbaum, 2009).

The hypothalamic–pituitary–adrenal (HPA) axis controls production of the stress hormone cortisol while the hypothalamic–pituitary–gonadal (HPG) axis controls production of sex hormones such as testosterone, estrogens, and progesterone, which have sex-specific functions (Sapolsky, 2004). The HPA and HPG are interconnected, and thus, a change in one axis may induce a change in the other axis (Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999). Since HCs alter naturally circulating levels of sex hormones (Beck, Cowsar, & Pope, 1980) – i.e., a change in the HPG axis – they may in turn alter the HPA axis. HC users showed significantly blunted cortisol responses to stressors compared to NC women (Roche, King, Cohoon, & Lovallo, 2013), and had peak cortisol levels only slightly above baseline levels (Kirschbaum, Pirke, & Hellhammer, 1995). Thus, HC use may interfere with the adrenocortical response to stress by decreasing the amount of bioavailable unbound cortisol (Kirschbaum et al., 1999).

# **Intrauterine Devices (IUD)**

There are two main types of IUDs: hormonal IUDs, which release a localized hormone (levonorgestrel), and copper IUDs, which do not contain hormones. Therefore, IUDs should not alter circulating hormones to the same extent as HCs. The levonorgestrel (LNG)-medicated intrauterine device has several mechanism of action. The main mechanism of the LNG-IUD is its local suppressive effect on the endometrium, which includes glandular atrophy and significant changes to endometrial stroma cells (i.e., decidualization; Silverberg, Haukkamaa, Arko, Nilsson, & Luukkainen, 1986). LNG also causes a down-regulation of endometrial estrogen

receptors, decreasing their responsivity to circulating estradiol (Critchley, Wang, Kelly, Gebbie, & Glasier, 1998), and leading to endometrial thinning (Andersson, Odlin, & Rybo, 1994). Additionally, LNG enhances the expression of glycodelin A in the endometrium (Ortiz & Croxatto, 2007). Glycodelin A is a protein that prevents sperm binding, further inhibiting fertilization. Another mechanism of action of LNG is making the cervical mucus thicker, which creates a more hostile environment for incoming sperm (Guillebaud, 2003). The ovulatory cycle is preserved in both cooper IUD users (Faundes et al., 1980) as well as in the majority of LNG-IUD users, and a decrease or lack of menstrual bleeding does not signify a lack of ovulatory cycle (Luukkainen, Lähteenmäki, & Toivonen, 1990). It is important to note that since the ovulatory cycle is still present in IUD users, researchers should still seek to acknowledge cycle phase of IUD users in studies. Since both IUD users and HC users seek out the use of contraceptives, and IUDs should not alter circulating sex hormones – or should not alter circulating sex hormones to the same extent as HCs – IUD users represent a useful group to compare with HC users.

# **Current Study**

The goal of the first study is to investigate the relationship between HC use, BPD symptoms, empathy, and oxytocin levels. I expect that HC use and low estrogen level will result in higher BPD scores and lower empathy scores as compared to high estrogen level. Additionally, I hypothesize that HC use decreases empathy through a mechanism that interferes with oxytocin levels. The objective of the second study is to extend previous findings on the association between menstrual cycle phase and partner preferences by incorporating research regarding the change in hormones associated with HC usage. The goal is to examine how hormonal change due to HC use influences women's partners preferences in terms of male

attractiveness ratings, as well as dominant behaviour in short- and long-term partners. I hypothesize that HC users will rate feminized faces as more attractive than masculinized faces, and that HC users will rate dominance in partners as less important than women who are NC. The aim of third study is to compare reactive cortisol in HC users and non-users, as well as compare depression and anxiety levels between these two groups. Finally, the fourth study examines numerous differences in terms of psychology and behaviour between combination pill users and intrauterine device users.

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# Chapter 2: Investigating the Relationship Between Hormonal Contraceptive Use and Borderline Personality, Empathy and Oxytocin Level

## Introduction

Previous findings support the observation that hormonal contraceptives (HC) use may be linked to an increase in borderline personality disorder (BPD) symptomology (DeSoto, Geary, Hoard, Sheldon, & Cooper, 2003). BPD is characterized by instability in interpersonal relationships, self-image, and affects. It is predominantly diagnosed in women, and 75% of BPD cases are female (DSM 5, 2013). The higher prevalence of BPD in women may potentially be moderated by sex hormones. Since HCs alter naturally occurring levels of sex hormones in females (Beck, Cowsar, & Pope, 1980), HC usage may induce BPD-like symptoms in users. DeSoto et al. (2003) found that variation in estrogen levels predicted the presence of BPD symptoms. BPD symptoms were most common in women using HCs as well as during times in the menstrual cycle when estrogen level is rising. Moreover, for women with already high preexisting levels of BPD, symptoms became significantly worse after HC initiation (DeSoto et al., 2003). HC use has also been linked to higher levels of jealousy (Cobey, Pollet, Roberts, & Buunk, 2011) and emotional reactivity (Armbuster, Kirschbaum, & Strobel, 2017) – traits that are closely related to BDP symptoms. Thus, there may be an association between HC use and BPD symptomology.

There may be a potential link between HCs and lower empathy levels (Radke & Derntl, 2016). Additionally, there may be a connection between BPD and empathy since individuals with BPD show lower levels empathy (Dziobek et al., 2011). Empathy is the ability to infer and share another individual's internal emotional states. In most models of empathy, researchers describe three core components of empathy: 1) emotion recognition (the recognition of emotions

in oneself and others through facial expressions, speech or other behaviours), 2) affective responsiveness (i.e., the ability to experience similar emotions as others while understanding that this is a simulation of emotional feeling and it is not one's actual emotion), and 3) perspectivetaking (i.e., the cognitive ability to take the perspective of another individual; Decety & Jackson, 2004). Sex differences on empathy levels are well established and demonstrate that women have higher empathy scores as compared to men on average (Eisenberg & Lennon, 1983). Furthermore, menstrual cycle phase and ovarian hormone concentrations are associated with empathic behaviour. Progesterone levels significantly correlated with empathetic behaviour in women in the luteal phase of their cycle (Derntl, Hack, Kryspin-Exner, & Habel, 2013). The effects of HC use on empathy are less well studied. Despite the relevance for women and their relationships, little is known about how HC interacts with social abilities. HC use may negatively influence emotion recognition (Hamstra, De Rover, De Rijk, & Van der Does, 2014). Yet, a different study reported an effect of pill phase on the affective responsiveness component of empathy. Among HC users, women currently taking the pill demonstrated better performance than HC users during their pill-free week (Radke & Derntl, 2016), which suggests that there is an association between changes in sex hormones and affective responsiveness.

Oxytocin has been shown to play a critical role in social and emotional processing as well as empathy (Rodrigues, Saslow, Garcia, John, & Keltner, 2009). Oxytocin is a peptide hormone and neurotransmitter, and it is known to exert a wide range of physiological effects. The effects of oxytocin range from specific physiological responses to the formation of complex social and bonding behaviours related to the reproduction and care of the offspring (Carter, Grippo, Pournajafi-Nazarloo, Ruscio, & Porges, 2008). Plasma oxytocin fluctuates throughout the menstrual cycle in naturally cycling (NC) women. However, in HC users, plasma oxytocin does

not show any significant cyclical variation throughout the menstrual cycle (Salonia et al., 2005). This lack of fluctuation in oxytocin should be examined more thoroughly in order to analyze any corresponding behavioural changes.

Traditionally, oxytocin has been described as being the "cuddle hormone" due to prior studies showcasing oxytocin as being heavily involved in pair bonding and monogamy (Carter et al., 2008). Yet, newer research demonstrates that oxytocin has a much more widespread range of effects on social behaviour. For example, after intranasal administration of oxytocin and provocation, participants displayed higher levels of aggression (Ne'eman, Perach-Barzilay, Fischer-Shofty, Atias, & Shamay-Tsoory, 2016). Therefore, oxytocin is not only involved in prosocial behaviour, but various forms of social behaviour, regardless of whether the behaviour is positive or negative. Increased aggression is a component in some symptoms of BPD. Since HC use has been linked to lower oxytocin levels, oxytocin may be involved in the relationship between HC and BPD (Scheele, Plota, Stoffel-Wagner, Maier, & Hurlemann, 2016). Likewise, BPD has also been linked to lower oxytocin levels (Bertsch, Schmidinger, Neumann, & Herpertz, 2013). If HCs are in fact associated with altered behaviour, it is crucial to understand the mechanisms behind its actions. Oxytocin represents one plausible mechanism of action for HC to incur behavioural and psychological changes in women due to its connection with empathy and BPD.

# **Objectives**

The goal of the present study is to investigate the relationship between HC use, BPD symptoms, empathy, and oxytocin levels. I expect that HC users will have higher BPD symptoms and lower empathy scores as compared to non-users. Additionally, I hypothesize that HC use is associated with higher BPD-like symptoms and lower empathy scores, and that it acts

through a mechanism that interferes with oxytocin levels. Therefore, I also hypothesize that HC users will have lower salivary oxytocin levels as compared to NC women.

# Methods

# **Participants**

Participants included 198 females, which were recruited using an undergraduate research participant pool where individuals receive course credit for participanting. Only participants who were NC or used combination pills were included in data analyses. Of the remaining participants, 106 participants did not use birth control of any kind and 67 used combination pills. Based on self-reported day in menstrual cycle and contraceptive use at the time of data collection, I divided participants into groups of combination pill users (HC; 39.88%), participants in their low estrogen phase (Low E; 41.67%), and participants in their high estrogen phase (High E; 18.45%). The mean age of the participants was 19.23 (range = 17-33). The majority of participants were Caucasian (50.58%), and there was a smaller proportion of participants identifying themselves as Asian (27.33%), Indo-Mediterranean (10.47%), African (5.23%), mixed (4.65%), Amerindian (1.16%), and other (0.58%). All data was collected in a single session. The Human Research Ethics Boards at the University of Alberta approved this study (Pro00078461).

#### Measurements

#### Reading the Mind in the Eyes (RMET)

The RMET questionnaire (Baron-Cohen, Wheelwright, Hill, Raste, & Plumb, 2001) consisted of 36 pictures of the eye region of the face (figure 2–1). Participants are instructed to select one word from a list of four that "best describes what the person in the picture is thinking or feeling". If needed, participants could consult a glossary that defined the words on the questionnaire. Participants received one point for each correct item, with no penalty for guessing

incorrectly. Therefore, they could obtain a maximum score of 36 and minimum score of 0. RMET measures theory of mind, a term that overlaps with empathy (Baron-Cohen et al., 2001). *Borderline Symptom List 23 (BSL-23)* 

Participants were administered the Borderline Symptom List 23 (BSL-23; Bohus et al., 2009). The BSL-23 is a self-report questionnaire that assesses BPD symptomatology. The BSL-23 has been shown to have high internal consistency (Cronbach's alpha of 0.93; Bohus et al., 2009) and good test-retest reliability over a one-week period (r = .82; Bohus et al., 2009). It has also shown good reliability for BPD diagnosis as well as a satisfactory sensitivity to change (Bohus et al., 2009). The measure consists of 23 items that ask participants to rate how much they have experienced each symptom of BPD over the past week on a 5-point Likert scale ranging from "0 - Not at all" to "4 - Very much."

#### BSL-Supplement: Items for Assessing Behaviour

The BSL-Supplement: Items for Assessing Behaviour (Bohus et al., 2001) is a self-report scale with 10 items that measures the frequency of specific borderline-like behaviours over the past week. The supplement assesses self-harming behaviours, suicidal intent and attempts, bingeing and purging behaviours, impulsivity, hostile outbursts, substance use, and sexual promiscuity. The behaviours assessed on the supplement do not overlap with the BSL-23 symptoms. The items are rated on a five-point frequency scale, with "0 - Not at all" to "4 - Daily or more often", and are analyzed as mean scores.

#### Buss Perry Aggression Questionnaire (BPAQ)

The BPAQ includes 29-items that examines total aggression as well as subscales of aggression, which include physical aggression, verbal aggression, anger, and hostility (Buss & Perry, 1992). Participants were instructed to rate how well each BPAQ item described them

using a 5-point scale, ranging from "1 - Extremely uncharacteristic of me" to "5 - Extremely characteristic of me."

# Empathy Quotient (EQ-Short)

The EQ-Short (Wakabayashi et al., 2006) consists of 22 statements, six of which are reversal items. Participants responded using a 4-point Likert scale for each question: "1 - Strongly agree", "2 - Slightly agree", "3 - Slightly disagree", or "4 - Strongly disagree."Participants received 2 points for displaying a "strongly" empathizing response and 1 point for displaying a "slightly" empathizing response.

# Oxford Happiness Questionnaire (OHQ)

The OHQ is a 29-item measure of broad measure of personal happiness that utilizes a six point rating scale of agreement ranging from "1 - Strongly agree" to "<math>6 - Strongly disagree" (Hills & Argyle, 2002). The OHQ has demonstrated high scale reliability ( $\alpha = 0.91$ ; Hills & Argyle, 2002).

# NEO–Personality Inventory (NEO–PI) – N3 Scale

The NEO–PI (Costa & MacCrae, 1992) measures the five-factor model of personality. The full version contains 181 items. Due to time constraint, only the N3 scale with 8-items was used. The N3 scale measures neuroticism related to depression (i.e., "Sometimes I feel completely worthless"). Items are answered along a 5-point Likert scale from "*1* – *Strongly disagree*" to "*5* – *Strongly agree*"

### Binge Eating Scale (BES)

The BES (Gormally, Black, Daston, & Rardin, 1982) is a self-report measure that assesses the extent and severity of binge eating problems. It consists of 16 sets of four statements relating to binge eating behaviour (e.g., "I don't feel any guilt or self-hate after I overeat" and

"Almost all the time, I experience strong guilt or self-hate after I overeat"). Participants are asked to choose one of each set of four statements which best describes their eating behaviour. *Experiences in Close Relationships-Relationship Structures Scale (ECR-RS)* 

The ECR-RS (Fraley, Heffernan, Vicary, & Brumbaugh, 2011) is a 9-item self-report questionnaire that measures two underlying attachment dimensions: avoidance (items 1–6) and anxiety (items 7–9) in mother, father, romantic partner, and best friend domains. Items are answered along a 7-point Likert scale, which ranges from "1 - Strongly disagree" to "7 - Strongly agree." The first four items were reverse scored. Higher scores reflect increased levels of insecure attachment in each individual relationship domain as well as a total score for all domains summed together.

# Beck Depression Inventory (BDI-IA)

The BDI-IA (Beck & Steer, 1993) is a 21-item multiple-choice self-report inventory used assess of the presence and degree of depression in adolescents and adults. The BDI-IA is scored by summing the ratings of all 21 items on the questionnaire. Each item is rated on a 4-point scale that ranges from 0 to 3, and the total scores can range from 0 to 63. BDI-IA total scores ranging from 0 to 13 represent "minimal" depression, total scores from 14 to 19 are "mild", total scores from 20 to 28 are "moderate", and total scores from 29 to 63 are "severe."

