

St. Luke's International University, Graduate School, Doctoral Dissertation, 2018.

**Physiological and Psychological Changes of Primipara
after First-time Interaction with an Infant during Pregnancy:
A Randomized Controlled Trial**

Nozomi SONODA

Table of Contents (Main Text)

Introduction	1
Background.	1
Objectives.	3
Significance of the study.	4
Definition of terms.....	4
Literature Review.....	6
Support for pregnant women during pregnancy.	6
Characteristics of pregnant women.....	8
Biological reaction by stimulation.	9
<i>Stress reaction.....</i>	<i>9</i>
<i>Comfort reaction.</i>	<i>9</i>
Consideration of physiological indices –cortisol and oxytocin-.....	10
<i>Characteristics of cortisol</i>	<i>10</i>
<i>Characteristics of oxytocin.....</i>	<i>12</i>
Suggestions from literature review for this research.	14
Methods.....	15
Study Design.....	15
Participants and Setting.....	15
Randomization.	15
Masking and Blinding.	15
Protocol.....	16
<i>Experimental group.</i>	<i>18</i>
<i>Control group.</i>	<i>20</i>
Outcome Measurements.	21
<i>Primary outcome.</i>	<i>21</i>
<i>Secondary outcomes.</i>	<i>21</i>
<i>Characteristics of participants.</i>	<i>22</i>

Sample Size.	23
Recruitment.	24
Analysis.	24
Ethical consideration.....	25
Results	27
Participant flow.....	27
Baseline data.	27
Implementation of Protocol.	28
Primary Outcome: Salivary Cortisol Level.....	31
<i>Selection of Samples to be Analyzed.....</i>	<i>31</i>
<i>Salivary Cortisol Level.</i>	<i>33</i>
<i>Comparison of Salivary Cortisol Level in Experimental group and Control group.....</i>	<i>33</i>
<i>Within-group Comparison of Salivary Cortisol Level before and after Intervention.....</i>	<i>34</i>
<i>Salivary Cortisol level and PHQ-9.....</i>	<i>35</i>
<i>Salivary Cortisol level and PBI.....</i>	<i>37</i>
Secondary Outcomes: State Anxiety Score.	42
<i>Selection of Samples to be Analyzed.....</i>	<i>42</i>
<i>State Anxiety Score.....</i>	<i>42</i>
<i>Comparison of State Anxiety Score in Experimental group and Control group.</i>	<i>43</i>
<i>Within-group Comparison of State Anxiety score before and after Intervention.....</i>	<i>44</i>
Secondary Outcomes: Salivary Oxytocin Level.....	45
<i>Selection of Samples to be Analyzed.....</i>	<i>45</i>
<i>Salivary Oxytocin Level.</i>	<i>46</i>
<i>Comparison of Salivary Oxytocin Level in the Experimental Group and Control Group..</i>	<i>46</i>
<i>Within-group Comparisons of Salivary Oxytocin Level before and after Intervention.</i>	<i>47</i>
<i>Polymorphisms of Oxytocin Receptors.....</i>	<i>48</i>
<i>Salivary Oxytocin level and PHQ-9.....</i>	<i>55</i>
<i>Salivary Oxytocin level and PBI.....</i>	<i>57</i>
Secondary Outcomes: Approach Feeling and Avoidance Feeling.	62

<i>Selection of Samples to be Analyzed.....</i>	<i>62</i>
<i>Approach Feeling.....</i>	<i>62</i>
<i>Comparison of Approach Feeling in Experimental group and Control group.</i>	<i>63</i>
<i>Within-group Comparison of Approach Feeling before and after Intervention.</i>	<i>65</i>
<i>Avoidance Feeling.....</i>	<i>65</i>
<i>Comparison of Avoidance Feeling in Experimental Group and Control Group.</i>	<i>66</i>
<i>Within-group Comparisons of Avoidance Feeling before and after Intervention.....</i>	<i>68</i>
<i>Score of Antagonism.....</i>	<i>69</i>
Process Evaluation	70
<i>Changed image of infant.....</i>	<i>70</i>
<i>Change for anxiety about living with infant.....</i>	<i>70</i>
<i>Change for anxiety about delivery.</i>	<i>70</i>
<i>Change for anxiety about family relationship.</i>	<i>71</i>
<i>Change for anxiety about economic situation.....</i>	<i>71</i>
<i>Burden.....</i>	<i>71</i>
<i>Satisfaction.</i>	<i>71</i>
<i>Description data for interaction with infant.</i>	<i>72</i>
Discussion.....	75
Primary Outcome: Salivary Cortisol Level.....	75
Secondary Outcome: State Anxiety Score.....	77
Secondary Outcome: Salivary Oxytocin Level.	78
Secondary Outcome: Approach Feeling and Avoidance Feeling.....	79
Process evaluation.....	81
Limitations and Future Studies.....	82
Conclusion.....	83
References	
Appendix	

Table of Contents (Tables)

Table 1 Participants Baseline Characteristics.	30
Table 2 Between Group Comparison of the Mean Level of Salivary Cortisol Level in Experimental Group and Control Group (ng/mL).	34
Table 3 Within-group Comparison of Salivary Cortisol Level before and after Intervention (ng/mL).	34
Table 4 Between Group Comparison of the Mean Level of Salivary Cortisol Level in the Experimental Group and Control Group Classified PHQ-9 (ng/mL).	36
Table 5 Between Group Comparison of the Amount of Change for Experimental Group and Control Group.	37
Table 6 Within-group Comparison of Salivary Cortisol Level before and after Intervention for in the PHQ-9 Minor and Moderate Experimental and Control Subgroups.(ng/mL).	37
Table 7 Between Group Comparison of the Mean Level of Salivary Cortisol Level in the Experimental Group and Control Group Classified PBI: CA (ng/mL).	39
Table 8 Between Group Comparison of the Amount of Change for Experimental and Control CA High and CA Low Subgroups.	39
Table 9 Within-group Comparison of Salivary Cortisol Level before and after Intervention in CA High and CA Low Subgroups. (ng/mL).	40
Table 10 Between Group Comparison of the Mean Level of Salivary Cortisol Level in the Experimental Group and Control Group Classified PBI: OP (ng/mL).	41
Table 11 Between Group Comparison of the Amount of Change for Experimental and Control OP High and Low Subgroups.	41
Table 12 Within-group Comparison of Salivary Cortisol Level before and after Intervention in OP High and OP Low Subgroups. (ng/mL).	42
Table 13 Between Group Comparison of State Anxiety Mean Scores in Experimental Group and Control Group.	44
Table 14 Between Group Comparison of the Amount of Change in the Experimental Group and Control Group.	44

Table 15 Within-group Comparison of State Anxiety Score before and after Intervention in the Experimental and Control Groups.	45
Table 16 Between Group Comparison of the Mean Level of Salivary Oxytocin Level in the Experimental Group and Control Group (pg/mL).....	47
Table 17 Within-group Comparison of Salivary Oxytocin Level before and after Intervention for the Experimental and Control groups (pg/mL).	47
Table 18 Between Group Comparison of the Mean Level of Salivary Oxytocin Level in the Experimental Group and Control Group Classified SNP (pg/mL).....	49
Table 19 Between Group Comparison of the Amount of Change for Experimental Group and Control Group who had GG.....	50
Table 20 Within-group Comparison of Salivary Oxytocin Level before and after Intervention in GG Experimental and Control Subgroups. (pg/mL).	50
Table 21 Between Group Comparison of the Amount of Change for Experimental Group and Control Group who had A Carrier.....	51
Table 22 Within-group Comparison of Salivary Oxytocin Level before and after Intervention in A Carrier Experimental and Control Subgroups. (pg/mL).	51
Table 23 Between Group Comparison of the Mean Level of Salivary Oxytocin Level in the Experimental Group and Control Group Classified SNP (pg/mL).....	53
Table 24 Between Group Comparison of the Amount of Change for Experimental Group and Control Group who had G Carrier.	53
Table 25 Within-group Comparison of Salivary Oxytocin Level before and after Intervention in G carrier Experimental and Control Subgroups. (pg/mL).	54
Table 26 Between Group Comparison of the Amount of Change for Experimental Group and Control Group who had AA.....	55
Table 27 Within-group Comparison of Salivary Oxytocin Level before and after Intervention in AA Experimental and Control Subgroups. (pg/mL).....	55
Table 28 Between Group Comparison of the Mean Level of Salivary Oxytocin Level in the Experimental Group and Control Group Classified PHQ-9 (pg/mL).	56
Table 29 Between Group Comparison of the Amount of Change for Experimental Group and Control Group.	57

Table 30 Within-group Comparison of Salivary Oxytocin Level before and after Intervention in the PHQ-p Minor and Moderate Experimental and Control Subgroup. (pg/mL).....	57
Table 31 Between Group Comparison of the Mean Level of Salivary Oxytocin Level in the Experimental Group and Control Group Classified PBI: CA (pg/mL).....	59
Table 32 Between Group Comparison of the Amount of Change for Experimental and Control CA High and CA Low Subgroups.	59
Table 33 Within-group Comparison of Salivary Oxytocin Level before and after Intervention in the CA High and CA Low Subgroups. (pg/mL).....	59
Table 34 Between Group Comparison of the Mean Level of Salivary Oxytocin Level in the Experimental Group and Control Group Classified PBI: OP (pg/mL)	61
Table 35 Between Group Comparison of the Amount of Change for Experimental and Control OP High and OP Low Subgroups.	61
Table 36 Within-group Comparison of Salivary Oxytocin Level before and after Intervention of OP High and OP Low Subgroups. (pg/mL).	61
Table 37 Between Group Comparison of Mean Scores of Approach Feeling in Experimental Group and Control Group.	64
Table 38 Between Group Comparison of the Amount of Change for Experimental Group and Control Group.	64
Table 39 Between Group Comparisons of the Amount of Change of each Item.....	64
Table 40 Within-groups Comparison of Approach Feeling before and after Intervention.....	65
Table 41 Between Group Comparison of Mean Scores of Avoidance Feeling in Experimental Group and Control Group.	67
Table 42 Between Group Comparison of the Amount of Change for Experimental Group and Control Group.	67
Table 43 Between Group Comparison of the Amount of Change of each Item.	68
Table 44 Within-group Comparisons of Avoidance Feeling before and after Intervention.....	69
Table 45 Between Group Comparison of Mean Scores of Antagonism in Experimental Group and Control Group.	69
Table 46 Within-group Comparisons of score of Antagonism before and after Intervention.	70

Table 47 Between Group Comparison of Process Evaluation in Experimental Group and Control
Group.72

Table 48 Descriptive data for interactions with infant.74

Table of Contents (Figures)

<i>Figure 1.</i> Intervention protocol.	17
<i>Figure 2.</i> Cryo Poter CS-80°C	17
<i>Figure 3.</i> Frozen Sample at -80°C	17
<i>Figure 4.</i> Communicate with infant.	19
<i>Figure 5.</i> Holding infant.	19
<i>Figure 6.</i> Watched 30 minutes DVD image of infant.	20
<i>Figure 7.</i> Flow diagram of participants.	29
<i>Figure 8.</i> Flow diagram of salivary samples: cortisol.	32
<i>Figure 9.</i> The distribution of salivary cortisol level of experimental group.	33
<i>Figure 10.</i> The distribution of salivary cortisol level of control group.	33
<i>Figure 11.</i> The distribution of State Anxiety Score in the experimental group.	43
<i>Figure 12.</i> The distribution of State Anxiety Score in the control group.	43
<i>Figure 13.</i> The distribution of salivary oxytocin level in the experimental group.	46
<i>Figure 14.</i> The distribution of salivary oxytocin level in the control group.	46
<i>Figure 15 .</i> The distribution of Approach Feeling in the experimental group.	62
<i>Figure 16.</i> The distribution of Approach Feeling in the control group.	63
<i>Figure 17.</i> The distribution of Avoidance Feeling in the experimental group.	66
<i>Figure 18.</i> The distribution of Avoidance Feeling in the control group.	66

Introduction

Background

Because of the declining birthrate and increasing nuclear families about half of women in Japan do not usually have interactions with infants until they have their own baby (Benesse Educational Research & Development Institute, 2007; Benesse Educational Research & Development Institute, 2013). The shortage of interaction with infants is regarded as a cause of child-rearing anxiety (Ministry of Health, Labour & Welfare, 2003).

A relationship of child-care anxiety with postpartum depression had also been reported (Sato, 2006; Sato, et al., 2006), and its influence on women and their families is immeasurable. With the declining birthrate and increasing nuclear families, it will be difficult to imitate a child-rearing model in daily life (Rubin, 1984 / Shindou, & Goto, 1997). Therefore, it is necessary to create opportunities for first-time pregnant women who are becoming mothers and who have no experience interacting with infants to be able to interact with infants.

There are reported initiatives that have set up opportunities for first-time pregnant women who have never interacted with infants (Omori, et al., 2005; Watanabe, et al., 2013). These initiatives were carried out as part of a pregnancy participation activity by an association of parents with children, and as part of prenatal classes during pregnancy. First-time pregnant women felt only a strong but vague image of a baby, which eventually changed to a concrete image (Omori, et al., 2005) of “the baby is cute” and by seeing and holding the infant (Watanabe, et al., 2013). Thus, it can be said that engaging with the infant is a prelude experience to actually knowing the infant. Furthermore, to reduce the possibility of “child-care anxiety” and to increase “attachment to the fetus”, images for postpartum and child rearing were prepared, in which “anxiety about child rearing was alleviated” (Watanabe, et al. 2013). It can be said that first-time mothers have their own experience of interacting with infants. However, as reports in this area are few, changes in first-time pregnant women resulting from their interaction with pregnant women and their infants remain unclear.

Therefore, it is necessary to clarify the specific changes in first-time pregnant women upon their interaction with pregnant woman, as well as changes in their relationships from their own experience through interaction with an infant. Clarifying these aspects using physiological markers and psychological data may provide scientific evidence and new findings regarding the interaction of first-time pregnant women with infants.

There are several physiological markers that are appropriate to measure to shed light on the status of first-time pregnant women when interacting with infants. One physiological index uses cortisol (Andrea, 2012), which is widely known as a stress hormone. Cortisol is secreted from the adrenal cortex resulting from physical and mental stress stimuli. It is detected both in the blood and saliva, with a 0.91 correlation between the sources, which means that either source provides valid information.

Another physiological index is oxytocin, which is widely known as an attachment and binding hormone. Oxytocin is synthesized in the neurons of the supraoptic nucleus of the hypothalamus and the paraventricular nucleus, secreted in pulses from the posterior pituitary into the blood, and found in saliva and urine (Stevens, et al. 2013). The presence of oxytocin had been reported not only during uterine contractions and the milk let-down reflex but also in relation to attachment to the fetus (Levine, et al. 2007) and association with child rearing behavior (Feldman, et al. 2007). It is secreted by stimulation of touch and human-human relationship (Holt-Lunstand, et al. 2008). Therefore, it could be possible to capture the changes occurring in first-time pregnant women when they interact with infants using cortisol, which is responsive to stress and oxytocin, which is related to attachment and child rearing behavior.