#### **Oxytocin Sampling and Analysis**

All salivary samples were collected between 1 pm and 3 pm in order to control for circadian rhythm effects. Samples were taken after completing the questionnaire so that all stimuli were the same before saliva collection. Participants were prohibited from eating and drinking while completing the questionnaire, meaning that they did not eat or drink for at least 30 minutes before saliva sample collection. Saliva samples were collected in pre-chilled

polypropylene 15 mL tubes, which were stored on dry ice throughout the duration of the session for 1 hour. Participants were instructed to produce 2 to 3 mL of saliva via passive drool. Samples were then kept on dry ice for approximately 10 minutes until they were transferred and frozen at -80°C to ensure that samples remained stable during long-term storage (Procyshyn, Spence, Read, Watson, & Crespi, 2017). Once all samples were collected and ready for processing, saliva samples were thawed and centrifuged. Raw saliva samples were used for processing since they met the minimum sensitivity of the ELISA (i.e., 15 pg/ml). Salivary oxytocin measurements were executed using 96-well Enzo Life Sciences enzyme-linked immunosorbent assay kits ADI-901-153 (ELISA; http://www.enzolifesciences.com/ADI-901-153A/ oxytocin-elisa-kit/ENZO Oxytocin ELISA).

All samples were processed in accordance with the manual's ELISA protocol. To create the oxytocin standards, the 10,000 pg/mL oxytocin standard solution was first warmed to room temperature, and seven tubes were labeled "*1*" through "*7*". Then, 900 µL of the standard diluent (i.e., assay buffer) was transferred into tube *1*. Next, 500 µL of standard diluent was pipetted into tubes *2* through *7*. 100 µL of the 10,000 pg/mL standard was added to tube *1*, which was then vortexed thoroughly. Then, 500 µL of tube *1* was added to tube *2*, and vortexed thoroughly. Afterward, 500 µL of tube *2* was added to tube *3*, and was vortexed. This processed was repeated for tubes *4* through *7*.  $\frac{100}{100}$  µL of the resulting concentration of oxytocin in tubes *1* through *7* was 1,000, 500, 250, 125, 62.5, 31.2, and 15.6 pg/mL respectively. The standards were used within 60 minutes of preparation.

To complete the assay procedure, all reagents were first brought to room temperature for at least 30 minutes prior to processing. Next, 100  $\mu$ L of standard diluent was pipetted into the non-specific binding (NSB) and the B<sub>0</sub> (i.e., contains 0pg/mL of standard oxytocin) wells. Then,

100  $\mu$ L of the standards *1* through 7 were pipetted into the appropriate wells, and 100  $\mu$ L of the samples was pipetted into the appropriate wells. Afterward, 50 µL of assay buffer was pipetted into the NSB wells, and 50  $\mu$ L of the blue conjugate was pipetted into each well, except the total activity (TA) and blank wells. Then, 50  $\mu$ L of the yellow antibody was pipetted into each well, except the blank, TA and NSB wells. The plate was tapped gently in order to mix contents. Subsequently, the plate was sealed and incubated at 4°C for 20 hours. After incubation, the contents of the wells were emptied, and a wash was completed by adding 400 µL of wash solution to each well. The wash process was carried out 2 more times for a total of 3 washes. After the final wash, the wells were emptied and aspirated by firmly tapping the plate dry on lint free paper towel to remove any remaining wash buffer. Next, 5  $\mu$ L of the blue conjugate was added to the TA wells, and 200 µL of the p-nitrophenylphosphate (pNpp) substrate solution was added to every well. The plate was then see incubated at room temperature for 1 hour without shaking. Finally, 50 µL of stop solution was added to every well, and the plate was read immediately. The plate reader was blanked against the blank wells, and the optical density of the samples was read at 405 nm. Oxytocin concentrations were later calculated from the standard curve. All samples were run in duplicate to ensure reliability, and the average of the two duplicate samples was used in data analysis as the salivary oxytocin level.

#### **Statistical Analyses**

All statistical analyses were executed in R (R Core Team, 2017). T-tests were used to calculate mean differences between the HC group and the NC group. Pearson's product-moment correlations were used to examine the strength of the linear relationship between two variables. Analysis of covariance (ANCOVA) was used to determine whether the means of the dependent variables were equal across the groups of HC users and non-users, while controlling for the

effects of salivary oxytocin level. Multivariate analysis of variance (MANOVA) was used to create a model with multiple dependent variables. Oxytocin levels had a positive skew, and so, a log transformation was applied to oxytocin levels to normalize the values. Non-linear relationships were tested by adding squared salivary oxytocin levels in order to create quadratic models. Principal component analysis (PCA) was used as a dimension-reduction tool since the dataset contained numerous variables. PCA reduced the large set of variables into a smaller set, which still contained most of the important information from the original dataset.

#### Results

#### HC Usage and Oxytocin Associations with Individual Measures

Pearson's product-moment correlations were used to assess any relationships between HC usage and any measures. HC use was not significantly correlated with any measures. Additionally, Pearson's product-moment correlations were used to examine any relationships between oxytocin level and any questionnaire measures. Oxytocin level was also not significantly correlated with any measures.

### **Individual Comparisons Between HC and NC Groups**

T-tests were used to examine group differences between measures. There were no significant mean differences between HC users and NC women on any measures (table 2–1). However, EQ scores (t = -1.89; p = 0.061), NEO-PI N3 scores (t = 1.66; p = 0.099), and OHQ scores (t = -1.91; p = 0.059) were approaching significance. The oxytocin levels between groups did differ with HC users having a mean salivary oxytocin level of 768.32 pg/mL and non-users having a mean level of 689.83 pg/mL; however, levels did not differ significantly (t = -0.61; p = 0.55). See figure 2–2 for a comparison of mean salivary oxytocin level between HC users and non-users.

#### **Grand Scale Differences Between HC and NC Groups**

Multivariate Analysis of Variance (MANOVA) was used to examine a model with all measures as dependent variables and HC usage as the independent variable. However, the model was not significant. Since the model based on the hypothesis was not significant, I used an exploratory approach and input variables that were approaching significance in the t-tests previously described. The MANOVA contained EQ, NEO-PI N3, BSL-23, BSL supplement, OHQ, and salivary oxytocin were used as the dependent variables and contraceptive use as the independent variable. The model was significant (F = 3.37; p = 0.0063; see figure 2–3 for a representation). Contrary to my hypothesis, HC users had lower BSL-23 scores and NEO-PI N3 scores as well as higher OHQ scores, EQ scores, and salivary oxytocin levels. Only the higher BSL supplement scores in HC users fit with my hypothesis and prior research.

#### Individual Comparisons Between HC, Low Estrogen, and High Estrogen Groups

ANOVAs were used to examine differences on measures between HC users, low estrogen (Low E) participants, and high estrogen (High E) participants at the time of data collection (table 2–2). Only Oxford Happiness Questionnaire (OHQ) scores significantly differed between groups ( $F_{2,158} = 5.75$ ; p = 0.0039). The oxytocin levels between groups did differ with HC users having a mean salivary oxytocin level of 768.32 pg/mL, low estrogen participants having a mean level of 786.48 pg/mL, and high estrogen participants having a mean level of 589.16 pg/mL; however, levels did not differ significantly ( $F_{2,70} = 0.95$ ; p = 0.39). See figure 2–4 for a comparison of mean salivary oxytocin level between HC users, low estrogen participants, and high estrogen participants.

#### Grand Scale Differences Between HC, Low Estrogen, and High Estrogen Groups

When using three groups (i.e., Low E, High E, and HC) in the same MANOVA as previously described, the model's significance increased (F = 2.63; p = 0.0038; see figure 2–5 for a representation). The low estrogen group and HC group scored more similarly on the measures, whereas the high estrogen group differs from the other two groups. The high estrogen group scored higher on the BSL-23 and supplement, BDI, and NEO-PI N3. The high estrogen group also scored lower on the OHQ, and salivary oxytocin levels.

#### **Comparison of Principal Components Between Groups**

PCA was performed using the R *prcomp* function on all measures. The loadings of each subscale on components 1, 2, and 3 can be found in table 2–3. Principal component 1 (PC1) demonstrated strong negative loadings for NEO-PI N3, BPAQ, ECR-RS, BES, BSL, and BDI, and a strong positive loading for OHQ (figure 2–6). PC1 accounted for 48% of the variance. Principal component 2 (PC2) demonstrated a strong positive loading from RMET and a strong negative loading for oxytocin levels, and it accounted for 12% of the variance. Principal component 3 (PC3) demonstrated a strong negative loading for EQ only, and accounted 11% of the variance.

After performing PCA, I used t-tests to see if HC and NC groups differed significantly between the three principal components. There were no significant differences between groups; however, differences between groups on PC3 were approaching significance (t = 1.90; p = 0.063) with the NC group scoring higher than the HC group ( $x_{NC} = 0.17$ ;  $x_{HC} = -0.31$ ). I also used ANOVAs to determine if the HC, low estrogen, and high estrogen groups differed significantly between the three main principal components. Scores between groups on PC3 were significantly different with the low estrogen group scoring higher ( $x_{HC} = -0.31$ ;  $x_{Low E} = 0.43$ ;  $x_{High E} = -0.16$ ;

 $F_{2,59} = 3.30$ ; p = 0.044), and differences between groups on PC1 were approaching significance with HC users scoring higher ( $x_{HC} = 0.50$ ;  $x_{Low E} = 0.27$ ;  $x_{High E} = -0.94$ ;  $F_{2,59} = 2.47$ ; p = 0.093).

#### **Examining Oxytocin as a Potential Covariate**

Including salivary oxytocin level as a covariate Analysis Of Covariance (ANCOVA) did not significantly change results in either the model comparing HC users and non-users, or in the model comparing HC users, low estrogen participants, and high estrogen participants. I also included models with a quadratic transformation on salivary oxytocin level to examine nonlinear effects. Again, these models were non-significant and did not change the results.

#### Discussion

The aim of the current study was to investigate differences between women using hormonal contraceptives (HC) and naturally cycling (NC) women in terms of psychological differences associated with borderline personality and empathy as well as examine oxytocin as a possible mechanism of action. The results directly contradict my hypothesis that HC users would have higher BDP scores, lower empathy scores, and lower salivary oxytocin levels. Instead, HC users had higher empathy scores, happiness scores, and salivary oxytocin levels. Additionally, HC users had lower neuroticism scores and borderline personality scores. Not only do the results oppose my initial hypotheses, but they also contradict many published studies examining the influence of HCs on psychology (e.g., DeSoto et al., 2003; Derntl et al., 2013; Pahnke et al., 2019). I had a comparable sample size to DeSoto et al. (2003) and performed an exhaustive data analyses; yet, I was still unable to reject the null hypothesis. My results suggest that HC users more closely resemble the low estrogen participants, and that these two groups have lower borderline-like symptoms, higher happiness scores, and higher salivary oxytocin levels as compared to high estrogen participants. Therefore, the menstrual phase associated with high levels of estrogen (i.e., late follicular phase) may cause a transient increase in borderline symptomology while decreasing happiness and oxytocin levels in women.

Previous research demonstrated that menstrual cycle phase was associated with empathy performance, and that women in the follicular phase had higher accuracy at emotion recognition (Derntl et al., 2013). Considering that HC use decreases circulating sex hormones, studies have also exhibited that HC users are less accurate in emotion recognition than non-users (Pahnke et al., 2019). However, I did not find this effect. HC users and non-users in my study scored similarly on the Reading the Mind in the Eyes Task (RMET), which is a task that requires participants to recognize emotions and thoughts in photographs of eyes. Furthermore, HC users actually scored higher in the Empathy Quotient, although the p-value was only approaching significance.

I also expected to replicate some of DeSoto et al.'s (2003) findings that HC users have increased borderline-like symptoms. Yet, in my study, HC users demonstrated less borderline symptoms on the BSL-23, which was my main measure of borderline symptomology. HC users did score higher on the BSL supplementary material; however, this is a shorter questionnaire and depends more on the external behaviour of the participant in the past week. Thus, my results again oppose previous research. DeSoto et al. (2003) found that participants who scored higher on BPD symptoms before initiation of HC use experience an exacerbation of BDP symptoms when they begin HC use. The worsening of pre-existing BPD symptoms after beginning HC usage may suggests that the initiation of HC use does not cause an increase in BPD symptoms in itself. Instead, HCs may worsen borderline symptoms in women with a predisposition to BPD. Since I only collected data in a single session and I did not collect data before HC initiation, this may explain some of the discrepancy between my findings and DeSoto et al.'s (2003) findings.

Previous research found that oxytocin interacts with the brain reward system to reinforce partner value representations and that this oxytocin mechanism was disturbed in HC users, and hypothesized that this may be due to the effects of oxytocin being antagonized by sex hormones (Scheele et al., 2016). Again, my findings contradict previous research as HC users in my study actually had increased salivary oxytocin levels, demonstrating that the effects of HC may not be straightforward. There may be many ways in which oxytocin interacts with HCs. For instance, oxytocin may be altered by endogenous sex hormones, it may be altered by exogenous sex hormones, or oxytocin receptors and receptor binding affinity may be altered due to HC usage. Further research should attempt to elucidate any possible interactions between HCs and oxytocin.

My study had several limitations. Firstly, I did not look at pre-HC use to post-HC use. Research incorporating a within-subject design would be greatly beneficial. However, withinsubject designs for HC use is still highly confounded since women who seek HC use already differ from women who choose not to use HCs. One way to avoid this is issue would be to use participants who want to use HCs and compare them to women who need to use HCs for medical reasons (e.g., dysmenorrhea, endometriosis, etc.). However, this type of research design is still confounded since women using HCs for medical reasons may differ from healthy women. Thus, finding appropriate groups of HC users and non-users to compare is challenging. Secondly, HCs may interact with behaviour and psychology in many different ways, meaning that HCs would interact with individual differences. Therefore, HC use effects may be flattened out by noise in the data due to individual variation. That is to say that if HCs increase BPD symptoms in some women while decreasing BPD symptoms in other women, these effects would cancel each other out and there would be no statistical differences between groups of users and non-users. Lastly, another important limitation in the current study is the use of a non-clinical sample. My sample included healthy young women attending university. Future research should examine how HCs influence women with BPD to see whether there is worsening of symptoms after initiation of HC use. Moreover, research could include participants with a variety of different psychological disorders to definitively establish that HCs do not exacerbate already existing symptoms.

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## playful

# comforting



## irritated

### bored

**Figure 2–1.** Example of Reading the Mind in the Eyes Test (RMET). Participants were provided with 36 different pictures of eyes (18 female and 18 male pictures) in random order and were instructed to choose the adjective that best described what they thought the person in the picture was thinking or feeling.

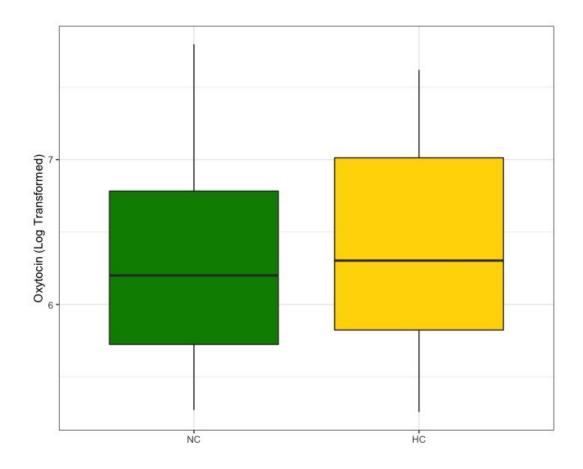
Variable	NC (n = 106)		HC $(n = 67)$		T-Test	
	Μ	SD	Μ	SD	<i>t</i> (df)	p-value
Age (Years)	19.15	2.01	19.36	2.05	-0.65(138.61)	0.52
RMET	74.37	12.07	75.42	11.15	-0.58(146.39)	0.56
Female Pictures Only	73.50	14.71	74.15	13.88	-0.29(144.25)	0.77
Male Pictures Only	75.14	13.59	76.56	12.04	-0.71(150.42)	0.48
BPAQ	70.53	19.04	70.22	19.85	0.10(132.52)	0.92
EQ	17.20	3.91	18.22	3.11	-1.89(160.47)	0.061•
BSL (Composite Scores)	30.51	23.56	26.98	24.78	0.93(133.02)	0.36
BSL-23	27.64	20.69	23.33	20.69	1.33(138.3)	0.19
BSL-Supplement	2.91	4.93	3.82	5.35	-1.13(132.06)	0.26
NEO-PI N3	25.36	7.74	23.31	7.82	1.66(134.98)	0.099•
BDI	35.40	10.48	32.97	10.48	1.37(153.68)	0.17
BES	28.49	9.08	29.52	10.21	-0.67(126.9)	0.51
OHQ	113.14	23.26	119.99	22.08	-1.91(139.21)	0.059•
ECR-RS (Composite Scores)	101.34	32.44	95.73	32.96	1.08(137.52)	0.28
Oxytocin (Log Transformed)	6.30	0.68	6.40	0.72	-0.57(43.57)	0.57

• p < .10 \* p < .05

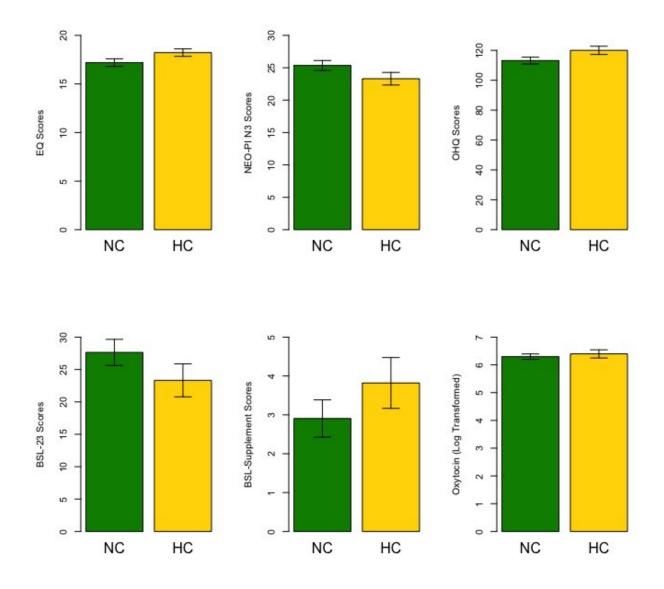
\*\* p < .01

\*\*\* p < .001

**Table 2–1.** T-tests between hormonal contraceptive (HC) users and naturally cycling (NC)women. There were no significant differences between groups. However, empathy quotientscores, NEO-PI N3 scores, and Oxford Happiness scores were approaching significance.



**Figure 2–2.** Log transformed salivary oxytocin levels (pg/mL) in naturally cycling (NC; green) women and hormonal contraceptive (HC; yellow) users. Oxytocin levels were not significantly different between groups (t = -0.57; p = 0.57).

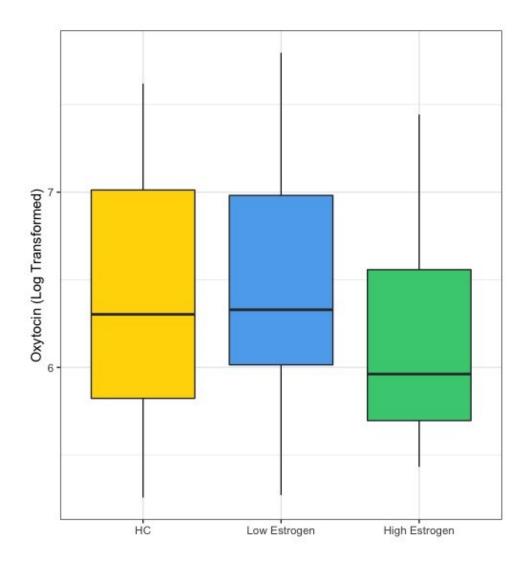


**Figure 2–3.** Representation of MANOVA where empathy (EQ), happiness (OHQ), neuroticism (NEO-PI N3), borderline symptoms (BSL-23 and BSL-Supplement), and salivary oxytocin levels (log transformed) are dependent variables and HC use (HC and NC) is the independent variable in the model. The HC group is depicted in yellow and the NC group is depicted in green.

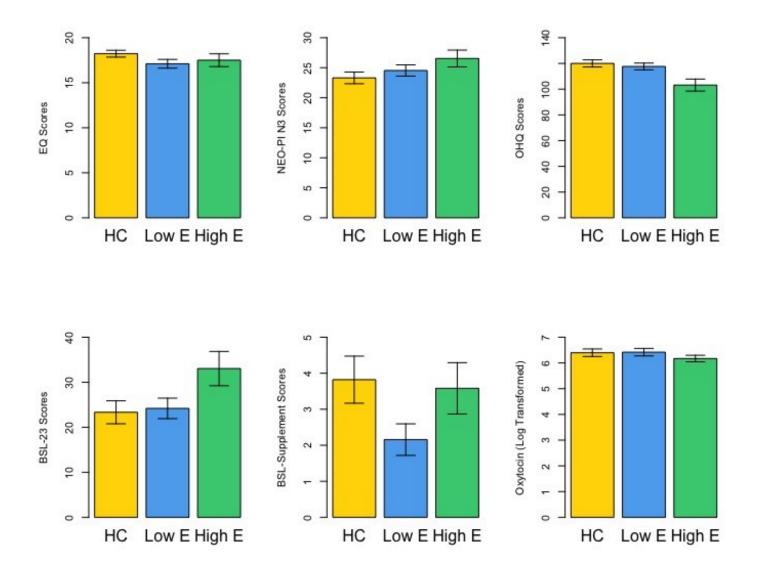
Variable	HC $(n = 67)$		Low E $(n = 70)$		High E $(n = 31)$		ANOVA	
	Μ	SD	Μ	SD	Μ	SD	<i>F</i> (df)	p-value
Age (Years)	19.36	2.05	19.20	2.24	18.97	1.52	0.39(2,165)	0.68
RMET	75.42	11.15	74.76	11.05	72.96	14.42	0.45(2,163)	0.64
Female Pictures	74.15	13.88	73.61	13.76	72.35	16.29	0.16(2,163)	0.85
Male Pictures	76.56	12.04	75.79	13.00	73.51	15.67	0.56(2,163)	0.57
BPAQ	70.22	19.85	69.17	17.91	71.80	20.36	0.20(2,158)	0.82
EQ	18.22	3.11	17.10	3.96	17.50	3.89	1.62(2,161)	0.20
BSL (Composite								
Scores)	26.98	24.78	26.29	20.56	36.61	23.21	2.43(2,163)	0.091•
BSL-23	23.33	20.69	24.19	18.88	33.03	21.17	2.72(2,163)	0.069•
BSL-Supplement	3.82	5.35	2.16	3.67	3.58	3.96	2.61(2,165)	0.076•
NEO-PI N3	23.31	7.82	24.54	7.78	26.53	7.69	1.78(2,161)	0.17
BDI	32.97	10.48	33.13	9.11	37.90	10.33	2.94(2,161)	0.056•
BES	29.52	10.21	27.57	8.43	29.00	8.79	0.78(2,161)	0.46
OHQ	119.99	22.08	117.64	21.89	103.18	24.76	5.75(2,158)	0.0039**
ECR-RS (Composite								
Scores)	95.73	32.96	97.21	32.14	108.21	32.10	1.59(2,159)	0.21
Oxytocin (Log								
Transformed)	6.40	0.72	6.42	0.73	6.17	0.62	0.95(2,70)	0.39

• p < .10 \* p < .05 \*\* p < .01 \*\*\* p < .001

Table 2–2. T-tests between hormonal contraceptive (HC) users, participants with low estrogen (Low E), and participants with high estrogen (High E) at time of data collection. Only Oxford Happiness Questionnaire (OHQ) scores significantly differed between groups ( $F_{2,158} = 5.75$ ; p =0.0039).



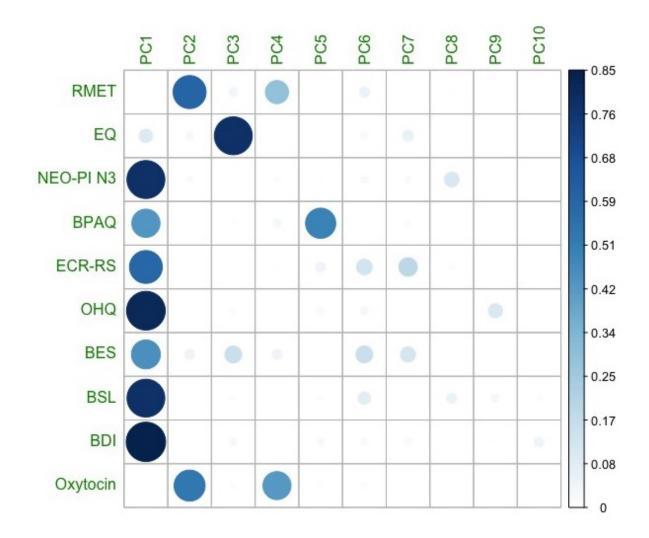
**Figure 2–4.** Log transformed salivary oxytocin levels (pg/mL) in hormonal contraceptive (HC) users (yellow), participants with high estrogen (green), and participants with low estrogen (blue) at time of data collection. Oxytocin levels were not significantly different between groups (t = 0.95; p = 0.39).



**Figure 2–5.** Representation of MANOVA where empathy (EQ), happiness (OHQ), neuroticism (NEO-PI N3), borderline symptoms (BSL-23 and BSL-Supplement), and salivary oxytocin levels (log transformed) are dependent variables and estrogen level (High E, Low E, and HC) is the independent variable in the model. The HC group is depicted in yellow, the Low E group is depicted in blue, and the High E group is depicted in green.

Measure	PC1 (48%)	PC2 (12%)	PC3 (11%)
RMET	0.039	0.697	0.191
EQ	0.141	0.147	-0.862
NEO-PI N3	-0.404	-0.122	0.010
BPAQ	-0.301	-0.037	0.086
ECR-RS (Composite Scores)	-0.350	0.079	0.083
OHQ	0.412	-0.001	-0.120
BES	-0.308	0.198	-0.382
BSL (Composite Scores)	-0.403	0.024	-0.095
BDI	-0.419	0.026	-0.157
Oxytocin (Log Transformed)	-0.002	-0.656	-0.112

Table 2–3. Principal components 1, 2, and 3 loadings across each variable.



**Figure 2–6.** Visualization of a correlation matrix between principal components (PC) and variables in the model.

# Chapter 3: Re-Examining the Influence of Hormonal Contraceptives on Partner Preference

#### Introduction

A growing body of literature has demonstrated shifts in women's attractiveness ratings in men across the menstrual cycle, with the time surrounding ovulation related with increased attraction to more masculine morphological and behavioural traits in men (Gangestad & Thornhill, 2008). In a within-subject design, changes in women's estradiol concentrations positively correlated with their changes in preferences for facial cues of high testosterone (i.e., masculinity) in male faces (Roney, Simmons, & Gray, 2011). Usage of exogenous hormones, such as those in hormonal contraceptives (HC), may modify women's mate preferences since they alter typical levels of sex hormones and their cyclical variation (Alvergne & Lummaa, 2010). This raises important issues for women who initiate HC use, cease HC use, or choose to use a different type of contraceptive during a relationship as it may alter attractiveness ratings and preferences towards their current partner.

Male face morphology may provide information on that individual's health and hormonal status. Testosterone levels at puberty are correlated with the amount of growth of male secondary sexual characteristics in terms of jaw, cheekbones, and brow ridges (Thornhill & Gangestad, 1996). It has been suggested that women's preferences for specific male traits vary throughout the ovulatory cycle in an adaptive fashion in naturally cycling (NC) women. Traits including masculinity, dominance, and symmetry are hypothesized to indicate higher genetic quality in males (Gangestad & Thornhill, 2008). Thus, an increase in preferences for these traits during fertile periods in the ovulatory cycle could be a possibly adaptive mechanism. Women's preferences for facial masculinity (Penton-Voak et al., 1999), masculine body shape (Little,

Jones, & Burriss, 2007), masculine voices (Feinberg et al., 2006), and the odor of masculine men (Havlíček, Roberts, & Flegr, 2005), are heightened during the time surrounding ovulation. Such cyclic changes in preferences could raise the likelihood that women would mate with men with higher genetic quality, increasing the probability that offspring would survive.

Since the menstrual cycle is regulated by consistent shifts in circulating hormone concentrations, studies have attempted to examine the hormonal signals that may regulate ovulatory shifts in women's mate preferences (Jones et al., 2005; Roney & Simmons, 2008). Additionally, cyclical shifts in preferences for male face shapes may also be influenced by the context of the relationship. Women selecting male face shapes for a "long-term partner" showed no cyclical preference change; however, women selecting for male face shapes for a "short-term sexual partner" showed cyclical preference shifts (Penton-Voak et al., 1999). Conversely, other research suggests that there is no relationship between menstrual cycle and facial masculinity preference. Of particular note, one study found no differences in masculinity preferences throughout the different phases of the cycle, as well as no differences in masculinity preferences between short-term and long-term mating contexts (Marcinkowska et al., 2016).

While some research supports the idea that women's partner preferences change along the menstrual cycle (Penton-Voak & Perrett, 2000), the evidence for HC users' partner preference is less clear. Some studies demonstrate that HC usage decreases women's preference for masculinized faces (Little, Burriss, Petrie, Jones, & Roberts, 2013) while other studies demonstrate no such effect (Bobst, Sauter, Foppa, & Lobmaier, 2014). Little et al. (2013) tested within-subject changes in masculine face preferences in women initiating pill use. They found that pill use initiation was significantly associated with a reduction in women's preferences for male facial masculinity but did not affect preferences for same-sex faces. On the other hand,

Bobst et al. (2014) found no association between hormone levels and facial masculinity preference in HC users.

Lukaszewski, and Roney (2009) utilized a different type of mate preference task named the Trait Preference Survey (TPS). Instead of rating masculinized/feminized photographs, the TPS asks women to rate traits associated with dominance (traditionally associated with masculinity), kindness, and trustworthiness (the last two are less traditionally associated with masculinity). The TPS examines women's preferences for these traits in both short-term and long-term partner contexts. Lukaszewski, and Roney (2009) found that women's preferences for dominance in short-term mates were highest on cycle days when LH and FSH are typically peaking. I hope to elaborate upon their findings by examining differences in HC users and nonusers in conjunction with the facial attractiveness task.

My primary objective of this study is to extend previous findings and incorporate research regarding the change in hormones associated with HC usage and how this change influences women's partners preferences in terms of male attractiveness ratings and dominant behaviour. I hypothesize that HC users will rate feminized faces as more attractive than masculinized faces. I also hypothesize that HC users will rate dominance in partners as less important than NC women.