Furthermore, from the reports of Omori, et al. (2005) and Watanabe, et al. (2013), interacting with infants presents the possibility that changes will occur in first-time pregnant women's images of and anxieties about their interactions infants. It is possible to objectively evaluate the psychological aspect by using the *Taiji Kanjyo Hyoutei Shakudo* (TKHS), a scale that measures the mother's positive Approach feelings and negative Avoidance feelings towards infants (Hanazawa, 1992), and the State Trait Anxiety Inventory (STAI – JYZ) (Hidano, et al., 2000), which is a measure of anxiety. It is highly likely that the first baby the first-time pregnant women

encounters can reveal their psychological changes that result from interacting in some way with the infant.

Based on the above-mentioned background, we aim to clarify the changes in first-time pregnant women by their interaction with infants using two different indicators, namely, physiological indicators and psychological indicators. This may also highly likely be a scientific basis for caring for first-time pregnant women and is expected to be the basis for support for future first-time pregnant women. Furthermore, new knowledge is anticipated which could become a basis of future research and development to further clarify the relationship between first-time pregnant women and infants.

Objectives

The objectives of this randomized, controlled, two-armed trial was to verify the change in the experimental group after 30 minutes' of interaction with infants compared to the control group after 30 minutes watching a DVD of infants.

The primary outcome was salivary cortisol level. The secondary outcomes were state anxiety score, salivary oxytocin level and approach feeling and avoidance feeling.

Furthermore, a process evaluation was conducted on the content, to better inform program changes for first-time pregnant women interacting with infants.

Hypotheses

1. The **salivary cortisol level** at 30 minutes after the intervention is significantly reduced in the experimental group compared with the control group.
2. The **State-Trait Anxiety Inventory score** after the intervention is significantly lower in the experimental group than in the control group.
3. The **salivary oxytocin level** increases 30 minutes after the intervention in the experimental group compared with the control group.
4. The **approach feeling** increases and the **avoidance feeling** decreases after the intervention in the experimental group compared with the control group.

5. Interaction with an infant increases **positive image of infant**, and decreases **anxiety about living with infant, anxiety about delivery, anxiety about family relationship and anxiety about economic situation**.

Significance of the study

Although there are studies that evaluate the fragility of relationships between first-time pregnant women and infants from a psychological point of view, the number is small, and the methodology and effects have not been clarified. Therefore, clarifying the changes brought about by the interaction between the infant and the first-time pregnant women can serve as a scientific basis for improving the psychological and physiological state of first-time pregnant women. The scientific evidence gained from this study regarding the positive effects of the experience of first-time pregnant women with infants can serve as one form of prenatal care to help other women in their pregnancies. Furthermore, the anticipated new knowledge from this study can provide a platform for future research and development in this field, and may further clarify the relationship and interaction between the first-time pregnant women and the infant.

Definition of terms.

First-time pregnant women who have no experience of interacting with infants:

At the time of study participation, first-time pregnant women refer to women who are pregnant for the first time and who have no routine experience of infant care (such as hugging, diaper change, bathing) necessary to care for an infant.

Women, who have some experience of hugging or playing with friends or acquaintances' children, are included in with first-time pregnant women who have no experience of interacting with infants.

For purposes of this study, henceforth first-time pregnant women who have no experience of interacting with infants are referred to as first-time pregnant women.

Interaction with infant:

This refers to watching an actual infant, listening to the infant's voice or sounds made, touching the infant, smelling the infant, communicating with the infant and communicating with infant's mother. Infants are two months to six months old.

Literature Review

Support for pregnant women during pregnancy.

During pregnancy, dynamic changes occurred for pregnant women. Therefore, pregnant women experience various stresses such as anxiety. O'Connor, et al. (2003) reported the relationship between anxiety during pregnancy and child growth and development. Children whose mothers experienced high levels of anxiety in late pregnancy exhibited higher rates of behavioral/emotional problems at 18 months of age after controlling for obstetric risks, psychosocial disadvantage, and postnatal anxiety and depression (girls: OR = 1.91, 95%CI = 1.26-2.89, boys: OR = 2.16, 95%CI = 1.41-3.30).

Interventions to reduce the anxiety during pregnancy are mainly education programs, which were effective for reducing anxiety (Akbarian, et al., 2018; Sanaati, et al., 2018). According to Akbarian, et al. (2018), pregnant women allocated to the Mental Health Training Program had a significant decrease of mean score of depression, anxiety and stress after a two-weeks intervention program compared to control group and intervention with couple group ($F=5.84, p=0.00$; $F=14.76, p<0.00$; $F=11.98, p<0.00$). Sanaati, et al. (2018) reported the effect of lifestyle-based training for pregnant women. Lifestyle-based training for women-only group and dyad group were significantly decreased in post-partum depression and state anxiety compared control group (adjusted difference women-only group: -5.5, -13.6, dyad-group: -3.2, -5.8).

Similarly, support for pregnant women are mainly education program such as classes for mothers in Japan. However, currently in Japan, the shortage of interactions with infants is a problem that causes child-rearing anxiety. The declining birthrate and increasing nuclear families, increases the difficulty for women of childbearing age to interact with infants in everyday life. About half of women do not have experience of interaction with infants until they have their own children: 47.1% in the 2006 survey and 42.8% in the 2011 survey (Benesse Educational Research & Development Institute, 2007; Benesse Educational Research & Development Institute, 2013). Within this

context, planned interactions between first-time pregnant women and infants have been implemented in the clinical setting, although the reports are few in number.

Kumagai, et al. (2004) conducted interactions between 13 first-time pregnant women who were hospitalized due to premature birth at 24 weeks gestation to 35 weeks gestation and also newborns in order to provide the first-time pregnant women with situation in the hospital so they could more positively grasp their own pregnancy. The content of the interaction was the experience of holding a newborn and the observation of bathing the newborn. Kumagai, et al. (2004) compared the intervention group (interact with infant) and control group (no infant interaction) scores of image of the newborn. The intervention group had a more specific image of the newborn compared to the control group.

Omori, et al. (2005) conducted interaction between 5- first-time pregnant women and infants to invited first-time pregnant women to 'Meeting a Baby' [*Akachan Dousoukai*]: the class for postpartum mothers with baby. Questionnaires were conducted before and after the interaction with an infant. The questionnaire results indicated that the image of the infant changed from a vague one to a concrete one, and participants' anxiety about child rearing was reduced by listening to the mother who was experienced in child rearing. Omori, et al. (2005) suggested that the interaction with an infant was useful for reducing first-time pregnant women's anxiety and providing a realistic perception of an infant.

Watanabe, et al. (2013) conducted interactions between first-time pregnant women and infants as part of a mother's class. Interviews with first-time pregnant women after class indicated that they increased their sense of attachment of their fetus and they increased their image of child rearing in general and especially child rearing after the postpartum period.

In other countries, there are no reports about the interactions of pregnant women with infants. However, there are some reports on interactions with the fetus and with her own infant, such as an increase in affection and growth optimization (Moberg, 1996; Guney & Ucar, 2018). Guney and Ucar (2018) reported that maternal-fetal attachment was positively affected by counting the fetal movement counts. The

comparison of experimental group who counted fetal movements and the control group, who did not count, indicated that the maternal-fetal attachment score was higher in the experimental group after four weeks ($p<0.00$).

According to Moberg (1996), mother-infant interactions are not only psychological but also have neuroendocrine mechanisms. It seems that tactile, olfactory, and visual sensory stimuli contribute to the adaptive changes in both mother and infant.

In summary, the research shows a diversity of interactions between first-time pregnant women and infants, not only in terms of gestation, but also the age of the infant and the purpose and method of interactions. In addition, these interactions also suggest various outcome possibilities, such as the image of one's own infant being realized through the experiences of actual relationships with infants, reducing anxiety of child rearing and a positive effect on the neuroendocrine system.

Characteristics of pregnant women.

Pregnancy causes not only physical changes but also psychological changes for women. Women in pregnancy have various anxieties: anxiety about continuity of pregnancy, anxiety about fetal development, anxiety about fetal abnormalities, anxiety concerning childbirth, anxiety concerning child rearing, and pregnancy (Hanazawa, 1992). Pregnant women have more anxiety compared with women who are not pregnant (Nakajima, et al, 1998; Matsuoka, et al, 2002; Sato, 2006). As a characteristic of anxiety during pregnancy, anxieties about “influence on mother”, “expectation of delivery”, “expectation of child rearing” and “change in appearance” are characteristic in late pregnancy. There is no difference in the focus of anxiety in late pregnancy by natural means compared to pregnancy by infertility treatment (Zhao, et al, 2006). Also, when comparing first-time pregnant women and multiparas, first-time pregnant women have more anxiety caused by their inexperience being pregnant (Nakajima, et al, 1998).

In summary pregnant women are more anxious compared to women who are not pregnant, and their anxiety becomes stronger when they are in the later phases of

pregnancy. Anxiety of “expectation of delivery” and “expectation of child rearing” are stronger in late in pregnancy. Anxiety caused by absence of experience is stronger among first-time pregnant women.

Biological reaction by stimulation.

Sensory receptors receive stimulation from the outside world and transmit it to the central nervous system. Sensory receptors include taste, hearing, vision, smell, and touch. The tactile receptors that are the sense of touch are most widely distributed throughout the body.

Stress reaction.

It is widely known that the stress load on the body can occur when the HPA system and the noradrenaline system are activated. When physical and psychological stress occur, corticotrophin-releasing hormone (CRH) secreted from the hypothalamus acts on the pituitary gland and adrenocorticotrophic hormone (ACTH) secreted from the anterior pituitary gland, and stimulates the secretion of cortisol. Cortisol is a glucocorticoid secreted from the adrenal cortex and is known to be the final product of hypothalamic-pituitary-adrenal axis (HPA system). The noradrenaline system activates the autonomic nervous system, especially the sympathetic nervous system. These two systems said to adapt to stress (Oyola and Handa, 2017).

Cortisol release is a response to both psychological and physical acute stress (Viru, 1992; Dickerson, et al., 2004; Kudielka, et al., 2004). Salivary cortisol level increased significantly in response to the Trier Stress Test and the peak was 20-30 minutes after the test (Kudielka, et al., 2004). Physical stress increased salivary cortisol level, as did sustained exercise load (Viru, 1992).

Comfort reaction.

Humans feel comfortable as a result of being stimulated from the outside world in a way that activates the reward system. Stimulation which activates the reward system

is said to be: food, sexual behavior, praise from others and maternal behavior (Nishitani, et al., 2013; Alonso-Alonso, et al., 2015).

The stimulation of the reward-center is mediated by the neural system of the brain regions. When the stimulation is sensed by a sensory receptor, the midbrain sends projections to target areas in the limbic forebrain, particularly the nucleus accumbens, as well as the prefrontal cortex, which is activated and the prefrontal cortex provides descending projections to the nucleus accumbens and the ventral tegmental area (Nestler, 2004; Scofield, et al., 2014; Wise, et al., 2014; Weiland, et al., 2014).

Consideration of physiological indices –cortisol and oxytocin-

Sonoda, et al. (2018) used salivary cortisol and salivary oxytocin as an evaluation index of the first-time pregnant women's interaction with infants. Cortisol is secreted during stress stimulation; it can objectively evaluate the first-time pregnant women's reaction to interacting with infants. Oxytocin is also secreted as a result of human-to-human interaction. It can be used as an index to evaluate the first-time pregnant women's interaction with infants.

In Sonoda, et al. (2018) research as a result of comparison between the intervention group who had interactions with infants and the control group who watched a DVD image of the landscape, a significant decrease in the salivary cortisol level and a fluctuation in the salivary oxytocin level in the intervention group was noted.

Characteristics of cortisol

Cortisol is widely used as a biological indicator of stress because it is secreted by stress stimulation (Clements, 2012). It is also used as an evaluation index for reduction of stress in interventions such as touch, massage, and yoga (Adib-Hajbaghery, Rajabi-Beheshtabad, & Abasi, 2013; Chol, & Lee, 2015; Pinar, & Afsar, 2015; Kusaka, Matsuzaki, Shiraishi, & Haruna, 2016; Rapaport, Schettler, & Bresey, 2012).

Cortisol is affected by such events as diurnal rhythms, pregnancy, diet, luxury goods, disease and psychological aspects. As a characteristic of diurnal rhythm cortisol, cortisol level becomes the lowest level one to two hours after falling asleep and rises during sleeping reaching a maximum level 30 minutes to two hours after awakening and then gradually decreases throughout the day. (Clements, 2012). Therefore, when measuring the cortisol level, it is necessary to consider the sampling time of diurnal rhythm. The concentrations of CRH, ACTH, plasma cortisol, and free cortisol are increased by pregnancy (Kudielka, et al., 2009; Migeon, et al., 1957; Tateoka, et al., 2004). Cortisol level accompanying pregnancy is about 2.5 times higher at the end of pregnancy than that of non-pregnant women (Migeon, et al., 1957). Salivary cortisol level in the afternoon in late pregnancy is reported as 3ng/mL (Tadokoro, 2016). Nicotine, caffeine and food intake associated with cortisol has also been reported (Clements, 2012; Kudielka, et al., 2009). Smoking (nicotine), caffeine intake and carbohydrate intake all raise salivary cortisol levels (Clements, 2012; Kudielka, et al., 2009).

Diseases affecting the secretion of cortisol, are widely known as the pituitary ACTH secretion syndrome (Cushing's disease) which is excessive secretion of cortisol and Addison's disease in which cortisol is decreased. Patients with hypertension, asthma and rheumatism are reported to have a low cortisol level (Clements, 2012).

Depression, anxiety and childhood attachment style have also been reported to be related to changes in cortisol levels (Dedovic, et al., 2009; Engert, et al., 2011; Kudielka, et al., 2009; Voetline, 2013). In low-risk pregnant women, depression and anxiety are correlated with higher salivary cortisol levels. High levels of salivary cortisol were noted among pregnant women at 30 to 32 weeks gestation who had high levels of depression and anxiety (Voetline, 2013). In addition, an association of attachment style in childhood with salivary cortisol has been reported (Kudielka, et al., 2009), and it was reported that cortisol tended to rise with stress among those pregnant women who have low care experience from their parents (Dedovic, et al., 2009; Engert, et al., 2011).

Characteristics of oxytocin

Oxytocin is a peptide hormone composed of nine amino acids, which is produced in neurons of the supraoptic nucleus and the paraventricular nucleus of the hypothalamus, and is secreted from the posterior pituitary into the blood in pulses. Oxytocin has a positive feedback system and its effect is shown by binding oxytocin with the oxytocin receptor on the surface of the target organ. Oxytocin secreted from the posterior pituitary into the blood is called peripheral oxytocin and is found in blood, saliva and urine (Stevens, Wiesman, Feldman, Hurley, & Taber, 2013).

Oxytocin has been reported to be related not only to uterine contraction and the milk-ejection reflex but also to child rearing behavior (Feldman, Gordon, Schneiderman, Weisman, & Zagoory-Sharon, 2010; Feldman, Weller, Zagoory-Sharon, & Levine, 2007). It is reported that oxytocin is higher in pregnant women than non-pregnant women, and oxytocin rises as pregnancy progresses (de Geest, Thiery, Piron-Pussuyt & Driessche, 1985; Lindow, Newham, Hedricks, Thompson, & Spuy, 1996; Takagi, et al., 1985; Takeda, Kuwabara, & Mizuno, 1985). It is reported that oxytocin has a diurnal rhythm, that rises from 8:00 am and peaks at midnight during 37 weeks gestation to 39 weeks gestation (Fuchs, Behrens, & Liu, 1992).

Relationships with partners, depression, anxiety, and oxytocin receptor gene polymorphisms have been related to oxytocin baseline. Oxytocin is higher in women who have more hugs with partners than usual (Light, Grewen, & Amico, 2005).

Oxytocin shows its effect by binding to the oxytocin receptor on the surface of the target organ, but the genetic base polymorphism is recognized in the oxytocin receptor. The oxytocin receptor gene polymorphism exists as three introns and four exons on the third chromosome. For example it was revealed that the genetic polymorphisms of *rs53576* and *rs2254298* of the oxytocin receptor are related to autism (Bakermans-Kranenburg, & van Ijzendoorn, 2008). Oxytocin receptor gene polymorphism has been shown to be associated with the progress of delivery and relationship with children. Oxytocin receptor gene *rs53576* with “G” was associated with the slow progress of delivery (Terkawi, 2012), and the oxytocin receptor gene *rs1042778* with type TT was associated with less interaction with children (Feldman,

2012). Feldman, et al. (2012) reported that was an association between interaction with infants and plasma oxytocin, OXTR *rs1042778*, and OXTR *rs2254298*. In particular, those who had “G” had significantly lower baseline plasma oxytocin level than those who has “A” (AA, AG) at OXTR *rs2254298*.

Suggestions from literature review for this research.

1. It has been suggested that interacts with infants by first-time pregnant women change the image of infant to a positive image, increase the attachment for the fetus and reduce the anxiety for child-care. But the changes for first-time pregnant women due to interactions with infant, remains unclear.
2. Pregnant women have more anxiety compared to women who are not pregnant, and their anxiety becomes stronger concerning childbirth and child rearing in the latter part of pregnancy. Therefore, it is appropriate for the target to be women in their late pregnancy and approaching delivery.
3. Cortisol is a hormone secreted when there is psychological stress. Therefore, cortisol could be evaluated in terms of changes caused by interactions with infants.
4. Oxytocin is a hormone that called attachment hormone because it promotes relationships between humans. Therefore, could be possible that it would increase in pregnant women when in interactions with infants.
5. Several factors related to the secretion of cortisol and oxytocin have been reported. Therefore, it is necessary to take those factors into account.

Methods

Study Design.

This study was a two-armed randomized control trial.

Participants and Setting.

The eligibility requirements of this study were low-risk Japanese primiparas and who: had a singleton pregnancy, were planning a vaginal delivery, were over 20 years old and who could participate in the study between 38 weeks 0 day and 38 weeks 6 days of pregnancy. Excluded were those who had any one of the following: an obstetric complication requiring treatment, a medical history of an endocrine disorder or psychiatric disorders, treatment for oral diseases including caries, smoking habit, involvement in daily life of infants care during pregnancy or performing nipple massage as self-care for labor induction.

This randomized controlled trial was conducted in four facilities: hospital, clinic, birth center and perinatal medical center in Tokyo, Japan between May 2017 and March 2018.

Randomization.

At the time participants agreed to participate in the study they were randomly assigned to either the experimental group to interact with infant or to the control group to watch a DVD image of infant. In order to equalize the number of participants in the experimental group and the control group, randomization was carried out using a web-based randomization system, with permuted blocks of four.

Masking and Blinding.

Participants and interveners: infants and mothers could not be masked because of the nature of this intervention. However the biochemists that measured salivary cortisol levels and salivary oxytocin levels of the physiological index, which were the primary and the secondary outcomes, were blinded.

Protocol.

Interventions were conducted between 9:00 and 15:00 in a quiet private room under a controlled temperature of 24-26°C by air conditioning at the facility. Participants had their medical history of rubella, measles, and mumps checked before the intervention **Appendix 1**. In addition, the researcher checked health condition on the day of the study **Appendix 2**. All participants were given instructions of the study protocol and saliva collection methods. The time for using intervention was 30 minutes. The participant sat on a chair to watch a silent movie (*Nature Therapy Seseragi*, JAN 4961501647780, Della Icn, Japan) 10 minutes prior to saliva collection for the base line (O1) to calm and without additional stress (**Figure 1**). Saliva was collected at two points: before (O1) and after the intervention (O2). To collect a sufficient volume of saliva for cortisol and oxytocin measurement at least 50µL of cortisol was needed and at least 1.0mL of saliva for oxytocin was needed. Participants were instructed to: 1) wash their mouth at the beginning on the study, 2) drink 100mL water 10 minutes before each saliva collection (Horiuchi, et al, 2016) and 3) look on a picture of salted plum as they stored saliva in their mouth. To avoid discrepancies in cortisol and oxytocin level measurement, the participant were asked to withhold from 1) dental treatment two days before participation in the study, 2) alcohol, spicy foods and sexual intercourse the day before the study, 3) caffeine intake 12 hours before the study and 4) food intake one hour before the study. Saliva was collected in a 15mL tube and then separated into two tubes: one was for the cortisol assay and the other was for the oxytocin assay. After saliva collections, the participants responded to the questionnaire before (O1) **Appendix 3** and after (O2) **Appendix 4** intervention.

After completion of the O2-questionnaire, buccal mucosa samples were obtained using a cotton swab to assay the single nucleotide polymorphism: SNPs of oxytocin.

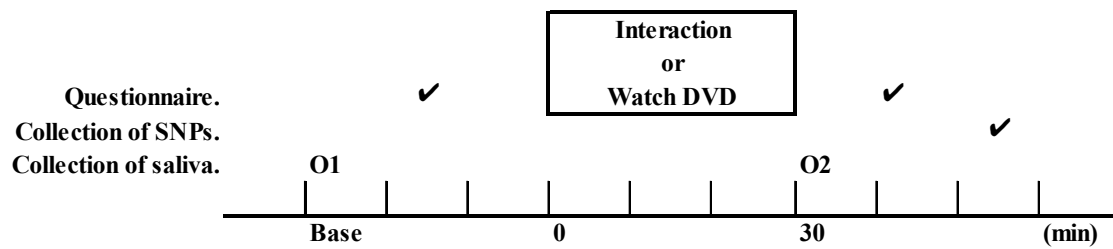


Figure 1. Intervention protocol.

After collection of saliva, the saliva samples were immediately stored in a freezer (Cryo Porter CS-80C, Scinics Corp., Tokyo, Japan) at -80°C (**Figure 2.**).



Figure 2.

Cryo Porter CS-80 $^{\circ}\text{C}$, Scinics Corp., Tokyo, Japan.



Figure 3.

Frozen Sample at -80°C .

The saliva samples were transported under frozen condition to Nagasaki University Graduate School of Medicine and Dentistry in Physio2 Laboratory and stored at -80°C before cortisol and oxytocin assay (**Figure 3.**).

After collection of buccal mucosa, the cotton swabs were dried up and transported to Nagasaki University Graduate School of Medicine and Dentistry in Physio2 Laboratory to assay.

Experimental group – interaction protocol.

First-time pregnant women interacted with infant. The intervention was as follows:

① Observation 5 minutes:

Eye to eye contact with infant, call his/her name, and touch his /her hand or leg.

② Communicate with infant 10 minutes: ***Figure 4.***

Touch, soothing, talking and eye to eye contact reserved instruction from his/her mother.

③ Holding infant 5 minutes: ***Figure 5.***

Holding infant and eye to eye contact. Soothing and talking to infant.

④ Holding infant 5 minutes:

After changing position, holding infant.

⑤ Observation 5 minutes:

Eye to eye contact, call his/her name, and touch his /her hand or leg.

As a point of attention, researcher took notes of the following items:

- Crying and vomiting of infant care with his/her mother.
- Not to acquire childcare skills, participant not to change diapers nor clothes.

Before recruitment into the study, mothers (with their informants) who agreed to participate in this study received training on how to interact with participants. In order to achieve equivalence of interventions, a third party checked for the following events every five to seven interventions **Appendix 5.**



Figure 4. ② Communicate with infant.



Figure 5. ③ Holding infant.

Control group – interaction protocol.

Using a similar situation as the experimental group, the control group participants, using a personal computer and headphones, watched a 30-minute DVD image of an infant (**Figure 6**).

The infant of DVD was 2-month girl, and program of control group was as follow.

- ① She spent time quietly on her bed: 15 minutes.
- ② Breast fed: 4 minutes.
- ③ Hugged her mother and finally fall asleep: 5 minutes.
- ④ Sleeping on her bed: 6 minutes.



Figure 6. Watched 30 minutes DVD image of infant.

Outcome Measurements.

Primary outcome.

The preliminary study of the study (Sonoda et al, 2018), indicated that the cortisol level in the intervention group was significantly decreased at the point of 30 minutes after the intervention. Therefore, the cortisol level was measured after 30 minutes' after the intervention.

Salivary cortisol level was analyzed using the Cortisol Salivary Immunoassay kit (Salimetrics, PA, USA) at the Nagasaki University Graduate School of Medicine and Dentistry in Physio2 Laboratory, following the manufacture's instruction. When the % coefficient of variation (%CV) of inter assay was > 10 , such data was not used.

Secondary outcomes.

State anxiety score was measured by STAI-form JYZ at the point of before(O1) and after (O2) intervention. Hidano, et al. (2000), developed the STAI-form JYZ and achieved a confirmed Cronbach α of .859-.923. State anxiety score consists of 20 items with a 4-point Likert scale. State anxiety score's range was 20-80, and 20-45 was judged as low anxiety and >55 was judged as high anxiety.

Salivary oxytocin level was analyzed by enzyme-linked immunosorbent assay (ELISA; ENZO Life Sciences, NY, USA) at the Nagasaki University Graduate School of Medicine and Dentistry in Physio2 Laboratory, following the manufacture's instruction. Before assay, the sample was adjusted following Carter, et al. (2007) and additionally aprotinin (Sigma-Aldrich Corporation, MO, USA) was added. When the % coefficient of variation (%CV) of inter assay was > 10 , such data was not used.

Taiji Kanjyo Hyoutei Shakudo could measure feelings about the image or imagination of the infant. *Taiji Kanjyo Hyoutei Shakudo* was developed by Hanazawa (1992). It has two scales, namely, approach feeling and avoidance feeling, each consisting of 14 items with a 4-point Likert scale. Approach feeling indicates the positive feelings for the infant (e.g. joyful, cheerful and beautiful), and the higher score means more positive feelings for infant. Avoidance feeling indicates the negative feeling for the infant, and the higher score means more negative feelings for the infant

(e.g. noisy, bothersome and frightening). The highest score for each scale is 42. The approach feeling and avoidance feeling were confirmed in terms of validity (checked correlation of question of infants, Approach feeling: $r=.76$, Avoidance feeling: $r=.68$) and reliability (checked by re-test method, Approach feeling: $r=.85$, Avoidance feeling: $r=.85$).

Characteristics of participants.

This questionnaire asked about: height, weight, age, marital status, living with partner, having own younger brother/sister, and experience of interactions with infants. The researcher gathered these data from the medical record: gestational date of delivery, height, weight, age, and medical history. Basic information related to cortisol and oxytocin level was also collected using a questionnaire. This included states of depression, anxiety, experiences of parental bonding and SNPs (*rs53576*, *rs2294298*).

Depression was assessed using the Japanese version of PHQ-9 (Muramatsu, et al, 2009), which consisted of nine items with a 4-point Likert scale. Muramatsu, et al. (2007) truncated the PHQ: Patient Health Questionnaire for Japanese, which then had a sensitivity 0.84, specificity 0.95, positive predictive value 0.87, negative predictive value 0.94 and kappa coefficient 0.79. A total score over 10 was classified as moderate severe to severe depression.

Trait anxiety was assessed using Hidano, et al. (2000) STAI-form JYZ, with a confirmed with Cronbach α .859-.923. Trait anxiety score consisting of 20 items has a 4-point Likert scale. Trait anxiety score's range was 20-80, and 20-45 was judged as low anxiety and over 55 was judged as high anxiety.

Experiences of parental bonding were assessed using the Japanese version of Parental Bonding Instrument (PBI). PBI was developed by Parker (1979) and shortened in the Japanese version by Ogawa (1991). It has two scales: care factor and over-protection factor. Each has confirmed validity for content validity, construct validity and concurrent validity and reliability (Cronbach's alpha was 0.83-0.92). Care factor (CA) consists of 12 items and over-protection factor (OP) consists of 13 items with a 4-point Likert scale. High score of care factor means the person received

warm-caring from their caregivers, and high score of over protection factor means the person received over protected care from their care givers.

The single nucleotide polymorphisms were assayed using buccal mucosa samples. From the buccal mucosa samples, two oxytocin receptor gene polymorphisms: *rs53576* and *rs2254298* were assayed by TaqMan single nucleotide polymorphism assay (Applied Biosystems, Thermo Fisher, MA, USA).

Process Evaluation.

Data for the process evaluation were gathered through before and after researcher developed questionnaires. The before questionnaire (O1) asked about: presentation of anxiety about living with infant, about delivery, about family relationship and about economic situation. The post intervention, questionnaire (O2) asked about: changes in the image of infant and change in anxiety about living with infant/ about delivery/ about family relationship/ about economic situations, burden and satisfaction. Opinions and comments for the program were from participants were from the open-ended section of the O2 questionnaire.

Sample Size

The sample sized was calculated based on Sonoda, et al. (2018). Since the change amount of the salivary cortisol level in the intervention group was 0.60 ng / mL and the change amount of the salivary cortisol level in the control group was 0.36 ng / mL, the difference to be detected was 0.20 ng / mL. SD in this study was estimated to be 0.3.

$$n = 2 \times (1.96 + 0.84)^2 \times 0.32 / 0.22$$

$$n = 35.28 \text{ people}$$

Therefore a total of at least 72 participants were needed for the study ($n=36$ experimental group; $n=36$ control group).

Recruitment

Recruitment of the participants was conducted at four facilities: hospital, clinic, birth center and perinatal medical center from May 2017 to March 2018. The number of participants was reached therefore recruitment was stopped in March 2018.

The researcher invited participation using **Appendix 6** with the cooperating institution from the four institutions. After consent from the institution, the researcher posted the disclosure of study information **Appendix 7** and leaflet **Appendix 8** at the outpatient wards before beginning recruitment.