#### Methods

#### **Participants**

Participants included 154 female undergraduate students receiving course credit for their introductory psychology course. Only females who used combination pills or who were NC were included in data analyses. Of the remaining participants, 86 participants did not use birth control of any kind and 46 used combination pills. Most participants were Caucasian (43.94%), and a

smaller number of participants were Asian (27.27%), Indo-Mediterranean (10.61%), mixed (9.09%), African (7.58%), and Amerindian (1.52%). Mean age of the participants was 18.87 years (range = 16 - 46). All data was collected in a single session and the Human Research Ethics Boards at the University of Alberta approved the study (Pro00085370).

#### Measures

#### Bem Sex-Role Inventory Short Form (BSRI)

The BSRI (Bem, 1974) is a self-report measure of sex role orientation. The short form of the BSRI contains 20 items. The masculinity scale consists of 10 traits traditionally viewed as more desirable for men to exhibit than for women to exhibit. Sample items from the masculinity scale include "*independent*", "*competitive*", and "*aggressive*". The femininity scale consists of 10 traits traditionally viewed as more desirable for women to exhibit than men. Sample items from the femininity scale include "*compassionate*", "*sympathetic*", and "*sensitive to the needs of others*" (Bem, 1974).

#### Sociosexual Orientation Inventory Revised (SOI-R)

The SOI-R (Penke & Asendorpf, 2008) is a 9-item self-report questionnaire that measures individual differences in the tendency to have casual, and uncommitted sexual relationships. The inventory can be further divided into three facets: behaviours, attitudes, and desires regarding casual sex (e.g., "Sex without love is OK"). Lower scores indicate a more restricted sociosexuality, which is associated with interest in exclusive, long-term relationships. *Facial Preference Task* 

The Facial Preference Task was presented as a forced choice task: participants were shown face pairs and where asked to choose the more attractive face. Face pairs consisted of a masculinized and a feminized version of the same face (figure 3–1). Participants were presented

with frontal photographs of twenty male faces and twenty female faces with neutral expressions presented in random order (retrieved from https://doi.org/10.6084/m9.figshare.4220517.v1; DeBruine & Jones, 2017). The order of which photograph was masculinized in each pair was also randomized. Each face was transformed towards the male average and towards the female average prototype, resulting in a masculinized and in a feminized version of each male face. Transformation was a shape transformation only; the masculinized and feminized versions differed only in shape but not in colour or skin texture. Phase in menstrual cycle has been shown to correlated with skin colour preference (Frost, 1994); thus, only photographs of Caucasian men and Caucasian women standardized were used in the task. A transformation was applied to skin colour of photographs so that all pictures had identical and standardized skin colouration. For each stimulus, a transformation of 25% masculinized and 25% feminized was applied to ensure that the difference between the two versions was not too obvious (for further information see DeBruine & Jones, 2017).

#### Trait Preference Survey (TPS)

The TPS (Lukaszewski & Roney, 2009) contains 40-items examining preference for (1) dominance (*aggressive, assertive, bold, brave, competitive, dominant, leader, masculine, powerful, strong, take-charge, tough*), (2) kindness (*affectionate, considerate, generous, gentle, helpful, kind, sensitive, supportive, sympathetic, thoughtful*), and (3) trustworthiness (*committed, dependable, devoted, honest, loyal, reliable, sincere, trustworthy*). Participants were instructed to rate each trait term for importance in evaluating either a partner for "a brief sexual affair" and for "a committed romantic relationship such as marriage". The rating scale ranged from "1 – Not important" to "7 – Very important". The items were not organized by category and appeared in a scrambled order. Only items representing the positive extreme of each trait construct were

included (e.g., participants assess the term 'strong' but not 'weak'), prior literature has indicated that participants examine personality in a dichotomous manner such that individuals who are perceived to be high on traits like dominance are therefore perceived to be low on opposite traits such as submissiveness (Trapnell & Wiggins, 1990).

#### **Statistical Analyses**

R (v. 3.6.0) was used to analyse all data. T-tests were used to calculate mean differences between the HC group and the NC group. Multivariate analysis of variance (MANOVA) was used to create a model with multiple dependent variables. Multivariate analysis of covariance (MANCOVA) was used to investigate whether there was any effect of covariates on the model.

#### Results

#### **Individual Comparisons Between HC and NC Groups**

T-tests results are shown in table 3–1. There were no significant differences between the HC group and NC group in terms of age, BEM, or the Facial Preference task. HC users scored higher on all facets of the SOI-R, with scores being statistically significant for the attitude scale  $(t_{90.38} = -2.44; p = 0.016)$ , the behaviour scale  $(t_{63.97} = -3.13; p = 0.0026)$ , and total score  $(t_{87.46} = -2.51; p = 0.014)$ . On the TPS, the only significant difference between groups was for long-term partner dominance  $(t_{98.32} = 2.02; p = 0.046)$  where non-users rated dominance more preferentially than HC users, which is in opposition with previous findings (Lukaszewski & Roney, 2009). After using False Discovery Rate (FDR) corrections on the t-tests, only the behaviour scale of the SOI-R remained significant (p = 0.04), while the attitude scale of the SOI-R (p = 0.07) and total SOI-R remained near significance (p = 0.07)

#### Grand Scale Differences Between HC and NC Groups

All TPS variables were entered into a MANOVA as the dependent variables and HC use was entered as the independent variable. Again, only preference for dominance in long-term partner was significant (F = 3.19; p = 0.045; figure 3–2).

#### **Controlling for Covariates in the Model**

Considering that HC users had higher SOI-R scores, I used SOI-R has a covariate in the MANOVA model described above since it may be the case that women who have higher SOI-R scores prefer more dominant men in long-term relationships. However, including SOI-R as a covariate decreased significance, suggesting that the difference was due to HC usage.

#### **Comparing HC, Low Estrogen, and High Estrogen Groups**

Further dividing NC women into groups of high estrogen and low estrogen based on selfreports of their last period did not significantly change results.

#### Discussion

Similarly to Marcinkowska et al. (2016), I did not find any differences between hormonal contraceptive (HC) users and naturally cycling (NC) women on the facial preference task. Also, further dividing NC women in groups of participants with low estrogen and high estrogen did not alter results. However, unlike Lukaszewski and Roney (2009), I saw a very different trend in women's preference for male traits in hypothetical short- and long-term relationships. Instead of demonstrating that NC women prefer dominance in the short-term, and kindness and trustworthiness in the short-term, I found no differences between NC women and HC users on any measures of the TPS except that NC women prefer dominant men in the long-term. Not only do my findings oppose Lukaszewski and Roney (2009), they oppose most evolutionary psychology theories on the menstrual cycle's influence on mate preference (Jones et al., 2008).

HC use could affect a variety of behaviours related to relationships, starting with a woman's attraction to her partner and sexual satisfaction with her partner, leading to other aspects of relationship functioning (Roberts et al., 2011), including jealousy (Cobey, Pollet, Roberts, & Buunk, 2011) and possibly even relationship dissolution. The congruency hypothesis (Roberts, Cobey, Klapilová, & Havlíček 2013) suggests that when a partnered woman either begins to use or ceases to use HCs, she may experience changes in her relationship since her preferences become incongruent with those prevalent at the time of her partner choice. Roberts et al. (2013) compared the relationship satisfaction of women and men with respect to current HC use, HC use when the relationship first began, and congruency in HC use between these two times. HC use congruency predicted women's sexual satisfaction with their partners. HC congruency effects were only seen in women's satisfaction and not in male partners' satisfaction. This suggests that the congruency effect is likely due to changes in women's partner preference when they change their HC use rather than to changes in men's attraction to their partners as a result of their partners' HC use. Neither previous HC use nor current HC use predicted sexual satisfaction individually. Only when current use and previous use were examined together did significant effects emerge. Thus, a possible reason for not seeing the predicted hypothesis in the current study could be due to not considering women's preferences before initiation of HC use. In future studies, researchers should aim to examine women before HC use initiation and during HC use in order to examine individual differences.

However, the validity of the congruency hypothesis has been called into question recently. Jern et al. (2018) demonstrated that in a large sample, there was no effect of a woman's initiation or cessation of HC use during her relationship on relationship quality. Therefore, more research is needed in order to determine whether the congruency hypothesis is a worthy line of

research to continue exploring. Nonetheless, examining women pre-HC use and post-HC use initiation may still be a useful methodology to follow changes resulting from HCs.

There may be some alternative explanations for the results seen in the current study. Firstly, since women do not have a masculine face preference (i.e., an unconscious preference) but did show a preference for dominance traits (i.e., a more conscious preference), women may be actively reasoning that they want a dominant partner and are thus biasing their answers according to what they think they should want. Secondly, HC decreases the value women place on attractiveness in their current partner (Russell, McNulty, Baker, & Meltzer, 2014). Since women using HCs do not value attractiveness to the same extent as NC women, HC users may be answering at the facial attractiveness task haphazardly since they place less value on physical appearance. Lastly, many studies establishing an effect of menstrual cycle phase on partner preference were executed in decades ago (e.g., Penton-Voak et al., 1999). Previous studies demonstrating an effect of menstrual phase may have been due to false positives, or possibly due an environmental change. Currently, North American culture is becoming increasingly more progressive in terms of attitudes towards casual sex, while also having a decreased desire for rearing offspring (Sng, Neuberg, Varnum, & Kenrick, 2017). Therefore, women today may be less prone to consider male genetic contribution in the long term. Even the attitudes towards having a long-term partner are now different. The phrase "long-term partner" is arbitrary and does not indicate a specific amount, which is a limitation in the current study as well as many other studies. "Long-term partner" may also carry a different meaning currently then it previously did decades ago when research on this subject first began.

Some of the limitations of the present study included the fact that I relied on women to self report their phase in the menstrual cycle, which may not be accurate. Since some hormones

concentrations exhibit transient peaks on specific cycle days (Reilly, 2000), errors of even one or two days in estimated day of cycle could possibly alter correlations between estimated hormones and partner preferences. Secondly, women were tested on a single day. Utilizing a within-cycle shift design would be more compelling with a within-subjects design in which the same women are tested multiple times across different cycle days. Next, although there is some research that suggests that laboratory-tested partner preferences reflect real-life partner choices (DeBruine et al., 2006), further evidence that preferences in NC women reflect real-life partner choice and that HC use disrupts natural preferences is needed since the facial preference task may not be fully ecologically valid. Lastly, since HCs alter endogenous sex hormone levels (van Heusdan & Fauser, 1999) as well as exogenous sex hormone levels (Cobey et al., 2011), it may be that the decrease in endogenous hormones neutralizes the increase in synthetic hormones, resulting in no change in face preference. Therefore, future research needs to elucidate whether changes occur due to increase exogenous hormones or decreased endogenous hormones.

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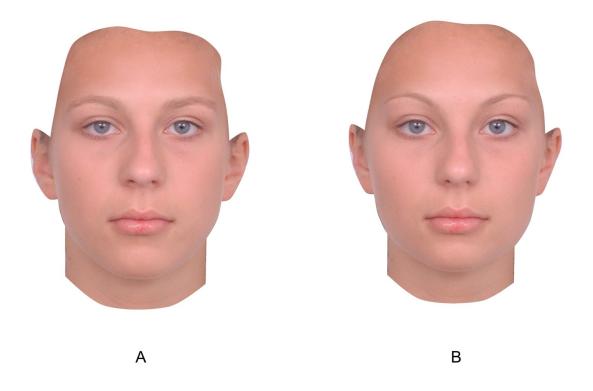
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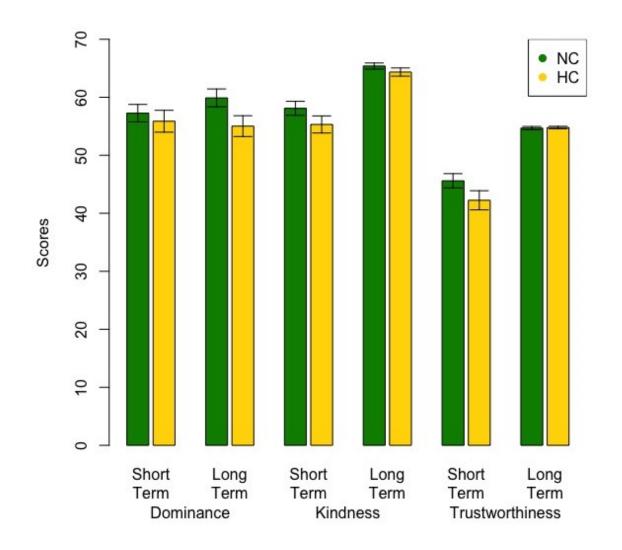
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**Figure 3–1.** Example of the Facial Preference Task. The task is presented as a forced choice task where participants must choose either picture A (50% masculinized) or B (50% feminized).

Variable	NC (n = 86)		HC $(n = 46)$		T-Test			
	М	SD	Μ	SD	<i>t</i> (df)	p-value	FDR Corrected p-value	
Age (Years)	19.13	3.86	18.39	1.08	1.65(107.54)	0.10	0.25	
BEM								
Female Scale	57.49	8.96	55.28	9.60	1.29(87.05)	0.20	0.35	
Male Scale	44.09	9.22	43.84	8.95	0.14(93.06)	0.89	0.89	
SOI-R	24.77	11.55	30.27	12.00	-2.51(87.46)	0.014**	0.07•	
Attitude Scale	11.50	7.22	14.80	7.43	-2.44(90.38)	0.016**	0.07•	
<b>Behaviour Scale</b>	4.49	2.52	6.51	3.91	-3.13(63.97)	0.0026***	0.04*	
Desire Scale	8.57	5.36	9.28	4.75	-0.78(102.67)	0.44	0.56	
Facial Preference Task	15.22	6.27	14.13	7.01	0.88(83.63)	0.38	0.53	
TPS Short Term								
Dominance	57.26	13.76	55.87	12.05	0.59(101.77)	0.56	0.65	
Kindness	58.10	10.82	55.31	9.88	1.47(98.23)	0.15	0.29	
Trustworthiness	45.60	11.21	42.24	11.21	1.63(93.02)	0.11	0.25	
TPS Long Term								
Dominance	59.89	13.62	55.02	12.79	2.02(98.32)	0.046*	0.16	
Kindness	65.38	4.78	64.35	4.78	1.18(92.69)	0.24	0.38	
Trustworthiness	54.68	2.59	54.78	1.41	-0.28(126.94)	0.78	0.84	

- p < .10\* p < .05 \*\* p < .01 \*\*\* p < .001



**Figure 3–2.** Differences between hormonal (HC; yellow) users and naturally cycling (NC; green) women on the Trait Preference Survey (TPS). Scores were only significantly different for long-term dominance ( $t_{98.32} = 2.02$ ; p = 0.046).

# Chapter 4: Association Between Stress Reactivity and Hormonal Contraceptive Use Introduction

In the past decade, there has been growing interest on the effects of hormonal contraceptives (HC) on women's behaviour and psychology. The interaction between HC usage and stress reactivity is an important line of research to pursue as stress affects women's everyday lives. Females represented a meager 17% of subjects in hypothalamic–pituitary–adrenal (HPA) axis studies until as recently as 1995 (Taylor et al., 2000). Female subjects were systematically excluded due to their cyclic HPG-axis variations and despite the fact that the inclusion of women in the psychoneuroendocrine studies of stress physiology has increased (Taylor et al., 2000), within-sex variations in sex hormones are assumed to exist but are rarely measured. This fact is increasingly important presently, where over 100 million women use HCs worldwide (Christin-Maitre, 2013), and other factors (i.e., hormonal contraceptives) are even less often considered.