Eligible pregnant women were selected by the researcher or institution staff. When eligible pregnant women after 34 weeks gestation visited the hospital for a health checkup or maternity class, the researcher provided a verbal explanation about the research objectives and methods using **Appendix 1** and **Appendix 9**. When the researcher gained the women's agreement two *written informed consent to participate in the study forms* were signed and the researcher kept one and the other was given to the participant.

The researcher recruited 2-6 month old infants and their mothers who were interventionees. The institution staff introduced the infants and their mothers to the research program. When the infant and his/her mother visited the hospital for the one-month checkup or vaccination, the researcher provided a verbal explanation about the research objectives and methods using **Appendix 10**. When the researcher gained the mother's agreement two *written informed consent to participate in the study forms* were signed and the researcher kept one and the other was given to the mother.

Analyses

All data were analyzed using descriptive statistics. The primary outcomes of the salivary cortisol level were compared between experimental group and control group. Also, salivary cortisol levels were compared within groups. The secondary outcomes: state anxiety score, salivary oxytocin and approach feeling and avoidance feeling were also compared between the experimental group and control group, and compared within groups. After performing the test of normality and drawing histograms, the

primary outcome and secondary outcomes were analyzed using the independent *t*-test and paired *t*-test. If needed the amount of change was calculated and compared groups.

As sub-group analysis, the following analysis was conducted. To compare and to confirm the affect of the degree of depression with salivary cortisol level and salivary oxytocin level, participants with PHQ-9 of 10 or more at baseline were classified moderate and those with nine or less were classified as minor. Comparisons between and within groups were conducted. To compare and to confirm the affect of PBI with salivary cortisol level and salivary oxytocin level, the median was calculated for both CA and OP. The scores higher than the median were defined as the High group and lower than the median were the Low group. Between group comparisons and within group comparisons were conducted. Regarding SNP, *rs2254298* was classified as GG and A carrier, and *rs53576* was classified as G carrier and AA. SNPs were related to oxytocin secretion, in order to confirm the affects on baseline and the reactivity; after the classification, comparison between groups and comparison within group were conducted.

For process evaluation, the frequency distribution was statistically analyzed. A chi-square test was conducted to confirm the relationship between the presence of intervention and the change in anxiety. Data from the open-ended comments, referring to the method of Kayama (2007), based differences and similarities of context described were coded by comparison. The naming of categories to express the codes' meaning were examined which raised the level of abstraction of the coding.

Statistical analyses were performed using SPSS Statistics version 25. (Static Base, Advanced Statistics, IBM Japan, Tokyo, Japan) with a two-sided 5% level of significance.

Ethical consideration.

This study was approved by the Research Ethics Committee of St. Luke's International University, Tokyo, Japan (No. 17A-004). This study was registered in the Clinical Trials Registry of University Hospital Information Network in Japan (UMIN000028471). This study was conducted in accordance with "Ethical Guidelines

on Medical Research for Human beings”. If an adverse event occurred due to the implementation of the study and health damage to the participant and infant occurred, the researcher and the hospital would respond so that appropriate treatment and other necessary measures could be taken. Health insurance would be applied to the provided treatment.

Recruitment and informed consent.

Participation in this study was voluntary the researcher provided verbal and written informed consent for participants before study participation. Even after consent to participate participants could withdraw it at any time before the publication of this study. Participants were assured that there was no disadvantage to them due to their decision to not participate or to decline to participate at any time in the course of the study.

Anonymization of personal information.

After acquiring study consent, a correspondence table between ID and name was created. The information gathering of this study: saliva sample tubes, DNA samples and questionnaire was done under consolidated anonymization. Data gathered in the study is save for at least five years, and kept secure under lock and key.

Response after the end of the study.

At the end of the study, a report of the study will be sent to the Research Ethics Committee of St. Luke’s International University. The results of the study will be published as a doctoral dissertation, and submitted to academic journals for publication and to academic societies for presentations.

Results

Participant flow.

The participant flow diagram is shown in **Figure 7**. Of the women who were eligible to be participants, 102 women gave consent and were randomly assigned using the web-based randomization system to one of two groups: 51 women were in experimental group and 51 women were in control group.

Of the 51 women assigned to the experimental group, 13 participants dropped out before the intervention. The reasons for dropped out were: consent withdrawal ($n=7$), delivery before intervention ($n=3$), obstetric complication ($n=1$) and infection of infant who was an interventionee ($n=1$). Finally, 38 women participated in the intervention; the missing rate was 25.49%.

Of the 51 women assigned to the control group, seven participants dropped out before the intervention and two women dropped out by the end of intervention. The reasons for dropped out before the intervention were: consent withdrawal ($n=1$), delivery before intervention ($n=4$), obstetric complication ($n=1$) and transferred to another facility ($n=1$). The reasons for dropping out by the end of intervention were: felt uncomfortable during saliva collection ($n=1$) and did not fulfill inclusion criteria ($n=1$). Finally, 41 women participated in the control group; the missing rate was 17.65%. The final number of participants was 80. Data for the primary and secondary outcomes came from 38 participants in the experimental group and 42 in the control group.

Baseline data.

Characteristics of the participants are shown in **Table 1**. There was no difference between the two groups except for the State Anxiety score. There was not a significant difference between the groups, however salivary cortisol level was higher in the control group and salivary oxytocin level was higher in the experimental group.

There were no participants who engaged in nipple stimulation as induction for delivery and there were no scars in the oral cavity.

Implementation of Protocol.

All participants (n = 38 experimental group; n = 42 control group) participated in the study between 9:00 and 15:00. The researcher gave all participants verbal instructions of the study protocol and saliva collection methods. All participants collected saliva at two points: before (O1) and after the intervention (O2). All participants sat on a chair to watch a silent movie (*Nature Therapy Seseragi*, JAN 4961501647780, Della Icn, Japan) 10 minutes prior to saliva collection for the base line (O1). After collecting saliva, researcher stored saliva -80°C immediately. All participants were not to have 1) dental treatment two days before participation in the study, 2) alcohol, spicy foods and sexual intercourse the day before the study, 3) caffeine intake 12 hours before the study and 4) food intake one hour before the study. After completion of the O2-questionnaire, buccal mucosa samples were obtained using a cotton swab by the appropriate method.

In the experimental group, 11 mother-infant pairs were the intervention dyads for first-time pregnant women. To interact same mother-infant pair, mother-infant pair were limited. The 11 mother-infant pairs were 2-6 months after birth: six were boys and five were girls. Six baby boys interacted with a total of 26 first-pregnant women (68.42%), five baby girls interacted with a total of 12 first-pregnant women (31.58%).

Three infant boys age two months interacted with the first-pregnant women. Three infant boys aged two months interacted with first-pregnant women. Four infants (2 boys and 2 girls) at age three months' interacted with first-time pregnant women. Ten infants (8 boys and 2 girls) at four months of age interacted with first-time pregnant women. Nine infants (8 boys and 1 girl) at age five months interacted with first-time pregnant women. Twelve infants (5 boys and 7 girls) when they were six months old interacted with first-time pregnant women.

The outline of mother's instructions are shown in **Appendix 5**. Interactions with infants indicated that the 1) definition of "Motherese" (baby-talk) was insufficient, 2) participants were fearful of touching the infant; 3) length of holding infant was only a few seconds, and 4) participants talked with mother about child care.

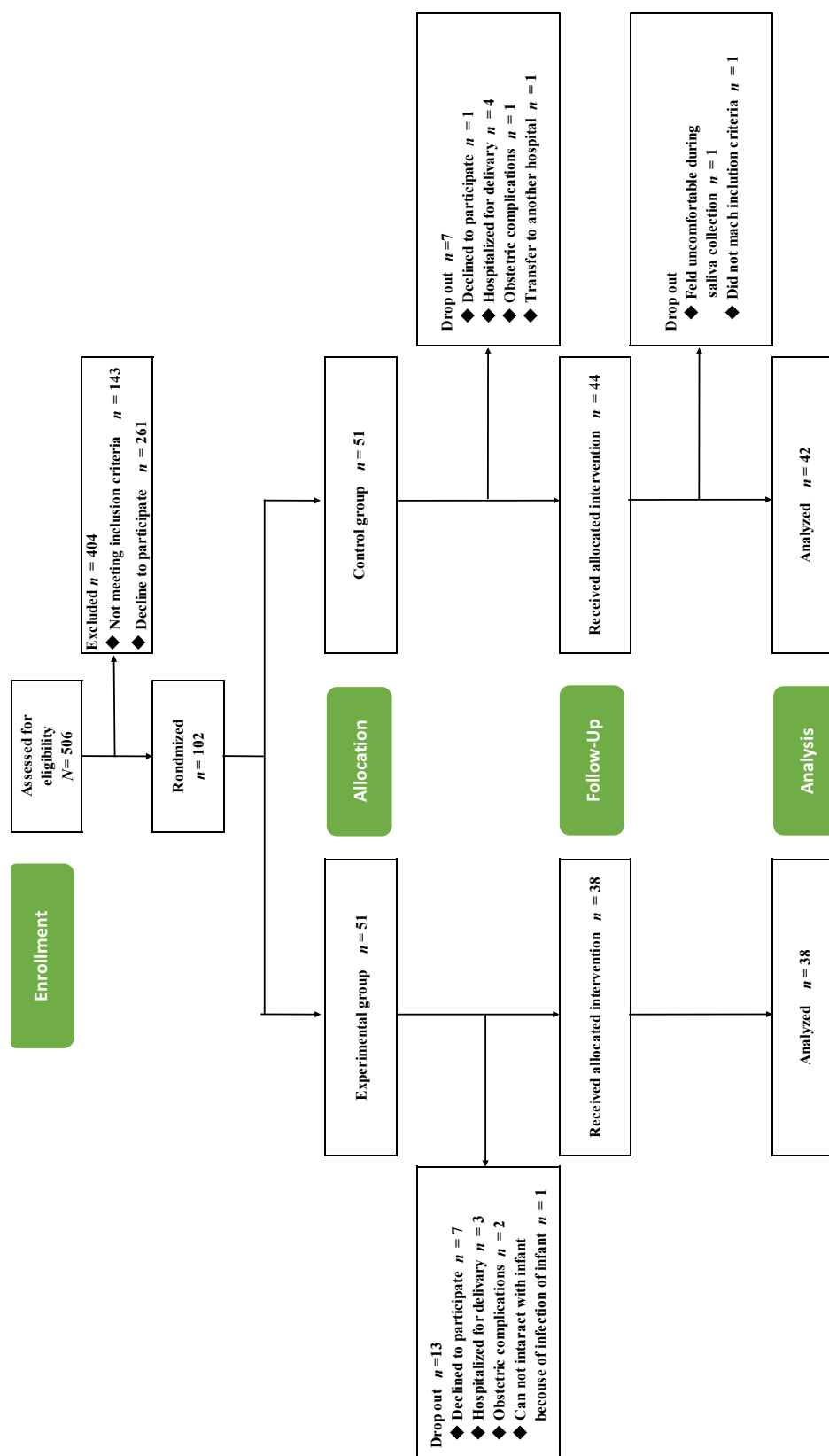


Figure 7. Flow diagram of participants.

Table 1***Participants Baseline Characteristics.***

Characteristics		Experimental <i>n</i> = 38		Control <i>n</i> = 42	
Age	<i>M</i> [<i>SD</i>]	32.39	[4.59]	32.57	[4.32]
Boby Mass Index before pregnancy	<i>M</i> [<i>SD</i>]	20.95	[2.70]	20.78	[2.19]
Infertility treatment	<i>n</i> (%)	8	(21.05%)	7	(16.67%)
Married	<i>n</i> (%)	38	(100.00%)	41	(97.62%)
Having own younger brother/sister	<i>n</i> (%)	23	(60.53%)	18	(42.86%)
Living with partner	<i>n</i> (%)	38	(100.00%)	41	(97.62%)
Experience of interaction with infants	<i>n</i> (%)	24	(63.16%)	18	(42.86%)
Delivery institution					
Hospital	<i>n</i> (%)	17	(44.74%)	21	(50.00%)
Clinic	<i>n</i> (%)	16	(42.11%)	13	(30.95%)
Birth Center	<i>n</i> (%)	5	(13.16%)	5	(11.90%)
Perinatal Medical Center	<i>n</i> (%)	0	(0.00%)	3	(7.14%)
PHQ-9 score ≥ 10 ^{a.}	<i>n</i> (%)	3	(7.89%)	5	(11.90%)
Parental Bonding Instrument (CA)					
High group ^{b.}	<i>M</i> [<i>SD</i>]	33.00	[2.51]	32.75	[2.05]
Low group ^{c.}	<i>M</i> [<i>SD</i>]	20.72	[6.47]	21.76	[6.04]
Parental Bonding Instrument (OP)					
High group ^{d.}	<i>M</i> [<i>SD</i>]	13.84	[4.35]	15.81	[5.50]
Low group ^{e.}	<i>M</i> [<i>SD</i>]	5.00	[2.58]	4.00	[2.03]
State Anxiety score ^{f.}	<i>M</i> [<i>SD</i>]	36.97	[6.90]	40.33	[7.33]
Trait Anxiety score ^{g.}	<i>M</i> [<i>SD</i>]	37.82	[7.21]	39.90	[6.73]
Having anxiety					
about child care	<i>n</i> (%)	34	(89.5%)	38	(90.5%)
about delivery	<i>n</i> (%)	22	(57.9%)	26	(61.9%)
about family relationship	<i>n</i> (%)	6	(15.8%)	6	(14.3%)
about economic conditions	<i>n</i> (%)	12	(31.6%)	12	(28.6%)
Taiji Kanjiyo Hyoutei Shakudo					
Approach Feeling	<i>M</i> [<i>SD</i>]	26.95	[6.45]	27.45	[6.88]
Avoidance Feeling ^{h.}	<i>M</i> [<i>SD</i>]	9.85	[5.07]	9.71	[5.52]
Salivary cortisol level ^{i.}	<i>M</i> [<i>SD</i>]	4.60	[1.12]	4.77	[1.44]
Salivary oxytocin level ^{j.}	<i>M</i> [<i>SD</i>]	75.81	[47.37]	68.68	[46.15]
SNP <i>rs</i> 2254298					
GG	<i>n</i> (%)	19	(50.00%)	19	(45.24%)
GA	<i>n</i> (%)	18	(47.37%)	16	(38.10%)
AA	<i>n</i> (%)	1	(2.63%)	7	(16.67%)
SNP <i>rs</i> 53576					
GG	<i>n</i> (%)	5	(13.16%)	11	(26.19%)
GA	<i>n</i> (%)	22	(57.89%)	14	(33.33%)
AA	<i>n</i> (%)	11	(28.95%)	17	(40.48%)

Note. a.: Experimental group *n*=35, Control group *n*=41b.: Experimental group *n*=20, Control group *n*=20c.: Experimental group *n*=18, Control group *n*=21d.: Experimental group *n*=19, Control group *n*=21e.: Experimental group *n*=19, Control group *n*=20f.: Experimental group *n*=36, Control group *n*=42g.: Experimental group *n*=38, Control group *n*=40h.: Experimental group *n*=37, Control group *n*=42i.: Experimental group *n*=37, Control group *n*=41j.: Experimental group *n*=28, Control group *n*=27

Primary Outcome: Salivary Cortisol Level

Selection of samples to be analyzed.

Salivary samples were collected at times O1 and O2 from 38 participants in the experimental group and 42 participants in the control group. Salivary cortisol level at time O1 could not be analyzed due to lack of saliva amount in one sample of the control group.

Salivary samples of the experimental group were O1: 38 samples and O2: 38 samples. In experimental group, a duplicate assay was conducted on 44 samples in total: O1 had 22 samples and O2 had 22 samples, and single assay was conducted on 32 samples in total: O1 had 16 samples and O2 had 16 samples. In duplicate assay, one sample of O1 was %CV>10, and it was excluded from statistical analysis. Therefore, statistical analysis for the experimental group included 37 paired samples of O1 and O2.

Salivary samples from the control group at time O1 was 41 samples and O2 was 42 samples. In the control group, duplicate assay was conducted on a total of 44 samples: O1 was 22 samples and O2 was 22 samples. Single assay was conducted on 39 samples in total: O1 was 19 samples and O2 was 20 samples. Therefore, statistical analysis for the control group was conducted with 41 paired samples of O1 and O2 (**Figure 8**).

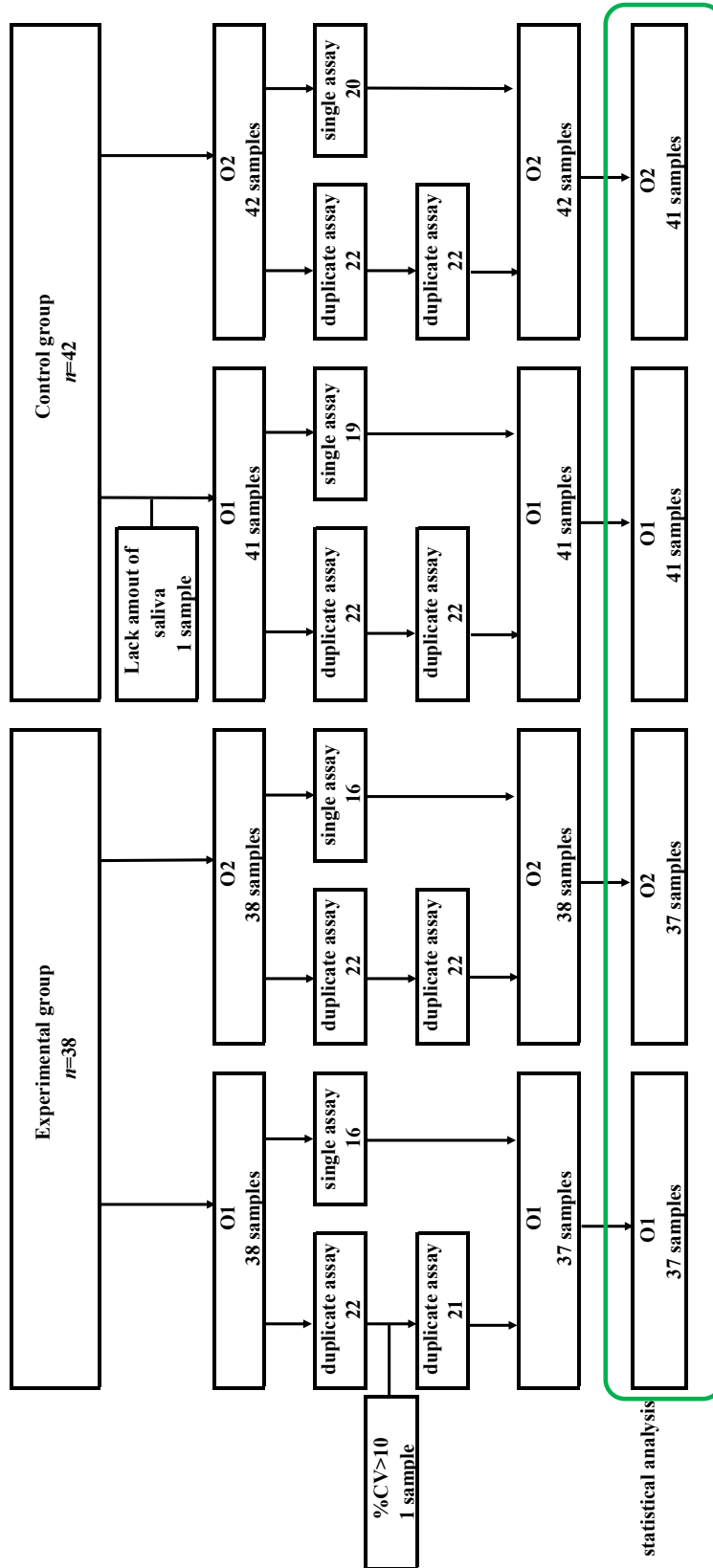


Figure 8. Flow diagram of salivary samples: cortisol.

Salivary Cortisol Level.

Data from the experimental group underwent the Shapiro-Wilk test for salivary cortisol level of O1 ($n=37$) and O2 ($n=37$). The p -value of O1 was $p = 0.40$ and the p -value of O2 was $p = 0.19$. The null hypothesis was not rejected.

In the control group, Shapiro-Wilk test for salivary cortisol level of O1 ($n=41$) and O2 ($n=41$) were conducted. The p -value of O1 was $p = 0.01$ and the p -value of O2 was $p = 0.18$. The null hypothesis of only O1 was rejected.

The distribution of salivary cortisol level of O1 and O2 in both groups is shown in the histogram, which was similar to a normal distribution (**Figure 9, Figure 10**)

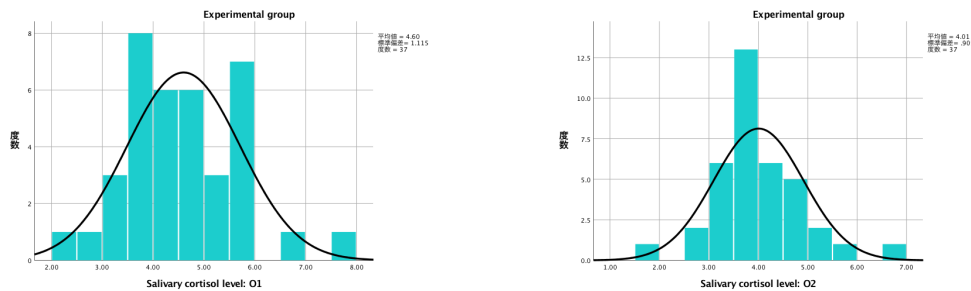


Figure 9. The distribution of salivary cortisol level of experimental group.

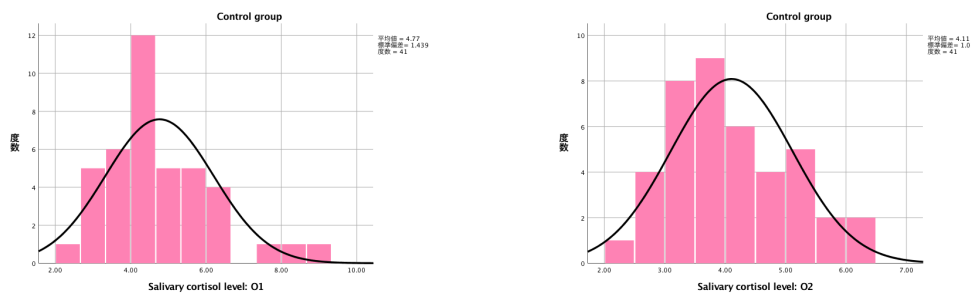


Figure 10. The distribution of salivary cortisol level of control group.

Comparison of Salivary Cortisol Level in Experimental group and Control group.

An independent t -test was conducted at time O2 between the experimental group ($n=37$) and the control group ($n=41$). The experimental group at O2 was 4.01 ± 0.91

(ng/mL) and the control group at O2 was 4.11±1.01 (ng/mL). The cortisol level of the experimental group was lower than the control group, however there was no significant difference between the two groups ($t=-0.459$, $p= 0.65$) (**Table 2**).

Table 2

Between Group Comparison of the Mean Level of Salivary Cortisol Level in Experimental Group and Control Group (ng/mL).

Cortisol level (ng/mL)	Experimental group			Control group			<i>t</i>	<i>p-value</i>
	<i>n</i>	<i>Mean</i>	<i>SD</i>	<i>n</i>	<i>Mean</i>	<i>SD</i>		
O1	37	4.60	1.12	41	4.77	1.44	-0.562	0.58
O2	37	4.01	0.91	41	4.11	1.01	-0.459	0.65

Note. Independent *t*-test was conducted.

Within-group Comparison of Salivary Cortisol Level before and after Intervention.

A paired *t*-test was conducted to compare within –group salivary cortisol levels at O1 and O2. The comparison of the salivary cortisol level at O1 and O2 in the experimental group ($n=37$) at O1 was 4.60±1.12 (ng/mL) and at O2 was 4.01±0.91 (ng/mL). Therefore, O2 was significantly lower than O1 ($t = 4.570$, $p = 0.00$) (**Table 3**). Similarly, the paired *t*-tests at O1 and O2 in the control group ($n=41$) were 4.77±1.44 (ng/mL) and 4.11±1.01 (ng/mL) with O2 was significantly lower than O1 ($t = 5.007$, $p = 0.00$) (**Table 3**).

Table 3

Within-group Comparison of Salivary Cortisol Level before and after Intervention (ng/mL).

	<i>n</i>	<i>Mean</i>	<i>SD</i>	<i>MD</i>	<i>SD</i>	<i>95%CI</i>	<i>t</i>	<i>df</i>	<i>p-value</i>
Experimental group									
O1	37	4.60	1.12						
O2	37	4.01	0.91	0.59	0.79	[0.33 - 0.86]	4.570	36	0.00
Control group									
O1	41	4.77	1.44						
O2	41	4.11	1.01	0.66	0.84	[0.39 - 0.93]	5.007	40	0.00

Note. Paired *t*-test was conducted.

Salivary Cortisol level and PHQ-9.

PHQ-9 score was measured for 35 participants in the experimental group and 41 participants in the control group. Mean score of PHQ-9 was 4.91 (*SD*: 2.91) in the experimental group and 5.49 (*SD*: 2.83) in the control group. Mean PHQ-9 score of the control group was higher than the experimental group, however there was no significant difference between the two groups ($t=-0.869$, $p=0.39$). PHQ-9 can be classified as “minor” or “moderate” depending on the total score of 10 points. In this study, “minor” was 32 participants in the experimental group and 36 participants in the control group. “Moderate” was three participants in the experimental group and five participants in the control group. The participants who were classified “moderate” were more in the control group, however there was no significant difference between the two groups ($\chi^2=0.263$, $p=0.61$). In the salivary cortisol level, statistical analysis was conducted on 37 paired samples in the experimental group and 41 paired samples in the control group. Finally, statistical analysis was conducted on 34 samples in the experimental group and 40 samples in the control group.

PHQ-9: minor group.

Comparison of the experimental group and the control group in the moderate sub-group, the salivary cortisol level at O2 was higher the control sub-group than experimental group, however there was not a significant difference between the groups ($t=-0.299$, $p=0.77$) (**Table 4**). Change amount of salivary cortisol levels in those who were classified as minor was larger the control sub-group compared the experimental sub-group, however there was no significant difference between two groups ($t=0.199$, $p=0.84$) (**Table 5**).

To compare the of the salivary cortisol level at O1 and O2, a paired t -test was conducted in the minor sub-groups. The comparison of the salivary cortisol level at O1 and O2 in the experimental sub-group ($n=31$) at O1 was 4.57 ± 1.18 (ng/mL) and at O2 was 4.02 ± 0.98 (ng/mL), O2 was significantly lower than O1 in both groups ($t = 3.856$, $p = 0.00$, $t = 4.520$, $p = 0.00$) (**Table 6**).

PHQ-9: moderate group.

Comparison of the experimental group and the control group in the moderate sub-group, the salivary cortisol level at O2 of the experimental sub-group was higher than the control sub-group, however there was no significant difference between the sub-groups ($t=0.175$, $p=0.87$) (**Table 4**). Change amount of salivary cortisol level of the moderate experimental sub-group was larger compared the moderate control sub-group, however there was no significant difference between the two groups ($t=-2.064$, $p=0.09$) (**Table 5**).

To compare the of the salivary cortisol level at O1 and O2, a paired t -test was conducted in the moderate sub-groups. The comparison of the salivary cortisol level at O1 and O2 in the experimental sub-group, O2 was lower than O1 ($t = 3.189$, $p = 0.09$) (**Table 6**). Similarly, a paired t -test at O1 and O2 in the control sub-group, O2 was significantly lower than O1 ($t = 3.646$, $p = 0.00$) (**Table 6**).

Table 4

Between Group Comparison of the Mean Level of Salivary Cortisol Level in the Experimental Group and Control Group Classified PHQ-9 (ng/mL).

Cortisol level (ng/mL)		Experimental group			Control group			t	p -value
		n	Mean	SD	n	Mean	SD		
minor	O1	31	4.57	1.18	35	4.68	1.30	-0.363	0.72
	O2	31	4.02	0.98	35	4.09	0.98	-0.299	0.77
moderate	O1	3	5.31	0.65	5	4.42	1.00	1.370	0.22
	O2	3	3.93	0.15	5	3.82	1.06	0.175	0.87

Note. Independent t -test was conducted.

Table 5

Between Group Comparison of the Amount of Change for Experimental and Control Groups.

	Experimental group			Control group			<i>t</i>	<i>p-value</i>
	<i>n</i>	<i>Mean</i>	<i>SD</i>	<i>n</i>	<i>Mean</i>	<i>SD</i>		
minor group	31	-0.55	0.80	35	-0.59	0.77	0.199	0.84
moderate group	3	-1.38	0.75	5	-0.59	0.36	-2.064	0.09

Note. Independent *t*-test was conducted.

Table 6

Within-group Comparison of Salivary Cortisol Level before and after Intervention for in the PHQ-9 Minor and Moderate Experimental and Control Subgroup. (ng/mL).

	<i>n</i>	<i>Mean</i>	<i>SD</i>	<i>MD</i>	<i>SD</i>	95%CI	<i>t</i>	<i>df</i>	<i>p-value</i>
PHQ: minor									
Experimental group									
O1	31	4.57	1.18	0.55	0.80	[0.26 - 0.84]	3.856	30	0.00
O2	31	4.02	0.98						
Control group									
O1	35	4.68	1.30	0.59	0.77	[0.33 - 0.86]	4.520	34	0.00
O2	35	4.09	0.98						
PHQ: moderate									
Experimental group									
O1	3	5.31	0.65	1.38	0.75	[-0.48 - 3.24]	3.189	2	0.09
O2	3	3.93	0.15						
Control group									
O1	5	4.42	1.00	0.60	0.37	[0.14 - 1.05]	3.646	4	0.02
O2	5	3.82	1.06						

Note. Paired *t*-test was conducted.