The relationship between the HPA and hypothalamic–pituitary–gonadal (HPG) axes – responsible for the situational release of cortisol and sex hormones, respectively – is well established (Viau, 2002). The HPA and HPG are interconnected; therefore, a change in one axis may induce a change in the other axis (Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999). Because HCs alter naturally circulating levels of sex hormones (i.e., a change in the HPG axis; Beck, Cowsar, & Pope, 1980), they may in turn stimulate a change in the HPA axis. Strong sex differences in cortisol stress reactivity have been reported: compared to women, men showed a two-fold higher cortisol reactivity after public speaking and mental arithmetic in front of an audience (Kirschbaum, Wüst, & Hellhammer, 1992). Moreover, men had increased salivary cortisol after stress tasks and that sex hormones modified sex-specific reactive and diurnal cortisol levels (Juster et al., 2016). Reschke-Hernandez, Okerstrom, Bowles Edwards, and Tranel

(2017) utilized two different types of stress tasks to examine whether stress reactivity differed in terms of the type of task: Trier Social Stress Test (TSST; involves public speaking and math) and the Iowa Singing Social Stress Test (I-SSST; similar to the TSST but includes a singing component). Men and women reported similarly high levels of subjective stress after both types of tasks. However, men and women exhibited different cortisol responses. Men demonstrated a more robust response to both tasks compared to women (Reschke-Hernandez et al., 2017).

Women's stress and cortisol reactivity vary depending on their phase in the menstrual cycle. Women using HCs have greater attenuation of cortisol stress reactivity, reasoned as an estradiol driven effect (Kirschbaum, Pirke, & Hellhammer, 1995). HCs contain high levels of synthetic estradiol that stimulate the production of cortisol-binding globulin, which in turn removes cortisol from circulation, thus resulting in lower concentrations of circulating cortisol (Kirschbaum et al., 1995). Subsequent studies assessed cortisol reactivity at different menstrual cycle phases to further examine HPA-axis and HPG-axis interactions. Women with low estrogen display reactivity patterns more similar to men, while women with high estrogen display reactivity patterns more similar to women using HCs (Kirschbaum et al., 1999). Therefore, acute stimulation of the HPA-axis is related to estrogen regulation in women. However, findings on the direct effects of HCs and stress reactivity are more contradictory (e.g., Crewther, Hamilton, Casto, Kilduff, & Cook, 2015; Hertel et al., 2017; Roche, King, Cohoon, & Lovallo, 2013) and less well understood (Merz et al., 2012). Roche et al. (2013) demonstrated that HC users had a blunted cortisol response to naltrexone and a stressor compared to non-users. Yet, Hertel et al. (2017) showed that HC users had elevated circulating cortisol levels.

Interestingly, some studies have demonstrated that psychopathy may be associated with stress (Welker, Lozoya, Campbell, Neumann, & Carré, 2014) as well as with sex hormone

concentrations (O'Leary, Loney, & Eckel, 2007). As these variables are interconnected, there may potentially be an interaction between HC, stress reactivity, and psychopathy traits. Cortisol production has been found to be a gender-specific marker for psychopathic personality traits. Male participants scoring higher in psychopathic personality traits lacked the stress induced increase in cortisol demonstrated by males who scored lower in psychopathic personality traits (O'Leary et al., 2007). Moreover, nonclinical variability in psychopathy may be predicted by baseline testosterone and cortisol levels. Welker et al. (2014) showed that cortisol moderated the relationship between testosterone and psychopathy in men. Testosterone and psychopathy had a positive relationship in men when cortisol levels were high, but had a negative relationship when cortisol levels were low (Welker et al., 2014). However, this relationship was only observed in male participants, and there was no relationship between testosterone and psychopathy needs further clarification in women using HCs.

The present study explored the following objectives: (1) compare reactive cortisol in HC users vs. NC women; (2) compare depression and anxiety levels between these two groups; (3) examine the potential association between HCs, stress, and psychopathy. I hypothesize that HC users will have increased stress response compared to women who do not use HC, and that their cortisol levels will more closely resemble that of men (i.e., higher salivary cortisol levels after the stress task). Also, I hypothesize that HC users will score high on the psychopathy measures.

## Methods

### **Participants**

Participants included 51 female students who were recruited using an undergraduate research participant pool where individuals receive course credit for participating. Only NC

women or women who used combination pills were included in data analyses. In the remaining sample, there were 31 NC participants and 17 participants used combination pills. The mean age of the participants was 18.81 years (range = 18–26). The ethnic background of participants in this study was 35.42% Caucasian, 33.33% Asian, and 12.50% African, 10.42% mixed, 4.17% Indo-Mediterranean, 4.17% other. The Human Research Ethics Boards at the University of Alberta approved this study (Pro00085688).

# **General Protocol**

Experiments were performed between 1:00 pm and 3:00 pm to minimize any circadian rhythm effects. During a 50 minute laboratory visit, participants (a) were requested to read and sign the consent form; (b) given a cup of water to drink in order to rinse mouths (c) provided 3 saliva samples at 3 different time points (baseline, 10 minutes post stress task, 20 minutes post stress task); (d) were exposed to the Mental Arithmetic Task; (e) were instructed to complete a questionnaire; and (f) were debriefed.

### Measures

## Visual Analogue Scales (VAS)

Participants were asked to rate their subjective stress before and after the mental arithmetic task on visual analogue scales (VAS). A VAS is a bipolar line that measures a characteristic across a continuum (Bond & Lader, 1974). Participants were instructed to mark a spot on the line that most closely resembled their subjective appraisal of their current stress. The scale was anchored from "0 - Feeling not stressed" at all to "30 - Feeling highly stressed." Depression Anxiety Stress Survey (DASS) – Short Form

The brief 21-item version of the Depression Anxiety Stress Scales (DASS; Lovibond & Lovibond, 1996) is a self-report measure of state negative affect with three 7–item self-report

scales (depression, anxiety and stress) taken from the full version of the DASS. Participants indicate the extent to which they experienced each of the symptoms depicted in the items during the previous week on a 4-point Likert-type scale between "0 - Did not apply to me at all" and "3 - Applied to me very much, or most of the time". The DASS-21 scales possess good convergent and discriminant validity, and high internal consistency in clinical and in non-clinical samples as well as in different ethnic groups in adults (Lovibond, 1998, Lovibond & Lovibond, 1996, Norton, 2007).

## State-Trait Anxiety Inventory (STAI)

Trait anxiety was assessed using the 20-item trait anxiety scale from the State-Trait Anxiety Inventory (STAI; Spielberger, 1983). Items are rated on a 4-point Likert scale with responses ranging from "I - Almost never" to "4 - Almost always" (e.g., "I worry too much over something that really doesn't matter" and "I feel that difficulties are piling up so that I cannot overcome them"). The STAI is a well-established anxiety measure with strong psychometric properties (Barnes, Harp, & Jung, 2002).

# State-Trait Anger Expression Inventory-2 (STAXI-2)

The State–Trait Anger Expression Inventory–2 (STAXI-2; Spielberg, 1999) is a psychometric assessment that measures the experience, expression, and control of anger in research and clinical settings. The inventory is divided into four subscales for expression and control (anger expression-in, anger expression-out, anger control-in, and anger control-out). All items are rated on a 4-point Likert scale ranging from "1 - Almost never" to "4 - Almost always".

# Levenson Self-Report Psychopathy Scale (LSRP)

Levenson Self-Report Psychopathy Scale (LSRP; Levenson, Kiehl, & Fitzpatrick, 1995)

is a 26-item scale containing a 16-item *Primary Psychopathy* subscale, which measures the distinctive psychopathic personality traits that differentiate psychopathy from the more general traits of antisocial behaviour (e.g., "I enjoy manipulating other people's feelings" and "I do not feel bad if my words or actions cause someone else to feel pain"; Hare, Hart, & Harpur, 1991). Items on the LSRP are rated on a 4-point Likert scale, ranging from "*1*–*Strongly disagree*" to "*4*–*Strongly agree*". The LSRP has demonstrated appropriate reliability and validity in prior research using general samples and college-based samples (Levenson et al., 1995).

#### **Mental Arithmetic Task**

Participants were confronted with a mental arithmetic task of 10-min duration, which induces stress and increases salivary cortisol (Noto, Sato, Kudo, Kurata, & Hirota, 2005). The task required serial subtraction of 7 from a 986. Participants were instructed to answer verbally at each subtraction. After confirming that the answer was correct, another subtraction of 7 from the correct number of the previous trial shown on the screen was performed. Participants were also told that they needed to answer as quickly as possible. If participants answer too slowly or incorrectly, they needed to start from the beginning. The researcher wore a lab coat, held a ticking stopwatch, and took notes during the duration of the task.

### **Cortisol Sampling and Analysis**

Saliva samples were collected in pre-chilled polypropylene 15 mL tubes via passive drool. Samples were kept on dry ice until they were stored and frozen at -80°C (Procyshyn, Spence, Read, Watson, & Crespi, 2017). All salivary samples were collected between 1 pm and 3 pm in order to control for circadian rhythm effects. Participants were given a glass of water before starting the study in order to produce cleaner samples. Afterwards, participants were prohibited from eating or drinking during the remainder of the study. Participants produced a

minimum of 2 mL of saliva at three different time points: baseline (i.e., immediately after signing the consent form and before completing the mental arithmetic task), 10 minutes after the mental arithmetic task, and 20 minutes after the second saliva sample. Salivary cortisol measurements were executed using Enzo Life Sciences enzyme-linked immunosorbent assay kits ADI-901-153 (ELISA; http://www.enzolifesciences.com/ADI-900-071/cortisol-elisa-kit/). All samples were run in duplicate to ensure reliability. Samples were processed in accordance with the manual's ELISA protocol (see chapter 2 methods for complete details on ELISA kit processing). After completion of the ELISA processing, samples were immediately read at 405 nm. Cortisol concentrations were later calculated from the standard curve.

# **Statistical Analyses**

R (v. 3.6.0) was used to analyse all data. T-tests were used to calculate mean differences between the HC group and the NC group. Pearson's product-moment correlations were used to examine the strength of the linear relationship between two variables. Analysis of covariance (ANCOVA) was used to examine HC use as an independent variable and include a covariate. To create a single variable examining the difference between baseline salivary cortisol level and post-stress salivary cortisol level, I subtracted baseline values from post-stress values and named this variable  $\Delta$ cortisol. The same process was also performed on subjective stress values (i.e., VAS Scores), and this variable was named  $\Delta$ VAS. Principal component analysis (PCA) was used as a dimension-reduction tool by reducing the large set of variables into a smaller set, which still contained most of the information from the original dataset.

#### Results

### **Individual Comparisons Between HC and NC Groups**

There were no mean differences between HC and NC groups on any variable (table 4–1). Specifically, baseline cortisol and post-stress cortisol did not significantly differ between HC users and non-users (figure 4–1), nor did baseline VAS and post-stress VAS.

# HC Usage, $\Delta$ Cortisol, and $\Delta$ VAS Associations with Individual Measures

Pearson's product-moment correlations were used to assess any relationships between HC usage and any measures. HC usage was not significantly correlated with any of the measures. However,  $\Delta$ VAS and  $\Delta$ cortisol were negatively correlated in the NC group (r = -0.65; p = 0.0032), but are not correlated in the HC group (r = 0.25; p = 0.38), which would suggest that NC women are not aware of their stress response. Also, the stress subscale from the DASS and  $\Delta$  cortisol were negatively correlated in the NC group (r = -0.47; p = 0.042), but were not correlated in the HC group (r = 0.96).

LSRP scores were not correlated with  $\Delta VAS$  or  $\Delta$  cortisol. However, in HC users only, LSRP scores were positively correlated with DASS scores (r = 0.70; p = 0.0035), specifically with the anxiety (r = 0.60; p = 0.015) and stress subscales (r = 0.75; p = 0.00081).

## Interaction Between HC Usage, \(\Delta\)Cortisol, and \(\Delta\)VAS

Analysis of Covariance (ANCOVA) revealed a significant interaction between HC use and  $\Delta$ VAS scores whilst controlling for  $\Delta$  cortisol (figure 4–2). There was a significant difference in mean  $\Delta$ VAS scores ( $F_{1,29} = 9.12$ , p = 0.005) between the groups. In HC users, as cortisol increases, subjective stress increases. However, in NC women, an increase in cortisol was not associated with an increase in subjective stress. Instead, subjective stress decreased as cortisol increased in NC women.

### **Principal Components Differ By Group**

First, PCA was performed using the R *prcomp* function on all measures in HC users only. A scree plot was used to determine the number of components to examine. Principal components (PC) with eigenvalues greater than 1 were kept for analysis. In the HC PCA, only the first two PCs were kept. The loadings of each subscale on components 1 and 2 can be found in table 4–2. Principal component 1 (PC1) had positive loadings for all variables and demonstrated strong positive loadings for the anxiety scale of the DASS, the stress scale of the DASS, and STAXI-2 (figure 4–3). PC1 accounted for 60% of the variance. Principal component 2 (PC2) had positive and negative loadings with the variables, and it demonstrated a strong positive loading for the depression scale of the DASS only. PC2 it accounted for 18% of the variance.

Next, a PCA was performed on all measures in NC women only. Again, a scree plot was used to determine which PCs should be examined and in the NC PCA, 3 PCs were kept. The loadings of each subscale on components 1, 2, and 3 can be found in table 4–3. PC1 demonstrated strong positive loadings for all DASS scales, and accounted for 47% of the variance (figure 4–4). PC2 demonstrated a strong positive loading for STAI, STAXI-2, and  $\Delta$ cortisol, and it accounted for 22% of the variance. Principal component 3 (PC3) demonstrated a very strong negative loading for LSRP only, and accounted 14% of the variance. Comparing figure 4–3 and figure 4–4 reveals that the correlation of the PCs in the HC PCA and NC PCA differ. In the HC PCA, most measures (except  $\Delta$ cortisol) correlated solely onto PC1. However, in the NC PCA, the measures are distributed more throughout PC1, PC2, and PC3.

### Discussion

I found that hormonal contraceptive (HC) users and naturally cycling (NC) women did not differ significantly on any measure, including subjective stress, salivary cortisol, or psychopathic traits. However, when using an ANCOVA with subjective stress as the dependent

variable, HC use as the independent variable and salivary cortisol as the covariate, I found that NC women had subjective stress scores decrease as their cortisol increased. My results oppose the hypothesis since this would suggest that NC women are not as aware of their stress response as contraceptive users. It may be that women who seek the usage of HCs are more self-aware of their physiological stress response. I also found that psychopathic traits were associated with stress and anxiety scores in HC users and this association was no observed in NC women, which suggests that there may in fact be a relationship between contraceptives, stress, and psychopathy.

An important limitation of the present study is the diurnal variability of cortisol production. Cortisol peaks and is less variable in the morning, and then stabilizes but becomes more variable in the afternoon (Fries, Dettenborn, & Kirschbaum, 2009). Even though salivary samples were always taken between 1 and 3 pm, there is still a great deal of variability in cortisol sampling. The variability may be due to individual HPA-axis differences as well as differences in the participants external environmental (i.e., drinking coffee, exercising, or other stimuli that could influence cortisol levels before arriving to the study). Also, the participants consisted of university students; thus, they may have abnormal circadian rhythms due to having odd wake and sleep schedules as compared to the general population.