Salivary Cortisol level and PBI.

The PBI score of the CA measured 38 participants in the experimental group and 41 participants in the control group. Median score of the CA was 28.0 in the experimental group and 30 in the control group. The participants whose score was more than 28.0 in the experimental group were classified as the High group and less than 28.0 were classified as the Low group. Mean score of CA High group and CA Low group were not significantly different between the experimental group and the

control group ($t = 0.345, p = 0.73, t = -0.519, p = 0.61$). Similarly, the PBI score of OP measured 38 participants in the experimental group and 41 participants in the control group. Median score of OP was 8.5 in the experimental group and 9.0 in the control group. Mean score of OP High group and OP Low group were not significantly different between the experimental group and the control group ($t = -1.246, p = 0.22, t = 1.394, p = 0.19$)

For the salivary cortisol level, statistical analysis was conducted on 37 paired samples in the experimental group and 41 paired samples in the control group. Finally, statistical analysis was conducted on 37 samples in the experimental group and 39 samples in the control group.

Salivary cortisol level and PBI: Care factor

Comparisons were conducted of the salivary cortisol level of O2 in the experimental group and the control group classified as CA High group. The salivary cortisol level of O2 in the experimental group was higher than the control group, however there was not a significant difference between the groups ($t = 0.166, p = 0.87$) (**Table 7**). Change amount of salivary cortisol level in the CA High sub-group was conducted. Change amount was larger in the control sub-group compared the experimental sub-group, however there was no significant difference between two groups ($t = 0.974, p = 0.34$) (**Table 8**). To compare the salivary cortisol level at O1 and O2, paired t -tests were conducted in the CA High sub-group. The comparison of the salivary cortisol level at O1 and O2 in the experimental group indicated that O2 was lower than O1 ($t = 1.906, p = 0.07$) (**Table 9**). Similarly, the paired t -tests at O1 and O2 in the control group showed that O2 was significantly lower than O1 ($t = 4.377, p = 0.00$) (**Table 9**).

Comparison of salivary cortisol level of O2 in the experimental group and the control group who were classified CA Low group showed that the salivary cortisol level of O2 in the control group was higher than the experimental group, however there was not a significant difference between the groups ($t = -0.677, p = 0.50$) (**Table 7**). Change amount of salivary cortisol level in the CA Low sub-group was larger in the

experimental sub-group compared to the control sub-group, however there was no significant difference between two groups ($t = -0.404, p = 0.69$) (**Table 8**). To compare the of the salivary cortisol level at O1 and O2, a paired t -test was conducted in the CA Low sub-group. The comparison of the salivary cortisol level at O1 and O2, indicated that O2 was significantly lower than O1 in both experimental group and control group ($t = 5.444, p = 0.00, t = 3.339, p = 0.00$) (**Table 9**).

Table 7

Between Group Comparison of the Mean Level of Salivary Cortisol Level in the Experimental Group and Control Group Classified PBI: CA (ng/mL).

		Experimental group			Control group			t	p -value
Cortisol level (ng/mL)		n	Mean	SD	n	Mean	SD		
CA High group	O1	20	4.45	1.19	19	4.62	1.16	-0.451	0.66
	O2	20	4.10	1.08	19	4.04	0.91	0.166	0.87
CA Low group	O1	17	4.78	1.02	21	4.87	1.70	-0.187	0.85
	O2	17	3.90	0.67	21	4.10	1.11	-0.677	0.50

Note. CA: Care factor of Parental Bonding Instrument. Independent t -test was conducted.

Table 8

Between Group Comparison of the Amount of Change for Experimental and Control CA High and CA Low Subgroups.

		Experimental group			Control group			t	p -value
		n	Mean	SD	n	Mean	SD		
CA High group		20	-0.35	0.82	19	-0.57	0.57	0.974	0.34
CA Low group		17	-0.88	0.67	21	-0.76	1.05	-0.404	0.69

Note. CA: Care factor of Parental Bonding Instrument. Independent t -test was conducted.

Table 9

Within-group Comparison of Salivary Cortisol Level before and after Intervention in CA High and CA Low subgroups. (ng/mL).

	<i>n</i>	<i>Mean</i>	<i>SD</i>	<i>MD</i>	<i>SD</i>	<i>95%CI</i>	<i>t</i>	<i>df</i>	<i>p-value</i>
PBI: CA High group									
Experimental group									
O1	20	4.45	1.19	0.35	0.82	[-0.03 - 0.73]	1.906	19	0.07
O2	20	4.10	1.08						
Control group									
O1	19	4.62	1.16	0.57	0.57	[0.30 - 0.85]	4.377	18	0.00
O2	19	4.04	0.91						
PBI: CA Low group									
Experimental group									
O1	17	4.78	1.02	0.88	0.67	[0.54 - 1.23]	5.444	16	0.00
O2	17	3.90	0.67						
Control group									
O1	21	4.87	1.70	0.76	1.05	[0.29 - 1.24]	3.339	20	0.00
O2	21	4.10	1.11						

Note. CA: Care factor of Parental Bonding Instrument. Paired *t*-test was conducted.

Salivary cortisol level and PBI: Over-protection factor

Comparison of salivary cortisol level of O2 in the experimental group and the control group whose classified OP High group, found there was not a significant difference between the groups ($t=0.065$, $p=0.95$) (**Table 10**). Change amount of salivary cortisol level in the OP High subgroup was larger in the control subgroup compared with the experimental subgroup, however there was no significant difference between the two groups ($t = 0.205$, $p = 0.84$) (**Table 11**). To compare the salivary cortisol level at O1 and O2, paired *t*-tests were conducted in the OP High subgroup. The comparison of the salivary cortisol level at O1 and O2, showed that O2 was significantly lower than O1 in both experimental group and the control group ($t = 4.423$, $p = 0.00$, $t = 4.864$, $p = 0.00$) (**Table 12**).

Comparison of salivary cortisol level of O2 in the experimental group and the control group who were classified OP Low group, found that the salivary cortisol level of O2 in the control group was higher than the experimental group, however there was not a significant difference between the groups ($t = -0.417$, $p = 0.68$) (**Table 10**). Change amount of salivary cortisol level in the OP Low subgroup was larger in the experimental subgroup compared to the control subgroup, however there was no

significant difference between two groups ($t = -0.256, p = 0.80$) (Table 11). To compare the of the salivary cortisol level at O1 and O2, a paired t -test was conducted in the OP Low sub-group. The comparison of the salivary cortisol level at O1 and O2; O2 was significantly lower in O1 in both experimental group and control group ($t = 2.441, p = 0.03, t = 2.507, p = 0.02$) (Table 12).

Table 10

Between Group Comparison of the Mean Level of Salivary Cortisol Level in the Experimental Group and Control Group Classified PBI: OP (ng/mL).

		Experimental group			Control group			t	p -value
Cortisol level (ng/mL)		n	Mean	SD	n	Mean	SD		
OP High group	O1	18	4.56	0.87	21	4.59	1.31	-0.081	0.94
	O2	18	3.87	0.55	21	3.86	0.85	0.065	0.95
OP Low group	O1	19	4.64	1.33	19	4.72	1.22	-0.197	0.85
	O2	19	4.13	1.15	19	4.28	1.08	-0.417	0.68

Note. OP: Over-protection factor of Parental Bonding Instrument. Independent t -test was conducted.

Table 11

Between Group Comparison of the Amount of Change for Experimental and Control OP High and Low Subgroup.

		Experimental group			Control group			t	p -value
		n	Mean	SD	n	Mean	SD		
OP High group		18	-0.68	0.65	21	-0.73	0.69	0.205	0.84
OP Low group		19	-0.51	0.91	19	-0.44	0.77	-0.256	0.80

Note. OP: Over-protection factor of Parental Bonding Instrument. Independent t -test was conducted.

Table 12

Within-group Comparison of Salivary Cortisol Level before and after Intervention in OP High and OP Low Subgroups. (ng/mL).

	<i>n</i>	<i>Mean</i>	<i>SD</i>	<i>MD</i>	<i>SD</i>	95%CI	<i>t</i>	<i>df</i>	<i>p-value</i>
PBI: OP High group									
O1	18	4.56	0.87	0.68	0.66	[0.36 - 1.01]	4.423	17	0.00
O2	18	3.87	0.55						
Control group									
O1	21	4.59	1.31	0.73	0.69	[0.41 - 1.04]	4.846	20	0.00
O2	21	3.86	0.85						
PBI: OP Low group									
O1	19	4.64	1.33	0.51	0.91	[0.07 - 0.95]	2.441	18	0.03
O2	19	4.13	1.15						
Control group									
O1	19	4.72	1.22	0.44	0.77	[0.07 - 0.81]	2.507	18	0.02
O2	19	4.28	1.08						

Note. OP: Over-protection factor of Parental Bonding Instrument. Paired *t*-test was conducted.

Secondary Outcomes: State Anxiety Score

Selection of samples to be analyzed.

The State Anxiety score was measured at two time-points: O1 and O2 in both groups. There were 35 women in the experimental group (missing rate: 7.89%), responding to the State Anxiety measure and 42 women in the control group at the O1. Similarly, at the O2, there were 36 women responding to the State Anxiety measure in experimental group (missing rate: 5.26%), and 41 in the control group (missing rate: 2.38%). Finally, statistical analysis was conducted on 35 women at times O1 and O2 in the experimental group and 41 women at times O1 and O2 in the control group.

State Anxiety Score.

Shapiro-Wilk test for State Anxiety score of O1 (*n*=35) and O2 (*n*=35) in the experimental group were conducted. The *p*-value of O1 was *p*= 0.77 and the *p*-value of after intervention was *p*= 0.63, and the null hypothesis was not rejected both O1 and O2.

In the control group, Shapiro-Wilk test for State Anxiety score of O1 (*n*=41) and O2 (*n*=41) was conducted. The *p*-value of O1 was *p*= 0.57 and the *p*-value of O2 was *p*= 0.24, and the null hypothesis was not rejected both O1 and O2.

The distribution of State Anxiety score of O1 and O2 in both groups is shown in the histogram, which was similar to normal distribution (**Figure 11.** and **Figure 12.**)

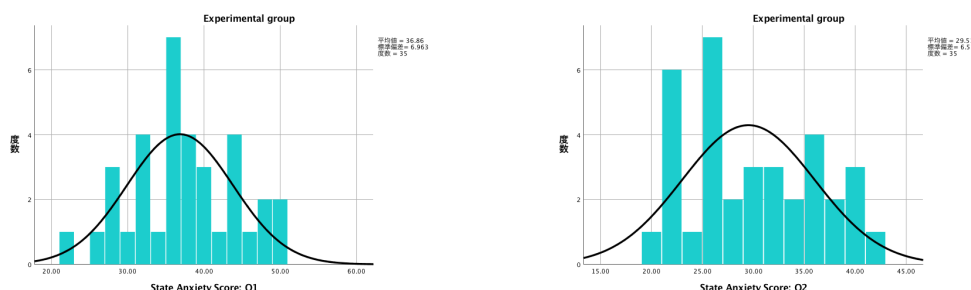


Figure 11. The distribution of State Anxiety Score in the experimental group.

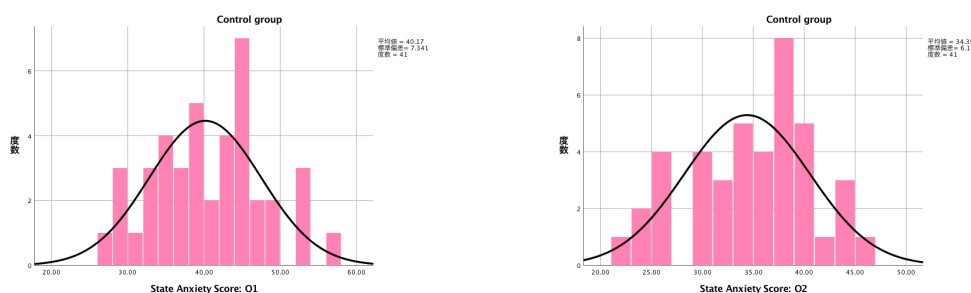


Figure 12. The distribution of State Anxiety Score in the control group.

Comparison of State Anxiety Score in Experimental group and Control group.

Independent *t*-test was conducted at the point of O2 between the experimental group ($n=35$) and control group ($n=41$). The experimental group score of O2 was 29.51 ± 6.51 and control group score of O2 was 34.39 ± 6.18 . The State Anxiety score of O2 was significantly lower in the experimental group ($t = -3.345, p = 0.00$) (**Table 13**)

However, the State Anxiety score of O1 was significantly lower in experimental group ($t = -2.008, p = 0.05$). For that reason, the amount of change, which was computed as the State Anxiety score of O2 minus the score of O1 was compared between the experimental group and control group. The amount of change in the experimental group was -7.34 ± 4.64 and the control group was -5.78 ± 6.08 ; the amount of change in the experimental group was larger than that of control group, however

there was no significant difference between the two groups ($t = -1.243$, $p = 0.22$) (Table 14).

Table 13

Between Group Comparison of State Anxiety Mean Scores in Experimental Group and Control Group.

State Anxiety score	Experimental group			Control group			<i>t</i>	<i>p-value</i>
	<i>n</i>	<i>Mean</i>	<i>SD</i>	<i>n</i>	<i>Mean</i>	<i>SD</i>		
O1	35	36.86	6.96	41	40.17	7.34	-2.008	0.05
O2	35	29.51	6.51	41	34.39	6.18	-3.345	0.00

Note. Independent *t*-test was conducted.

Table 14

Between Group Comparison of the Amount of Change in the Experimental Group and Control Group.

Change amount of score	Experimental group			Control group			<i>t</i>	<i>p-value</i>
	<i>n</i>	<i>Mean</i>	<i>SD</i>	<i>n</i>	<i>Mean</i>	<i>SD</i>		
	35	-7.34	4.64	41	-5.78	6.08	-1.243	0.22

Note. Independent *t*-test was conducted.

Within-group Comparison of State Anxiety score before and after Intervention.

Paired *t*-test of the State Anxiety score of O1 and O2 in the experimental group ($n=35$) was 36.86 ± 6.96 and 29.51 ± 6.51 respectively. Therefore, the within group of O2 was significantly lower than the point of O1 ($t = 9.363$, $p = 0.00$) (Table 15).

Similarly, paired *t*-test in the control group ($n=41$) of O1 was 40.17 ± 7.34 and O2 was 34.39 ± 6.18 , which was significantly lower than the point of O1 ($t = 6.091$, $p = 0.00$) (Table 15).

Table 15

Within-group Comparison of State Anxiety Score before and after Intervention in the Experimental and Control Groups.

	<i>n</i>	<i>Mean</i>	<i>SD</i>	<i>MD</i>	<i>SD</i>	<i>95%CI</i>	<i>t</i>	<i>df</i>	<i>p-value</i>
Experimental group									
O1	35	36.86	6.96						
O2	35	29.51	6.51	7.34	4.64	[5.75 - 8.94]	9.363	34	0.00
Control group									
O1	41	40.17	7.34						
O2	41	34.39	6.18	5.78	6.08	[3.86 - 7.70]	6.091	41	0.00

Note. Paired *t*-test was conducted.

Secondary Outcomes: Salivary Oxytocin Level.

Selection of Samples to be Analyzed.

Salivary samples were collected at O1 and O2 from 38 participants in the experimental group and 42 participants in the control group. Salivary oxytocin level could not be analyzed in 13 samples at O1 ($n=7$) and O2 ($n=6$) due to lack of saliva amount in experimental group; there were 25 unusable samples in the control group at O1, ($n=12$) and O2 ($n=13$).

Salivary samples at O1 for the experimental group were 31 samples and at O2 there were 32 samples. In the experimental group, duplicate assay was conducted with 19 samples: O1 was 9 samples and O2 was 10 samples. Single assay was conducted on a total of 43 samples: O1 was 21 samples and O2 was 22 samples. In duplicate assay, 3 samples at O1 had $\%CV>10$ and 1 sample at O2 was $\%CV>10$ and were excluded from statistical analysis. Therefore, statistical analysis was conducted on 24 samples from O1 and 24 samples from O2 in the experimental group.

Salivary samples of the control group from O1 were 30 samples and O2 yielded 29 samples. In the control group, duplicate assay was conducted on 20 samples in total: O1 had 10 samples and O2 had 10 samples. Single assay was conducted on 39 samples in total: O1 was 20 samples and O2 was 19 samples. Therefore, statistical analysis was conducted on 24 samples from O1 and 24 samples from O2 in the control group.

Salivary Oxytocin Level.

The Shapiro-Wilk test for salivary oxytocin level from O1 ($n=24$) and O2 ($n=24$) in the experimental group were conducted. The p -value of O1 was $p=0.14$ and the p -value of O2 was $p=0.04$, therefore the null hypothesis of O2 was only rejected.

In the control group, the Shapiro-Wilk test for salivary oxytocin level of O1 ($n=24$) and O2 ($n=24$) was conducted. The p -value for O1 was $p=0.00$ and the p -value of O2 was $p=0.04$, therefore the null hypothesis was rejected.

The distribution of salivary oxytocin level of O1 and O2 in both groups is shown in the histogram, which was similar to normal distribution (**Figure 13.** and **Figure 14.**)

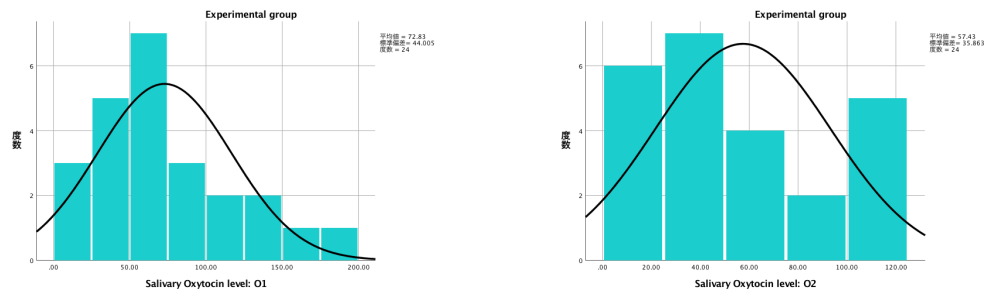


Figure 13. The distribution of salivary oxytocin level in the experimental group.

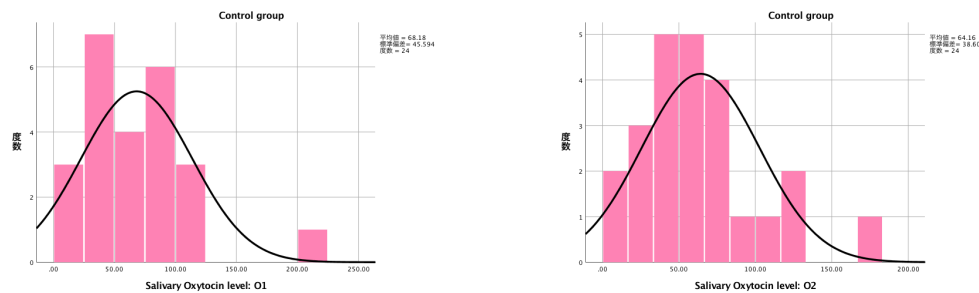


Figure 14. The distribution of salivary oxytocin level in the control group.

Comparison of Salivary Oxytocin Level in the Experimental Group and Control Group.

To compare salivary oxytocin levels an independent t -test was conducted on O2 between the experimental group and control group. The experimental group of O2 ($n=24$) was 57.43 ± 35.86 (pg/mL) and the control group of O2 ($n=24$) was 64.16 ± 38.80

(pg/mL). Salivary oxytocin level of the experimental group was lower than that of control group, however there was no significant difference between the two groups ($t = -0.626, p = 0.53$) (Table 16).

Table 16

Between Group Comparison of the Mean Level of Salivary Oxytocin Level in the Experimental Group and Control Group (pg/mL).

Oxytocin level (pg/mL)	Experimental group			Control group			<i>t</i>	<i>p-value</i>
	<i>n</i>	<i>Mean</i>	<i>SD</i>	<i>n</i>	<i>Mean</i>	<i>SD</i>		
O1	24	72.83	44.01	24	68.18	45.59	0.360	0.72
O2	24	57.43	35.86	24	64.16	38.60	-0.626	0.53

Note. Independent *t*-test was conducted.

Within-group Comparisons of Salivary Oxytocin Level before and after Intervention.

Paired *t*-tests of salivary oxytocin level of O1 and O2 in the experimental group ($n = 24$) for O1 was 72.83 ± 44.01 (pg/mL) and O2 was 57.43 ± 35.86 (pg/mL). Therefore, O2 was significantly lower than O1 ($t = 3.588, p = 0.00$) (Table 17).

Similarly, the paired *t*-test between O1 and O2 in the control group ($n = 24$) for O1 was 68.18 ± 45.59 (pg/mL) and for O2 was 64.16 ± 38.60 (pg/mL); O2 was lower than O1, however there was no difference between O1 and O2 in the control group ($t = 1.008, p = 0.32$) (Table 17).

Table 17

Within-group Comparison of Salivary Oxytocin Level before and after Intervention for the Experimental and Control groups (pg/mL).

	<i>n</i>	<i>Mean</i>	<i>SD</i>	<i>MD</i>	<i>SD</i>	95%CI	<i>t</i>	<i>df</i>	<i>p-value</i>
Experimental group									
O1	24	72.83	44.01	15.40	21.03	[6.52 - 24.28]	3.588	23	0.00
O2	24	57.43	35.86						
Control group									
O1	24	68.18	45.59	4.01	19.51	[-4.22 - 12.25]	1.008	23	0.32
O2	24	64.16	38.60						

Note. Paired *t*-test was conducted.

Polymorphisms of Oxytocin Receptors.

All samples (experimental group: $n = 38$ and control group: $n = 42$) were analyzed for SNPs detection. There were 24 samples of salivary oxytocin level in both groups.

rs2254298

In the experimental group, there were 19 of the GG (50.00%), 18 of the GA (47.37%) and 1 of the AA (2.63%). In the control group, there were 19 of the GG (45.24%), 16 of the GA (38.10%) and 7 of the AA (16.67%). From the type of GG and A carrier, there were 19 of the GG (50.00%) and 19 of the A carrier (50.00%) in the experimental group. In the control group, there were 19 of the GG (45.24%) and 23 of the A carrier (54.77%). There was no significant difference in number of all types in either groups ($\chi^2 = 0.181$, $p = 0.67$).

In the salivary oxytocin samples, which were adapted to statistical analysis of O1, GG were 12 samples and A carrier were 12 samples in the experimental group. The salivary oxytocin level of GG was 72.13 pg/mL (SD : 45.30) and of A carrier was 73.53 pg/mL (SD : 44.68). The salivary oxytocin level of A carrier was a little bit higher than GG, however there was no significant difference between the groups ($t = -0.076$, $p = 0.94$).

Similarly, in the salivary oxytocin samples, which were adapted to statistical analysis at time O1, GG was seven samples and A carrier was 17 samples in the control group. The salivary oxytocin level of GG was 61.70 pg/mL (SD : 33.58) and of A carrier was 70.85 pg/mL (SD : 50.39). The salivary oxytocin level of A carrier was a higher than GG, however there was no significant difference between the two groups ($t = -0.439$, $p = 0.67$).

rs 2254298: GG

Comparison of the experimental group and the control group in the GG, the salivary oxytocin level of O1 was 72.13 pg/mL (SD : 45.30) in the experimental group ($n=12$) and 61.70 pg/mL (SD : 33.58) in the control group ($n=7$). Salivary oxytocin

level of the experimental group was higher than the control group, however there was no significant difference between the groups ($t=0.528$, $p=0.60$). Similarly, salivary oxytocin level of O2 was 53.41 pg/mL (SD : 31.44) in the experimental group ($n=12$) and 60.92 pg/mL (SD : 32.86) in the control group ($n=7$). Salivary oxytocin level of O2 was higher in the control group than experimental group, however there was no significant difference between the two groups ($t=-0.494$, $p=0.63$) (**Table 18**). Change amount of salivary oxytocin level in those who had GG indicated that level of the experimental group was -18.72 pg/mL (SD : 22.87) and -0.78 pg/mL (SD : 14.56) in the control group. Change amount of salivary oxytocin level who had G carrier was larger in the experimental group compared the control group, however there was no significant difference between the two groups ($t=-1.855$, $p=0.08$) (**Table 19**).

To compare the change differences of the salivary oxytocin level at O1 and O2, a paired t -test was conducted in the GG subgroup. The comparison of the salivary oxytocin level at O1 and O2 in the experimental subgroup ($n = 12$) at O1 was 72.13 ± 45.30 (pg/mL) and at O2 was 53.41 ± 31.44 (pg/mL). Therefore, O2 was significantly lower than O1 ($t = 2.834$, $p = 0.02$) (**Table 20**). Similarly, the paired t -tests at O1 and O2 in the control subgroup ($n = 7$) were 61.70 ± 33.58 (pg/mL) and 60.92 ± 32.86 (pg/mL) with O2 lower than O1 ($t = 0.141$, $p = 0.89$) (**Table 20**).

Table 18

Between Group Comparison of the Mean Level of Salivary Oxytocin Level in the Experimental Group and Control Group Classified SNP (pg/mL).

Oxytocin level (pg/mL)		Experimental group			Control group			t	p -value
		n	Mean	SD	n	Mean	SD		
GG	O1	12	72.13	45.30	7	61.70	33.58	0.528	0.60
	O2	12	53.41	31.44	7	60.92	32.86	-0.494	0.63
A carrier	O1	12	73.53	44.68	17	70.85	50.39	0.148	0.88
	O2	12	61.44	40.81	17	65.50	41.60	-0.261	0.80

Note. SNP: rs2254298. Independent t -test was conducted.

Table 19

Between Group Comparison of the Amount of Change for Experimental Group and Control Group who had GG.

	Experimental group			Control group			<i>t</i>	<i>p-value</i>
	<i>n</i>	<i>Mean</i>	<i>SD</i>	<i>n</i>	<i>Mean</i>	<i>SD</i>		
Change amount of oxytocin level	12	-18.72	22.87	7	-0.78	4.56	-1.855	0.08

Note. SNP: rs2254298. Independent *t*-test was conducted.

Table 20

Within-group Comparison of Salivary Oxytocin Level before and after Intervention in GG Experimental and Control Subgroups. (pg/mL).

	<i>n</i>	<i>Mean</i>	<i>SD</i>	<i>MD</i>	<i>SD</i>	95%CI	<i>t</i>	<i>df</i>	<i>p-value</i>
Experimental group									
O1	12	72.13	45.30						
O2	12	53.41	31.44	18.72	22.88	[4.18 - 33.25]	2.834	11	0.02
Control group									
O1	7	61.70	33.58						
O2	7	60.92	32.86	0.78	14.55	[-12.69 - 14.24]	0.141	6	0.89

Note. SNP: rs2254298. Paired *t*-test was conducted.

rs 2254298: A carrier

Comparison of the experimental group and the control group in the A carrier, the salivary oxytocin level of O1 was 73.53 pg/mL (*SD*: 44.68) in the experimental group (*n*=12) and 70.85 pg/mL (*SD*: 50.39) in the control group (*n*=17). Salivary oxytocin level of the experimental group was higher than the control group, however there was no significant difference between the groups (*t*=0.148, *p*=0.88). Similarly, salivary oxytocin level of O2 was 61.44 pg/mL (*SD*: 40.81) in the experimental group (*n*=12) and 65.50 pg/mL (*SD*: 41.60) in the control group (*n*=17). Salivary oxytocin level of O2 was higher the control group than the experimental group, however there was no significant difference between the groups (*t*=-0.261, *p*=0.80) (**Table 18**). Change amount of salivary oxytocin level in those who had A carrier was calculated, change amount of salivary oxytocin level of the experimental group was -12.08 pg/mL (*SD*: 19.42) and -5.35 pg/mL (*SD*: 21.47) in the control group. Change amount of salivary

oxytocin level who had A carrier was larger the experimental group compared the control group, however there was no significant difference between the two groups ($t=-0.865$, $p=0.40$) (**Table 21**).

To compare the change difference of the salivary oxytocin level at O1 and O2, paired t -tests were conducted in the A carrier subgroups. The comparison of the salivary oxytocin level at O1 and O2 in the experimental subgroup ($n = 12$) at O1 was 73.53 ± 44.68 (pg/mL) and at O2 was 61.44 ± 40.81 (pg/mL). Therefore, O2 was significantly lower than O1 ($t = 2.156$, $p = 0.05$) (**Table 22**). Similarly, the paired t -tests at O1 and O2 in the control subgroup ($n = 17$) were 70.85 ± 50.39 (pg/mL) and 65.50 ± 41.60 (pg/mL) with O2 lower than O1 ($t = 1.027$, $p = 0.32$) (**Table 22**).

Table 21

Between Group Comparison of the Amount of Change for Experimental Group and Control Group who had A carrier.

Change amount of oxytocin level	Experimental group			Control group			t	p -value
	n	Mean	SD	n	Mean	SD		
	12	-12.08	19.42	17	-5.35	21.47	-0.865	0.40

Note. SNP: rs2254298. Independent t -test was conducted.