In a study examining a time of day effect in HC users, salivary cortisol responses to stress were largest in men and progressively smaller in NC women and in HC users (Lovallo, Cohoon, Acheson, Vincent, & Sorocco, 2019). In the morning sessions, HC users had significantly elevated rest day cortisol levels along with a complete absence of response on the stress day. In the afternoon sessions, both HC users and non-users had normal rest-day cortisol levels and normal responses to the stressors. Cortisol stress responses in HC users are absent in the morning

and normal by the afternoon. Therefore, studies of stress reactivity should account for time of day in evaluating cortisol responses in women using HCs.

A second limitation from the current study is that I did not administer HC in a randomized controlled setting but instead used naturally occurring groups that voluntarily did or did not use HC. This carries the risk that I may have overseen influences from factors other than the HCs. Future studies should either employ a random experimental administration of HC while ensuring participants use non-hormonal contraceptives to reduce the risk of pregnancy. Future research may also benefit from directly measuring progesterone and estradiol levels of the participants.

An alternative explanation of why I did not see the hypothesized differences between groups may be due HCs interaction with behavioural stress responses rather than hormonal stress responses. Taylor et al. (2000) introduced the tend-and-befriend pattern of behaviour first associated with women's stress response, but later expended it to include men as well (Taylor, 2006). The tend-and-befriend pattern is an addition to the fight-or-flight response and has underlying mechanisms involving oxytocinergic, opioid or dopaminergic actions. Evidence for tend-and-befriend reactions to stress has been found. Researchers identified higher levels of trust, trustworthiness, and sharing during acute stress in men (von Dawans, Fischbacher, Kirschbaum, Fehr, & Heinrichs, 2012). For instance, the cortisol response level in the fast-friends-procedure, a stress paradigm that examines social interactions in the lab (Aron, Melinat, Aron, Vallone, & Bator, 1997), was positively correlated with the psychological closeness to the interaction partner (Berger, Heinrichs, von Dawans, Way, & Chen, 2016). In a separate study, women had increased preference towards feminized male faces after stress, which suggests that the stress response increases the focus on female attributes (Ditzen, Palm-Fischbacher, Gossweiler, Stucky, &

Ehlert, 2017). Additionally, acute stress has even been demonstrated to increase empathy (Wolf et al., 2015). While I may not have seen a change in cortisol reactivity, there may be a behavioural change associated with it in terms of fight-or-flight, tend-and-befriend, empathy, etc. Future studies should examine how these behavioural and psychological facets change after stress paradigms in HC users vs. non-users.

In summary, the current study provides some support for the hypothesis that HCs may influence stress and cortisol reactivity. Although mean salivary cortisol did not differ between groups, there is an interaction between HCs, stress, and cortisol reactivity once entered into the model. However, the interaction I found was not what was expected. Women using HCs actually seemed more aware of their stress response than NC women, which may be due to women with a better understanding of their stress response seeking out the use of HCs. I also found a positive correlation between psychopathic personality traits and stress, and this association was only observed in HC users.

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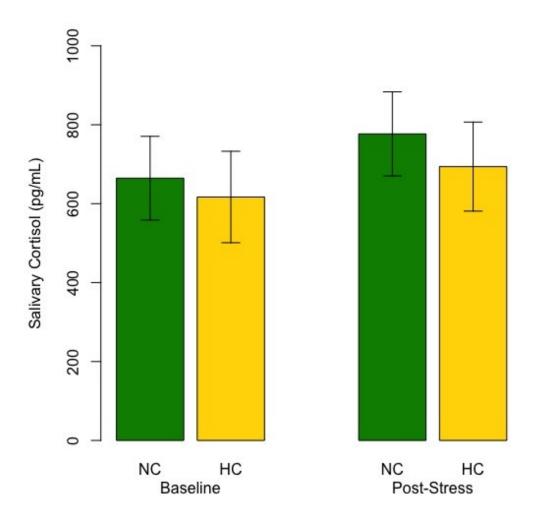
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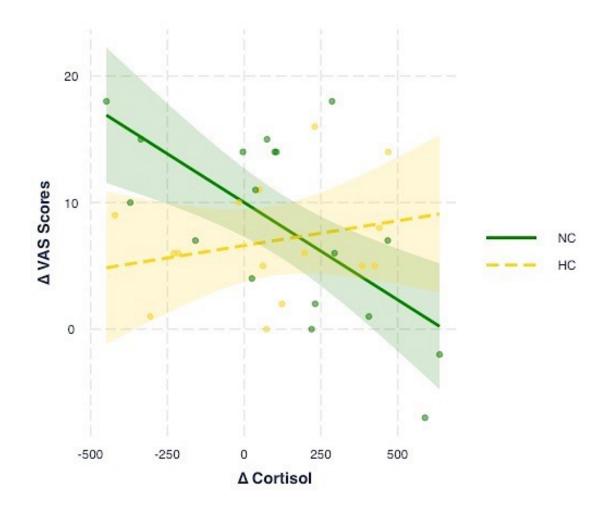
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Variable	NC (n = 31)		HC (r	n = 17)	T-Test	
	Μ	SD	Μ	SD	<i>t</i> (df)	p-value
Age (years)	18.58	1.18	19.24	1.92	-1.28(22.75)	0.21
STAI	96.66	18.50	103.75	13.48	-1.47(39.51)	0.15
STAXI-2	60.45	11.18	59.73	11.09	0.20(28.61)	0.84
LSRP	48.50	7.37	50.00	8.76	-0.58(27.10)	0.57
DASS	23.26	12.85	23.38	10.84	-0.03(35.34)	0.97
Depression Scale	7.29	5.34	7.63	4.18	-0.24(37.62)	0.81
Anxiety Scale	7.35	4.70	7.24	3.53	0.10(41.40)	0.92
Stress Scale	8.61	4.59	9.06	4.60	-0.32(33.01)	0.75
ΔVAS	7.70	6.48	6.81	4.39	0.55(41.31)	0.59
Baseline VAS	10.03	7.19	11.53	6.48	-0.74(36.11)	0.47
Post-Stress VAS	18.03	6.16	18.06	6.90	-0.01(27.83)	0.99
∆Cortisol	140.84	316.86	76.99	275.25	0.64(32.96)	0.53
<b>Baseline</b> Cortisol	664.66	474.15	616.94	463.24	0.30(32.60)	0.76
Post-Stress Cortisol	776.82	464.42	693.93	451.24	0.53(32.29)	0.60

 Table 4–1. T-tests between combination pill (CP) users and naturally cycling (NC) women.



**Figure 4–1.** Salivary cortisol levels in hormonal contraceptive (HC) users and naturally cycling (NC) women at baseline and after the mental arithmetic task. Although, cortisol levels were not significantly different at either phase, cortisol level is decreased slightly in HC users.



**Figure 4–2.** Hormonal contraceptive (HC) use and salivary cortisol level interact and influence subjective stress levels ( $\Delta$ VAS). In HC users (yellow), as cortisol increases, subjective stress increases. However, in naturally cycling (NC; green) women, an increase in cortisol was not associated with an increase in subjective stress, rather, subjective stress decreased as cortisol increased. The confidence intervals are represented as the shaded regions.

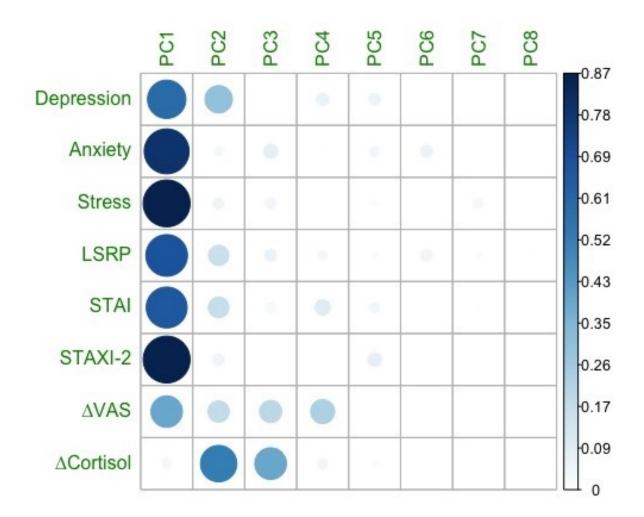
Measure	PC1 (60%)	PC2 (18%)	
DASS			
Depression Scale	0.345	0.452	
Anxiety Scale	0.404	-0.139	
Stress Scale	0.421	-0.174	
LSRP	0.374	-0.335	
STAI	0.368	0.341	
STAXI-2	0.421	-0.183	
$\Delta VAS$	0.283	0.353	
ΔCortisol	0.082	-0.600	

 Table 4–2. Principal components 1 and 2 loadings across each variable for hormonal contraceptive (HC) users.

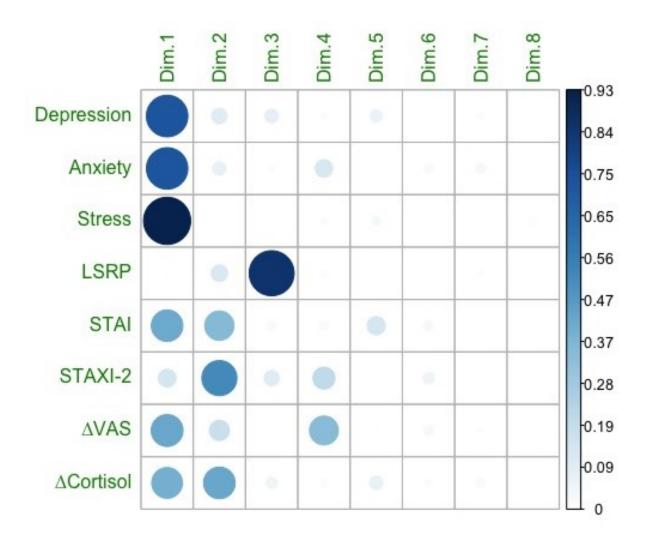
Measure	PC1 (47%)	PC2 (22%)	PC3 (14%)
DASS			
Depression Scale	0.439	0.238	0.261
Anxiety Scale	0.438	-0.206	-0.102
Stress Scale	0.498	-0.023	-0.065
LSRP	0.033	0.255	0.870
STAI	0.333	0.454	-0.156
STAXI-2	0.188	0.542	-0.291
$\Delta VAS$	0.337	-0.310	-0.083
ΔCortisol	-0.325	0.489	-0.210

 Table 4–3. Principal components 1, 2, and 3 loadings across each variable for naturally cycling

(NC) women.



**Figure 4–3.** Visualization of a correlation matrix between principal components (PC) and variables in the model in hormonal contraceptive (HC) users only.



**Figure 4–4.** Visualization of a correlation matrix between principal components (PC) and variables in the model in naturally cycling (NC) women only.

# Chapter 5: A Comparison of Naturally Cycling Women, Combination Pill Users, and Intrauterine Device Users

## Introduction

Intrauterine devices (IUDs) are small, plastic or metal devices inserted into the uterus that provide extended protection against pregnancy, primarily by interfering with sperm and eggs. The two main types of IUDs include hormonal IUDs, which release a localized hormone called levonorgestrel, and copper IUDs, which do not contain hormones. The levonorgestrel (LNG)medicated intrauterine device block sperm from getting to the egg by: 1) Releasing levonorgestrel (a progestogen) to keep ovaries from releasing eggs (unlike HC pills that release hormones into the bloodstream, levonorgestrel is a localized hormone released solely in the uterus); 2) thickening the mucus of the cervix so the sperm cannot reach the egg; 3) inhibiting the sperm's ability to swim toward the egg (Guillebaud, 2003). On the other hand, the copper IUD does not release any hormones. The copper IUD functions by releasing copper ions into the cervix, which transforms the uterus into a more hostile environment for incoming sperm. Due to the copper ions, the cervix produces a thick mucus that sperm are unable to navigate through to reach the ovum (Ortiz & Croxatto, 2007). Because both IUD users and HC users seek out the use of contraceptives and IUDs should not alter circulating sex hormones, IUD users represent an interesting group to compare with HC users. There may be a personality, mood, behavioural, or other differences associated with choosing a certain type of contraceptive over the other. Informed choices on contraceptives are correlated with increased satisfaction and compliance with the method used (Frost, Singh, & Finer, 2007). Therefore, we should provide women with a more complete knowledge of possible physical and psychological side effects pertaining to all types of contraceptive methods, including IUDs.

IUDs were introduced shortly after hormonal contraceptives (HCs) in the 1960s (Sonfield, 2007). IUDs were initially portrayed as an easier-to-use and more convenient alternative to HCs. By the early 1970s, almost 10% of U.S. women practicing contraception were relying on IUDs (Sonfield, 2007). However, in 1973, there were serious health problems with the Dalkon Shield – a new and heavily marketed, plastic IUD that had rapidly become the most popular model in America. By 1974, after multiple deaths from septic miscarriages, sales were first suspended in the United States and were then suspended globally. For a decade, the media emphasized numerous studies linking IUDs to pelvic inflammatory disease and consequent infertility. Although better and safer IUDS were later created, IUDs have not fully recovered their reputation as a major contraceptive option for American women (Sonfield, 2007).

Currently, IUDs are used by 4.7% of North American women in relationships (figure 5– 1; United Nations, 2015). Yet, in other parts of the world, IUDs are significantly more popular: 17.4% of Asian and 11.3% of European of married or in-union women use IUDs (United Nations, 2015). Specifically, in Europe, much of the discrepancy between rates of HC use and IUD use is due to more positive attitudes among health care practitioners and contraceptive users about the IUDs' safety, side effects, and other benefits (Sonfield, 2007).

IUD usage has many benefits over HC usage. IUDs are safe, rapidly reversible, inexpensive, highly effective, and long acting (Hubacher, Chen, & Park, 2009). IUDs are easier and more convenient to use as women only need to attend one appointment to have an IUD inserted rather than take a pill daily and continuously for years, which reduces the chance of human error. IUDs are a long-term solution to preventing pregnancy and need only be changed every 4 years on average for hormonal IUDs, and up to every 20 years for certain copper IUDs (Sivin, 2007). Yet, IUDs have some negative side effects that may deter contraceptive users including increased menstrual bleeding and inter-menstrual bleeding, as well as increased pain; however, side effects decrease over time (Hubacher et al., 2009).

There are many factors involved in women choosing a certain type of contraceptive over another. Women frequently note efficacy, accessibility, convenience, and adverse side effects as important considerations when choosing contraceptive methods (de Irala et al., 2007). Socioeconomic status (SES) can likely be ruled out as factor since Canadian health insurance provides similar coverage for HCs and IUDs. On the other hand, reproductive intentions, marital status, personal beliefs, and sexual lifestyles largely influence women's choice of contraceptive method (Oddens & Lehert, 1997).

In this study, I explored potential influences and interactions between type of contraceptive used (either HC or IUD) and multiple psychological measures. Because of the dearth of research on differences in terms of personality, psychology, and behaviour between users of differing types of contraceptives, I hypothesize that there will be multiple differences between HC users and IUD users; however, the exact differences between these groups are ambiguous.

# Methods

### **Participants**

Data on IUD users was collected throughout several other studies. 741 female participants were recruited using an undergraduate research participant pool where individuals receive course credit for participating. The mean age of the participants was 18.99 (range =16 – 46). 377 (51.15%) participants were Caucasian, 178 (24.15%) were Asian, 53 (7.19%) were mixed ethnicity, and 47 (6.38%) were African. 423 (57.09%) participants did not use birth control of any kind and were considered naturally cycling (NC), 236 (31.85%) used combination pills, and 38 (5.13%) used intrauterine devices. The Human Research Ethics Boards at the University of Alberta approved the studies (Pro00015728, Pro00078461, Pro00085370, Pro00085688).

# Measures

Participants were administered a questionnaires that included questions on demographic information, the Reading the Mind in the Eyes (RMET), the Borderline Symptom List 23 (BSL-23), the BSL-Supplement: Items for Assessing Behaviour, the Buss Perry Aggression Questionnaire (BPAQ), the Empathy Quotient (EQ-Short), the Oxford Happiness Questionnaire (OHQ), the NEO–Personality Inventory (NEO–PI) – N3 Scale, the Binge Eating Scale (BES), the Experiences in Close Relationships-Relationship Structures Scale (ECR-RS), and the Beck Depression Inventory (BDI-IA; see chapter 2 for full descriptions of these measures); Trait Preference Survey (TPS), Bem Sex-Role Inventory Short Form (BSRI), Sociosexual Orientation Inventory Revised (SOI-R), Facial Preference Task (see chapter 3); the Depression Anxiety Stress Survey (DASS) – Short Form, the State-Trait Anxiety Inventory (STAI), and the State-Trait Anger Expression Inventory-2 (STAXI-2; see chapter 4 for full descriptions of these measures). Along with the measures from previous chapters, participants were also administered the following five measures.

# Schizotypal Personality Questionnaire-Brief Revised (SPQ-BR)

The SPQ-BR (Cohen, Matthews, Najolia & Brown, 2010) was used to measure schizotypy in participants. The SPQ-BR contains 32 items rated on a 5-point Likert scale from *"Strongly disagree"* to *"Strongly agree"*. SPQ-BR scores can range from 0 to 160, where higher scores reflect increased levels of schizotypy. The SPQ-BR can be divided and categorized into three dependent measures: cognitive-perceptual (includes subscales of ideas of reference, odd

beliefs or magical thinking and unusual perceptual experiences), interpersonal (includes subscales of constricted affect and excessive social anxiety), and disorganized scores (includes subscales of odd or eccentric behaviour and odd speech).

# Autism Quotient (AQ)

The AQ (Baron-Cohen, Wheelwright, Skinner, Martin, & Clubley, 2001) quantifies where an individual is situated on the continuum from autism to normality. The AQ consists 50 statements. Participants are asked to indicate whether they "*strongly agree*", "*slightly agree*", "*slightly disagree*" or "*strongly disagree*" with each statement. Each item is scored as either zero or one, with one point being awarded if the participant chooses the "autistic trait" response. For half of the items, the "autistic trait" response is "*slightly/strongly agree*", and for the other half the "autistic trait" response is "*slightly/strongly disagree*". A total score is calculated by summing all items together, and scores can range from 0 to 50.

### **Big-Five Mini-Markers**

Saucier's (1994) Big-Five Mini-Markers were used to measure the Big-Five personality factors (i.e., agreeableness, conscientiousness, extraversion, neuroticism, and openness). The Mini-Markers consist of 40 adjectives (8-items for each personality factor). Participants are instructed to rate themselves on each trait using a 9-point Likert scale, ranging from "1 - Extremely inaccurate" to "9 - Extremely Accurate". Scores for individual personality factors are summed together to obtain personality scores. The Mini-Marker factors have demonstrated acceptable internal consistency ( $\alpha = .78 - .86$ ; Saucier, 1994).

Cognitive and Affective Mindfulness Scale – Revised (CAMS-R)

The 10-item version of the CAMS-R (Feldman, Hayes, Kumar, Greeson, & Laurenceau, 2007) measures a broad conceptualization of mindfulness (e.g., "It is easy for me to concentrate

on what I am doing", and "It's easy for me to keep track of my thoughts and feelings"). Participants are asked to rate each statement on a 4-point Likert scale, ranging from "1 - Rarelyor not at all" to "4 - Almost always". Higher scores indicate increased mindfulness. The CAMS-R has demonstrated acceptable internal consistency ( $\alpha = .74 - .77$ ) as well as appropriate convergent and discriminant validity with measures of mindfulness, well-being, emotionregulation, distress, and problem-solving (Feldman et al., 2007).

# Mindfulness Attention Awareness Scale (MAAS)

The MAAS (Brown & Ryan, 2003) is a 15-item measure of trait mindfulness. The MAAS measures open and receptive attention, and awareness of on-going experiences (e.g., "I find it difficult to stay focused on what's happening in the present," "I could be experiencing some emotion and not be conscious of it until some time later," and "I rush through activities without being really attentive to them"). Participants are instructed to rate each item on a scale from "1 - Almost always" to "6 - Almost never". Total scores are calculated by summing the scores of each item, with higher scores indicating greater mindfulness.

## Short Dark Triad (SD3)

The SD3 (Jones & Paulhus, 2014) measures three socially aversive traits – machiavellianism, narcissism, and psychopathy – which have been grouped together and are known as the Dark Triad. The SD3 contains 27 items (9 of each trait) and participants are asked to rate each item on a Likert scale from "1 - Strongly Disagree" to "5 - Strongly agree". Five items are reversed scored. Scores can be calculated for each individual trait and total scores can be calculated by summing all subscale scores together.

### **Statistical Analyses**

I first compared naturally cycling (NC) women to women who used contraceptives (either HCs or IUDs). Next, when divided the contraceptive users into two groups – combination pill users and intrauterine device users – and compared these two groups together. Because I performed multiple t-tests, I used a False Discovery Rate (FDR) correction on the p-values.

## Results

### **Individual Comparisons Between HC and NC Groups**

Table 5–2 compares NC women to contraceptive users (CU; combination pills or IUDs). Compared to NC women, CU scored significantly higher on male pictures in RMET ( $t_{679.37} = -2.91$ ; p = 0.0037), the BSL supplementary scale ( $t_{293.35} = -3.26$ ; p = 0.0013), the SOI-R ( $t_{120.32} = -3.95$ ; p = 0.00013), and conscientiousness ( $t_{283.97} = -2.22$ ; p = 0.027). CU scored significantly lower on neuroticism ( $t_{490.27} = 2.23$ ; p = 0.026), autism traits ( $t_{298.56} = 2.08$ ; p = 0.038), and the SPQ interpersonal skills ( $t_{305.91} = 2.45$ ; p = 0.015). After using the FDR corrections on the p-values, total SOI-R scores, SOI-R behaviour scores, SOI-R attitude scores, BSL behaviour scores, AQ social skills scores, RMET for male pictures scores, and age remained significant for comparisons between HC users and NC women.

# Individual Comparisons Between HC and IUD Groups

Next, I subdivided the contraceptive users into a group of combination pill (CP) users and compared them to IUD users (table 5–3). Compared to CP users, IUD users scored higher on the BSL-23 ( $t_{28.67} = -2.26$ ; p = 0.032), the DASS stress scale ( $t_{19.58} = -2.43$ ; p = 0.025), the SOI-R behaviour scale ( $t_{13.96} = -2.81$ ; p = 0.014), and extraversion ( $t_{25.15} = -2.72$ ; p = 0.012). IUD users scored significantly lower on the LSRP ( $t_{18.01} = 2.28$ ; p = 0.035), the AQ communication skills scale ( $t_{26.23} = 2.32$ ; p = 0.028), and SPQ interpersonal skills ( $t_{21.47} = 2.98$ ; p = 0.0071). After

using the FDR corrections on the p-values, no scores remained significantly different after FDR corrections when comparing HC users and IUD users.

### Discussion

To my knowledge, this is the first study examining such a wide variety of psychological and behavioural differences between combination pill (CP) users and intrauterine device (IUD) users. While I did expect to see psychological and behavioural differences between naturally cycling (NC) women and combination pills users due to a change in circulating sex hormones, I did not expect to find differences in IUD users. My results suggests that in comparison to NC women, contraceptive users are better at recognizing emotion in males, are more open-minded about casual sex, are less neurotic, are more conscientious, and have better interpersonal skills, and have less traits relating to autism, but they have more borderline-like symptoms. My results also suggests that in comparison to combination pill users, IUD users are more open-minded about casual sex, are more extraverted, have less psychopathic traits, have better communication skills, and interpersonal skills. However, IUD users have increased borderline-like symptoms and increased stress. It is important to recognize that combination pill users and IUD users differ in many ways as certain personality types may be drawn to different types of contraceptives. Thus, personality may be important to consider when doctors prescribe contraceptives as it may increase or decrease the likelihood of consistent and proper usage.

While most researchers support the notion that IUDs do not alter circulating sex hormones, Järvelä and Jouppila (1998) found a decrease in serum progesterone concentrations in women using levonorgestrel-releasing IUDs, but no differences in serum estrodial concentrations between users and non-users. Therefore, researchers may want to reconsider the influence of IUDs on circulating hormones. Moreover, the ovulatory cycle is preserved in copper IUD users

(Faundes et al., 1980) as well as in the majority of LNG-IUD users (Luukkainen, Lähteenmäki, & Toivonen, 1990). As such, it is critical to note since the ovulatory cycle is still present in IUD users, researchers should still seek to acknowledge cycle phase of IUD users in studies.

Some imitations of the current study included the fact that I are using entirely correlational data, and that the data was taken from several different studies and pieced together. Therefore, I was not able to determine whether combination pills or IUDs have a causal influence on the psychology or behaviour or the women of use them. I also relied on self-reports from the participants, and so this may not accurately represent contraceptive users outside of a lab environment and introduces bias. Additionally, this is merely an exploratory study meant as a first step in the process of examining any potential differences between numerous psychological and behavioural measures. Therefore, future studies are necessary in order to draw any definitive conclusions on such differences between HC users and IUD users.

Future research should incorporate a within-subjects design to examine psychological traits of women before they commence combination pill or IUD usage. Additionally, future research should use much larger samples. While I did include 38 IUD users in the sample, studies would benefit from a greater number of IUD users in order to distinguish robust psychological and behavioural differences. Moreover, including a greater number of IUD users would also allow researchers to study the influence of different types of IUDs by dividing IUD users into groups of copper IUD users and hormonal IUD users, which would provide further clarification on differences pertaining to personality, psychology and behaviour.

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Area	Any Method (%)	Pill (%)	IUD (%)	
Worldwide	63.6	8.8	13.7	
Africa	33.4	8.7	3.8	
Asia	67.8	6.4	17.4	
Europe	69.2	21.9	11.3	
Latin America & Caribbean	72.7	15.0	6.4	
Northern America	74.8	16.5	4.7	
Oceania	59.4	21.6	1.1	

**Table 5–1.** United Nations data on the estimates of contraceptive prevalence by method amongmarried or in-union women aged 15 to 49 (percentage) in 2015.

Variable	NC (n = 423)		CU (n = 318)		T-Test			
	M	SD	Μ	SD	<i>t</i> (df)	p-value	FDR Corrected p-value	
Age (Years)	18.76	2.52	19.28	2.26	-2.89(707.61)	0.0039**	0.03	
RMET	26.76	4.05	27.19	3.63	-1.48(668.4)	0.14	0.37	
Female Pictures	13.20	2.34	13.19	2.22	0.089(654.28)	0.93	0.94	
Male Pictures	13.55	2.43	14.05	2.03	-2.91(679.37)	0.0037**	0.03	
BPAQ	69.96	18.22	69.86	17.91	0.070(624.35)	0.94	0.94	
EQ	17.48	3.64	17.69	3.31	-0.81(656.84)	0.42	0.62	
BSL (Composite Scores)	29.06	22.11	30.55	23.41	-0.60(313.07)	0.55	0.75	
BSL-23	26.77	19.96	26.47	20.16	0.14(320.18)	0.89	0.94	
<b>BSL-Supplement</b>	2.52	4.07	4.13	4.93	-3.26(293.35)	0.0013**	0.014*	
NEO-PI N3	26.06	7.02	24.67	7.10	2.23(490.27)	0.026*	0.11	
BDI	35.40	12.26	33.88	10.63	0.92(188.95)	0.36	0.60	
BES	28.39	7.87	29.74	8.73	-1.84(467.49)	0.066•	0.23	
OHQ	114.40	21.65	116.32	20.99	-1.02(494.33)	0.31	0.55	
ECR-RS (Comp. Scores)	99.81	32.50	95.95	29.51	1.41(504.67)	0.16	0.40	
Dominance	48.15	11.32	49.03	11.68	-0.46(133.5)	0.65	0.84	
LSRP (Comp. Scores)	48.63	7.94	48.71	8.56	-0.073(186.14)	0.94	0.94	
DASS	19.28	12.76	21.01	12.05	-0.97(183.95)	0.33	0.57	
Depression Scale	5.97	5.13	6.12	4.39	-0.23(192.42)	0.82	0.94	
Anxiety Scale	5.97	4.81	6.86	4.54	-1.33(186.71)	0.19	0.42	
Stress Scale	7.45	4.72	8.15	4.81	-1.03(179.22)	0.30	0.55	
BEM								
Female Scale	57.49	8.96	55.18	9.81	1.48(131.11)	0.14	0.37	
Male Scale	44.09	9.22	44.73	9.04	-0.43(136.74)	0.67	0.84	
SOI-R	24.77	11.55	33.34	14.10	-3.95(120.32)	0.00013***	0.003**	
Attitude Scale	11.50	7.22	16.00	7.88	-3.58(131.33)	0.00048***	0.007**	
<b>Behaviour Scale</b>	4.49	2.52	7.61	4.64	-4.86(90.807)	4.97e-06***	0.0002***	
Desire Scale	8.57	5.36	9.94	5.27	-1.56(138.89)	0.12	0.37	
PMM								
Agreeableness	57.23	9.65	56.37	9.89	0.78(277.25)	0.44	0.64	
Conscientiousness	48.72	10.13	51.25	10.02	-2.22(283.97)	0.027*	0.11	
Extraversion	42.35	11.24	43.85	10.82	1.21(290.86)	0.23	0.45	
Neuroticism	41.86	9.78	42.15	10.24	-0.26(269.15)	0.80	0.94	
Openness	52.56	8.54	51.08	8.39	1.54(287.61)	0.12	0.37	
AQ	19.37	5.37	18.13	5.19	2.08(298.56)	0.038*	0.14	
Attention to Detail	5.82	2.10	5.79	2.02	0.15(312.7)	0.88	0.94	
Attention Switching	5.30	2.00	5.01	1.91	1.35(311.95)	0.18	0.42	
Communication	2.78	1.89	2.27	1.88	2.47(308.97)	0.014*	0.07	
Imagination	2.19	1.65	2.33	1.52	-0.82(320.89)	0.41	0.62	
Social Skills	3.30	2.02	2.66	1.72	3.12(327.11)	0.002**	0.02	

Variable	NC $(n = 423)$		CU (n = 318)		T-Test			
	Μ	SD	М	SD	<i>t</i> (df)	p-value	FDR Corrected p-value	
SPQ-BR	90.66	15.43	88.42	16.58	1.27(295.26)	0.20	0.42	
Cognitive Perceptual	38.09	7.32	37.92	7.81	0.20(296.47)	0.84	0.94	
Disorganized	20.58	4.33	20.37	4.57	0.43(300.4)	0.67	0.84	
Interpersonal	32.01	6.94	30.12	7.11	2.45(305.91)	0.015*	0.07	
Masculine Face Preference	18.34	3.19	17.97	3.99	0.61(119.7)	0.54	0.75	
SD3	67.32	12.42	69.16	12.70	-0.86(129.78)	0.39	0.62	
CAMS	26.26	3.77	26.17	3.52	0.24(319.54)	0.81	0.94	
MAAS	54.76	10.42	53.29	10.16	1.30(312.38)	0.20	0.42	

\* p < .05 \*\* p < .01 \*\*\* p < .001

Table 5–2. T-tests between contraceptive users (CU; combination pill and intrauterine device users together) and naturally cycling (NC) women.

Variable	CP (n =	236)	IUD (n	= 38)	T-Test			
	M	SD	Μ	SD	<i>t</i> (df)	p-value	FDR Corrected p-value	
Age (Years)	18.88	1.61	20.39	2.73	-3.33(41.29)	0.0018**	0.082•	
RMET	27.20	3.46	27.53	2.72	-0.65(55.40)	0.52	0.68	
Female Pictures	13.15	2.15	13.50	1.87	-1.01(51.46)	0.32	0.65	
Male Pictures	14.05	2.07	14.03	1.73	0.056(52.97) 0.96	0.96	0.99 0.68	
BPAQ	69.14	18.00	71.97	19.23	-0.84(47.67)	0.41		
EQ	17.71	3.44	18.11	3.07	-0.72(52.65)	0.48	0.68	
BSL (Comp. Scores)	27.65	23.29	39.10	20.42	-2.26(28.68)	0.032*	0.18	
BSL-23	23.88	19.98	34.60	17.52	-2.46(28.67)	0.020*	0.18	
BSL-Supplement	3.84	4.85	4.50	4.15	-0.64(28.99)	0.53	0.68	
NEO-PI N3	24.48	7.29	25.38	6.57	-0.64(35.13)	0.52	0.68	
BDI	32.97	10.48	36.10	12.22	-0.77(11.10)	0.46	0.68	
BES	29.64	8.77	31.04	9.64	-0.70(31.58)	0.49	0.68	
OHQ	116.88	21.14	118.92	20.98	-0.46(33.25)	0.65	0.79	
ECR-RS (Comp. Scores)	95.76	30.56	98.36	27.36	-0.44(33.46)	0.67	0.79	
Dominance	47.84	11.22	53.82	11.67	-1.53(14.86)	0.15	0.51	
LSRP (Comp. Scores)	49.28	8.85	45.22	4.32	2.28(18.01)	0.035*	0.18	
DASS	19.13	12.46	25.00	9.09	-1.92(20.00)	0.070•	0.27	
Depression Scale	5.79	4.53	6.75	4.14	-0.72(16.55)	0.48	0.68	
Anxiety Scale	6.22	4.25	8.00	5.41	-1.08(13.70)	0.30	0.64	
Stress Scale	7.30	4.90	10.25	3.62	-2.43(19.58)	0.025*	0.18	
BEM								
Female Scale	55.28	9.60	52.82	13.34	0.58(12.59)	0.57	0.72	
Male Scale	43.84	8.95	48.09	10.99	-1.19(13.43)	0.26	0.60	
SOI-R	30.27	12.00	40.55	16.32	-1.96(12.77)	0.072•	0.27	
Attitude Scale	14.80	7.43	18.18	9.01	-1.15(13.44)	0.27	0.61	
<b>Behaviour Scale</b>	6.51	3.91	10.64	4.48	-2.81(13.96)	0.014*	0.16	
Desire Scale	9.28	4.75	11.73	6.02	-1.26(13.13)	0.23	0.60	
PMM								
Agreeableness	56.90	9.62	54.58	12.55	0.76(22.62)	0.45	0.68	
Conscientiousness	51.16	9.40	50.61	11.15	0.20(21.93)	0.85	0.92	
Extraversion	42.69	10.76	49.78	10.00	-2.72(25.15)	0.012*	0.16	
Neuroticism	41.91	9.58	42.00	11.97	0.029(21.52)	0.98	0.99	
Openness	51.03	8.61	52.61	7.37	-0.81(26.81)	0.43	0.68	
AQ	18.20	5.27	16.75	5.46	0.99(19.80)	0.33	0.65	
Attention to Detail	5.80	2.19	5.88	1.41	-0.18(27.61)	0.86	0.92	
Attention Switching	5.06	1.86	4.88	1.82	0.37(20.12)	0.71	0.82	
Communication	2.28	1.88	1.44	1.26	2.32(26.23)	0.028*	0.18	
Imagination	2.32	1.47	2.31	1.66	0.019(18.72)	0.99	0.99	
Social Skills	2.65	1.69	2.25	1.73	0.86(19.59)	0.40	0.68	

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Variable	CP(n = 236)		<b>IUD</b> $(n = 38)$		T-Test			
	M SI		М	SD	<i>t</i> (df)	p-value	FDR Corrected p-value	
SPQ-BR	89.16	16.03	79.44	16.45	2.21(19.55)	0.039*	0.18	
Cognitive Perceptual	38.09	7.59	34.81	8.57	1.45(18.73)	0.16	0.53	
Disorganized	20.55	4.43	19.06	4.54	1.23(19.53)	0.23	0.60	
Interpersonal	30.50	7.01	25.56	6.07	2.98(21.47)	0.0071**	0.16	
Masculine Face Preference	17.67	4.37	18.45	3.42	-0.64(18.71)	0.53	0.68	
SD3	67.55	10.12	75.00	18.90	-1.21(10.20)	0.25	0.60	
CAMS	26.05	3.47	27.47	3.91	-1.34(17.24)	0.20	0.60	
MAAS	53.47	10.41	52.63	9.82	0.32(20.44)	0.75	0.85	

p < .10\* p < .05 \*\* p < .01 \*\*\* p < .001

Table 5–3. T-tests between combination	pill (	CP)	) users and intrauterine device (IUD) use	rs.
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### **Chapter 6: Discussion**

Including sex differences in research has become more popular as of late (Taylor et al., 2000). While understanding sex differences in research is crucial, they are not the only differences that researchers should take into consideration. We need a more comprehensive understanding of cyclical hormonal changes as well as how contraceptives interact with such hormones in order to better interpret findings from a multitude of studies. More than a third of women in North America use hormonal contraceptives (HC; Rotermann, Dunn, & Black, 2015). Yet, the literature on the effects that HC use has on brain and behaviour in women is slowcoming and lacks consensus. While medical practitioners warn women against the potential physical side effects of HC use, they rarely – if at all – warn women against any behavioural or psychological side effects. Women use HCs for numerous reasons, which include preventing pregnancy, alleviating menstrual pain, and reducing endometriosis symptoms (Mishell, 1982). Even adolescent and pre-adolescent females are sometimes prescribed HC, yet they are often not well informed on HCs effects outside of preventing pregnancy. Currently, there is a lack of studies examining the longitudinal effects of HC use, and whether HC effects differ in females who are not yet fully matured. Since HC use is highly prevalent globally (United Nations, 2015) and in women of all ages, any associations that HC use has with behaviour, psychology, and hormone levels must be considered, since these associations would affect millions of women compounded over many generations.

# **Summary of the Results**

Investigating the Relationship Between Hormonal Contraceptive Use and Borderline Personality, Empathy and Oxytocin Level

The aim of the study was to investigate differences between women using HCs and naturally cycling (NC) women in terms of psychological differences associated with borderline personality and empathy as well as examine oxytocin as a possible mechanism of action. My results directly contradict my hypothesis that HC users would have increased BDP symptoms, decreased empathy, and decreased salivary oxytocin. Instead, HC users had increased empathy scores, happiness scores, and salivary oxytocin levels compared to NC women; however, the differences in scores did not reach significance. Additionally, HC users had decreased neuroticism scores and borderline personality scores. Moreover, HC users and non-users in my study scored the same in the Reading the Mind in the Eyes Task (RMET), which is a task that requires participants to recognize emotions and thoughts in photographs of eyes. HC users actually scored higher in the Empathy Quotient, although the p-value was only approaching significance (p = 0.061). I also expected to replicate findings that HC users have increased borderline-like symptoms (DeSoto, Geary, Hoard, Sheldon, & Cooper, 2003). Yet, in my study, HC users had decreased scores on the BSL-23, which was the main measure of borderline symptomology. Thus, my results again oppose previous research. Lastly, prior research found that oxytocin interacts with the brain reward system to reinforce partner value representations and that this oxytocin mechanism was disturbed in HC users, and hypothesized that this may be due to the effects of oxytocin being antagonized by sex hormones (Scheele, Plota, Stoffel-Wagner, Maier, & Hurlemann, 2016). Again, my findings contradict previous research as HC users in my study actually had increased salivary oxytocin levels – although, not significantly  $(x_{NC} = 689.83 \text{ pg/mL}; x_{HC} = 768.32 \text{ pg/mL}; t = -0.57; p = 0.57)$  – demonstrating that the effects of HC may not be straightforward and are complex.

# Re-Examining the Influence of Hormonal Contraceptives on Partner Preference

I did not find any difference between HC and NC or time in menstrual phase on facial attractiveness task. However, unlike Lukaszewski and Roney (2009), I saw a very different trend in women's preference for male traits in hypothetical short- and long-term relationships. Instead of demonstrating that NC women prefer dominance in the short-term, and kindness and trustworthiness in the short-term, I found no differences between NC women and HC users on any measures of the TPS except that NC women prefer dominant men in the long-term. This result was not due to HC users scoring higher on the SOI-R (a measure of open mindedness towards casual sex), which I thought might influence preference for dominance. These results suggest that HC slightly disrupts adaptive changes in women's mate preferences. However, my results are less convincing than previous research (e.g., Gangestad, Garver-Apgar, Simpson, & Cousins, 2007; Jones et al., 2005; Penton-Voak et al., 1999) and suggests that the disruption in partner preferences is weak and may more likely be due to personality differences in women who choose to use HC.

#### Association Between Stress Reactivity and Hormonal Contraceptive Use

The study provided some support for the hypothesis that HCs may influence stress and cortisol reactivity. Although mean salivary cortisol did not differ between groups (t = 0.64; p = 0.53), there was an interaction between HCs, stress, and cortisol reactivity. However, the interaction I found was not what was expected. Women using HCs actually seemed more self-aware of their stress response than NC women, which may be due to women with a better understanding of their stress response seeking out the use of HCs. I also found a positive correlation between psychopathic personality traits and stress (r = 0.75; p = 0.00081), and this association was only observed in HC users. This finding provides some support for my hypothesis that stress, psychopathy, and HC usage may be linked.

106

A Comparison of Naturally Cycling Women, Combination Pill Users, and Intrauterine Device Users

Results from the study suggests that in comparison to NC women, contraceptive users are better at recognizing emotion in males, are more open-minded about casual sex, are less neurotic, are more conscientious, have better interpersonal skills, and have less traits relating to autism, but they also have more borderline-like symptoms. My results also suggests that in comparison to combination pill users, IUD users are more open-minded about casual sex, are more extraverted, have less psychopathic traits, have better communication skills, and interpersonal skills. However, IUD users have increased stress, and even further increased borderline-like symptoms.

# Conclusion

Research in the field of HCs is complex and often inconsistent. In the current studies, I try to provide clarity on some of the many psychological, hormonal, and behavioural influences of HCs. Yet, many of the current findings do not support my initial hypotheses, nor do they support various findings in the literature. Although contradictory findings are prevalent in this field of research, inconsistent results should not compel researchers to cease studies. Instead, researchers should endeavor to continue studies on HC usage while also using different methodology in order to gain insight and knowledge into the psychobehavioural and hormonal influence of HCs.

While it is important to compare HC users and NC women, it is of additional importance to examine differences within HC users. There are numerous types of HCs, many of which have different hormonal combinations from one another. Therefore, in order to fully understand the effects of HCs on women's emotional and cognitive functioning, future research must aware of

107

the different types of HCs and their constituents. Future studies should also incorporate withinsubject designs as well as cross-cultural comparisons. Moreover, future research should look at long-term effects of HC use and determine if duration of usage has any effect on women. Additional research on HC initiation or cessation before or during a long-term relationship could provide insight into the impact of HCs on women's behaviour and partner preferences. Despite some my attempts at controlling for potential differences between pill users and non-users, more research on whether general differences between those using and not using HCs account for the above effects is necessary. An effective way to experimentally demonstrate the effects of HC use would be to use a placebo-controlled double-blind study with random assignment to either a contraceptive pill or placebo, while using non-hormonal birth control for both groups to minimize ethical considerations (Roberts et al., 2011). However, an important caveat is that nonhormonal birth control is often less effective at preventing pregnancy (Trussell, 2004), and so one group would be more susceptible to the risk of pregnancy. An alternative method to examine evidence of HC changes would be to test for effects of HC use on nonhuman primates. Some research suggests that synthetic sex hormones can influence nonhuman female primates' sexual behaviour (Baum, Everitt, Herbert, & Keverne, 1977), and can hinder their ability to attract mates (Linn & Steklis, 1990). Studies on female gorillas (Sarfaty, Margulis, & Atsalis, 2012) and chimpanzees (Nadler, Dahl, Gould, & Collins, 1993) demonstrate changes in reproductive and social behaviour due to HC use. Future research should continue using this type of comparative research by examining the psychological, behavioural and genetic influences of HC use on female nonhuman primates and how results may generalize to human women. Nonetheless, research on nonhuman primates should be examined with caution since animal research does not always translate accurately to humans.

HC use is associated with several non-contraceptive health benefits. For instance, HC users are at a reduced risk of both ovarian (Lurie et al., 2007) and endometrial cancer (Burkman, 2001) compared to non-users. HC use decreases menstrual blood flow and is linked with a decrease in the risk of anaemia in women (Milman, Clausen, & Byg, 1998). Since HCs inhibit ovulation, the incidence of ovarian cysts is drastically reduced, and the occurrence of dysmenorrhea (i.e., menstrual cramps) decreases significantly (Mishell, 1982). HCs also decrease the likelihood that women will develop rheumatoid arthritis (Mishell, 1982). Finally, there is evidence that HCs reduce acne in some women (Koulianos, 2000). However, HCs also carry the risk of a variety of negative physical side effects such as an increased risk of venous thromboembolism (Lidegaard, Edström, & Kreiner, 2002), ischemic stroke (Kemmeren et al., 2002), myocardial infarction (Lewis, Heinemann, Spitzer, MacRae, & Bruppacher, 1997), as well as weight gain (Molland et al., 1996). HC users are at a greater risk of getting gallstones (Etminan, Delaney, Bressler, & Brophy, 2011), and experience more sleep disruption than non-users (Shine Burdick, Hoffmann, & Armitage, 2002).

Although a complete understanding of the potential effects of HC use on physiology, psychology, and behaviour is imperative, any HC effects should be weighed against its many benefits. HCs offer ease of use, tolerability and, when used correctly they are highly effective at preventing pregnancy (Trussell, 2004). Effective contraceptive methods have given women control over their fertility, which in turn has aided in many personal and economic achievements for women (Goldin & Katz, 2002). Regardless, researchers should endeavour to fully understand the psychological and behavioural effects of HC use in order to educate users on possible side effects and allow women to make more informed decisions regarding the type and timing of their

109

HC use. The knowledge gained from research on HC could also help in the development of new contraceptive methods with less impact on personality, behaviour, and psychology.

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