Table 22

Within-group Comparison of Salivary Oxytocin Level before and after Intervention in A carrier Experimental and Control Subgroups. (pg/mL).

	n	Mean	SD	MD	SD	95%CI	t	df	p -value
Experimental group									
O1	12	73.53	44.68	12.08	19.42	[-0.25 - 24.42]	2.156	11	0.05
O2	12	61.44	40.81						
Control group									
O1	17	70.85	50.39	5.35	21.47	[-5.69 - 16.39]	1.027	16	0.32
O2	17	65.50	41.60						

Note. SNP: rs2254298. Paired t -test was conducted.

rs53576

In the experimental group, there were five of the GG (13.16%), 22 of the GA (57.89%) and 11 of AA (28.95%). In the control group, there were 11 of the GG (26.19%), 14 of the GA (33.33%) and 17 of the AA (40.48%). From the type of G carrier and AA, there were 27 of the G carriers (71.05%) and 11 of the AA (28.95%) in the experimental group. In the control group, there were 25 of the G carriers (59.52%) and 17 of the AA (40.48%). There was no significant difference of number of all types in either groups ($\chi^2=1.166$, $p=0.28$).

In the salivary oxytocin samples which were adopted to statistical analysis of O1, G allele was 16 samples and AA was eight samples in the experimental group. The salivary oxytocin level of G carrier was 78.78 pg/mL (SD : 47.78) and of AA was 60.93 pg/mL (SD : 35.05). The salivary oxytocin level of G carrier was higher than AA, however there was no significant difference between the two groups ($t=0.934$, $p=0.36$). Similarly, in the salivary oxytocin samples which were adopted to statistical analysis of O1, G carrier was 16 samples and AA was eight samples in the control group. The salivary oxytocin level of G carrier was 70.27 pg/mL (SD : 48.00) and of AA was 63.99 pg/mL (SD : 43.17). The salivary oxytocin level of G carrier was a higher than AA, however there was no significant difference between the two groups ($t=0.312$, $p=0.76$).

rs53576: G carrier

Comparison of the experimental group and the control group in the G carrier, the salivary oxytocin level of O1 was 78.78 pg/mL (SD : 47.78) in the experimental group ($n=16$) and 70.27 pg/mL (SD : 48.00) in the control group ($n=16$). Salivary oxytocin level of the experimental group was higher than the control group, however there was no significant difference between the groups ($t=0.502$, $p=0.62$). Similarly, salivary oxytocin level of O2 was 62.85 pg/mL (SD : 39.35) in the experimental group ($n=16$) and 64.25 pg/mL (SD : 39.14) in the control group ($n=16$). Salivary oxytocin level of O2 was higher the control group than experimental group, however there was no significant difference between the groups ($t=-0.101$, $p=0.92$) (**Table 23**). Change

amount of salivary oxytocin level for those who had G carrier was calculated: change amount of salivary oxytocin level of the experimental group was -15.93 pg/mL (*SD*: 23.91) and -6.03 pg/mL (*SD*: 23.22) in the control group. Change amount of salivary oxytocin level who had G carrier was larger the experimental group compared the control group, however there was no significant difference between the two groups ($t=-1.189$, $p=0.24$) (**Table 24**).

To compare the differences of the salivary oxytocin level at O1 and O2, paired *t*-tests were conducted in the G carrier subgroups. The comparison of the salivary oxytocin level at O1 and O2 in the experimental group ($n = 16$) at O1 was 78.78 ± 47.78 (pg/mL) and at O2 was 62.85 ± 39.35 (pg/mL). Therefore, O2 was significantly lower than O1 ($t = 2.665$, $p = 0.02$) (**Table 25**). Similarly, the paired *t*-tests at O1 and O2 in the control group ($n = 16$) were 70.27 ± 48.00 (pg/mL) and 64.25 ± 39.14 (pg/mL) with O2 lower than O1 ($t = 1.038$, $p = 0.32$) (**Table 25**).

Table 23

Between Group Comparison of the Mean Level of Salivary Oxytocin Level in the Experimental Group and Control Group Classified SNP (pg/mL).

Oxytocin level (pg/mL)		Experimental group			Control group			<i>t</i>	<i>p</i> -value
		<i>n</i>	<i>Mean</i>	<i>SD</i>	<i>n</i>	<i>Mean</i>	<i>SD</i>		
G carrier	O1	16	78.78	47.78	16	70.27	48.00	0.502	0.62
	O2	16	62.85	39.35	16	64.25	39.14	-0.101	0.92
AA	O1	8	60.93	35.05	8	63.99	43.17	-0.156	0.88
	O2	8	46.59	26.57	8	64.00	40.17	-1.002	0.32

Note. SNP: rs53576. Independent *t*-test was conducted.

Table 24

Between Group Comparison of the Amount of Change for Experimental Group and Control Group who had G carrier.

Change amount of oxytocin level	Experimental group			Control group			<i>t</i>	<i>p</i> -value
	<i>n</i>	<i>Mean</i>	<i>SD</i>	<i>n</i>	<i>Mean</i>	<i>SD</i>		
	16	-15.93	23.91	16	-6.03	23.22	-1.189	0.24

Note. SNP: rs53576. Independent *t*-test was conducted.

Table 25

Within-group Comparison of Salivary Oxytocin Level before and after Intervention in G carrier Experimental and Control Subgroups. (pg/mL).

	<i>n</i>	<i>Mean</i>	<i>SD</i>	<i>MD</i>	<i>SD</i>	<i>95%CI</i>	<i>t</i>	<i>df</i>	<i>p-value</i>
Experimental group									
O1	16	78.78	47.78	15.93	23.91	[3.19 - 28.67]	2.665	15	0.02
O2	16	62.85	39.35						
Control group									
O1	16	70.27	48.00	6.03	23.22	[-6.35 - 18.40]	1.038	15	0.32
O2	16	64.25	39.14						

Note. SNP: rs53576. Paired *t*-test was conducted.

rs53576: AA

Comparison of the experimental group and the control group in the AA, the salivary oxytocin level of O1 was 60.93 pg/mL (*SD*: 35.05) in the experimental group (*n*=8) and 63.99 pg/mL (*SD*: 43.17) in the control group (*n*=8). Salivary oxytocin level of the control group was higher than the experimental group, however there was no significant difference between the groups (*t*=-0.156, *p*=0.88). Similarly, salivary oxytocin level of O2 was 46.59 pg/mL (*SD*: 26.57) in the experimental group (*n*=8) and 64.00 pg/mL (*SD*: 40.17) in the control group (*n*=8). Salivary oxytocin level of O2 was higher in the control group than the experimental group, however there was no significant difference between the two groups (*t*=-1.022, *p*=0.32) (**Table 23**). Change amount of salivary oxytocin level for AA was calculated, change amount of salivary oxytocin level of the experimental group was -14.34 pg/mL (*SD*: 15.03) and 0.01 pg/mL (*SD*: 8.17) in the control group. Change amount of salivary oxytocin level of those who had AA was significantly larger in the experimental group compared to the control group (*t*=-2.372, *p*=0.03) (**Table 26**).

To compare the change differences of the salivary oxytocin level at O1 and O2, paired *t*-tests were conducted in the AA subgroup. The comparison of the salivary oxytocin level at O1 and O2 in the experimental subgroup (*n*=8) at O1 was 60.93±35.05 (pg/mL) and at O2 was 46.59±26.57 (pg/mL). Therefore, O2 was significantly lower than O1 (*t* = 2.698, *p* = 0.03) (**Table 27**). Similarly, the paired

t -tests at O1 and O2 in the control subgroup ($n=8$) were 63.99 ± 43.17 (pg/mL) and 64.00 ± 40.17 (pg/mL) with O2 barely lower than O1 ($t = -0.003$, $p = 1.00$) (Table 27).

Table 26

Between Group Comparison of the Amount of Change for Experimental Group and Control Group who had AA.

Change amount of oxytocin level	Experimental group			Control group			t	p -value
	n	Mean	SD	n	Mean	SD		
	8	-14.34	15.03	8	0.01	8.17	-2.371	0.03

Note. SNP: rs53576. Independent t -test was conducted.

Table 27

Within-group Comparison of Salivary Oxytocin Level before and after Intervention in AA Experimental and Control Subgroups. (pg/mL).

	n	Mean	SD	MD	SD	95%CI	t	df	p -value
Experimental group									
O1	8	60.93	35.05						
O2	8	46.59	26.57	14.34	15.03	[1.77 - 26.91]	2.698	7	0.03
Control group									
O1	8	63.99	43.17						
O2	8	64.00	40.17	0.01	8.17	[-6.84 - 6.82]	-0.003	7	1.00

Note. SNP: rs53576. Paired t -test was conducted.

Salivary Oxytocin level and PHQ-9.

The salivary oxytocin level, statistical analysis conducted 24 paired samples in both groups. Finally, in this analysis, statistical analysis was conducted on 23 samples in the experimental group and 24 samples in the control group.

PHQ-9: minor group

Comparison of the experimental group and the control group in the minor group, the salivary oxytocin level of O2 was higher the control group than experimental group, however there was not significant difference between the two groups ($t = -0.602$, $p = 0.55$) (Table 28). Change amount of salivary oxytocin level for the minor group was

significantly larger in the experimental group compared the experimental group ($t=-2.082, p=0.04$) (**Table 29**).

To compare the amount of the change difference of the salivary oxytocin level at O1 and O2, paired t -tests were conducted in the minor sub-group. The comparison of the salivary oxytocin level at O1 and O2 in the experimental group revealed that O2 was significantly lower than O1 ($t=3.210, p = 0.00$) (**Table 30**). Similarly, the paired t -tests at O1 and O2 in the control subgroup showed that O2 was lower than O1 ($t = 0.570, p = 0.58$) (**Table 30**).

PHQ-9: moderate group

For the comparison of the experimental group and the control group in the moderate group, the salivary oxytocin level of O2 was higher the control group than experimental group, however there was not a significant difference between groups ($t=-0.467, p=0.67$) (**Table 28**). Change amount of salivary oxytocin level for moderate was larger in the control group compared to the experimental group, however there was no significant difference between groups ($t=0.293, p=0.78$) (**Table 29**).

To compare the amount of the change difference of the salivary oxytocin level at O1 and O2, paired t -tests were conducted in the moderate sub-group. The comparison of the salivary oxytocin level at O1 and O2 in the experimental subgroup and the control subgroup, indicated that O2 was lower than O1 in the subgroups ($t=4.164, p = 0.15, t = 0.928, p = 0.42$) (**Table 30**).

Table 28

Between Group Comparison of the Mean Level of Salivary Oxytocin Level in the PHQ-9 Experimental Subgroup and Control Subgroup (pg/mL).

		Experimental group			Control group			<i>t</i>	<i>p-value</i>
		<i>n</i>	<i>Mean</i>	<i>SD</i>	<i>n</i>	<i>Mean</i>	<i>SD</i>		
minor	O1	21	74.13	46.73	20	68.39	48.35	0.386	0.70
	O2	21	58.67	38.27	20	66.09	40.55	-0.602	0.55
moderate	O1	2	54.21	6.20	4	67.11	33.65	-0.508	0.64
	O2	2	47.59	3.95	4	54.55	29.33	-0.467	0.67

Note. Independent t -test was conducted.

Table 29

Between Group Comparison of the Amount of Change for Experimental Group and Control Group.

	Experimental group			Control group			<i>t</i>	<i>p-value</i>
	<i>n</i>	<i>Mean</i>	<i>SD</i>	<i>n</i>	<i>Mean</i>	<i>SD</i>		
minor	21	-15.46	22.07	20	-2.30	18.07	-2.082	0.04
moderate	2	-6.62	2.26	4	-12.56	27.06	0.293	0.78

Note. Independent *t*-test was conducted.

Table 30

Within-group Comparison of Salivary Oxytocin Level before and after Intervention in the PHQ-9 Minor and Moderate Experimental and Control Subgroup. (pg/mL).

	<i>n</i>	<i>Mean</i>	<i>SD</i>	<i>MD</i>	<i>SD</i>	95%CI	<i>t</i>	<i>df</i>	<i>p-value</i>
PHQ: minor									
Experimental group									
O1	21	74.13	46.73	15.46	22.07	[5.41 - 25.50]	3.210	20	0.00
O2	21	58.67	38.27						
Control group									
O1	20	68.39	48.35	2.30	18.07	[-6.15 - 10.76]	0.570	19	0.58
O2	20	66.09	40.55						
PHQ: moderate									
Experimental group									
O1	2	54.21	6.20	6.62	2.25	[-13.58 - 26.82]	4.164	1	0.15
O2	2	47.59	3.95						
Control group									
O1	4	67.11	33.65	12.56	27.06	[-30.50 - 55.62]	0.928	3	0.42
O2	4	54.55	29.33						

Note. Paired *t*-test was conducted.

Salivary Oxytocin level and PBI.

For the salivary oxytocin level, statistical analysis was conducted on 24 paired samples in the experimental group and 24 paired samples in the control group. Finally, statistical analysis was conducted on 24 samples in the experimental group and 23 samples in the control group.

Salivary oxytocin level and PBI: Care factor

For the comparison of the CA High experimental subgroup and control subgroup of salivary oxytocin level of O2, salivary oxytocin level in the control group was higher than the experimental group, however there was not a significant difference between the groups ($t=-0.859, p=0.40$) (**Table 31**). Change amount of salivary oxytocin level was larger in the experimental subgroup compared to the control subgroup, however there was no significant difference between two groups ($t = -1.638, p = 0.12$) (**Table 32**). To compare the change difference of the salivary oxytocin level at O1 and O2, paired t -tests were conducted in the CA High sub-groups. For the comparison of the salivary oxytocin level at O1 and O2 in the experimental group, O2 was lower than O1 ($t = 2.012, p = 0.07$) (**Table 33**). Similarly, for the paired t -tests at O1 and O2 in the control group there was not a significant difference between O1 and O2 ($t = 0.104, p = 0.92$) (**Table 33**).

For the comparison of the experimental group and the control group in the CA Low group, salivary oxytocin level at O2 in the experimental group was higher than the control group, however there was not a significant difference between the groups ($t = 0.212, p = 0.83$) (**Table 31**). Change amount of salivary oxytocin level in the experimental subgroup was lower compared to the control subgroup, however there was no significant difference between two groups ($t = -0.997, p = 0.33$) (**Table 32**).

To compare the of the salivary oxytocin level at O1 and O2, paired t -tests were conducted in the CA Low sub-groups. The comparison of the salivary oxytocin level at O2 was significantly lower than O1 in the experimental group ($t = 4.314, p = 0.00$) (**Table 33**). Similarly, for the paired t -tests at O1 and O2 in the control group the O2 was lower than O1 ($t = 1.044, p = 0.32$) (**Table 33**).

Table 31

Between Group Comparison of the Mean Level of Salivary Oxytocin Level in the PBI: CA Experimental Subgroup and Control Subgroup (pg/mL).

		Experimental group			Control group			<i>t</i>	<i>p-value</i>
Oxytocinlevel (pg/mL)		<i>n</i>	<i>Mean</i>	<i>SD</i>	<i>n</i>	<i>Mean</i>	<i>SD</i>		
CA High group	O1	12	75.67	52.67	11	75.70	59.70	-0.001	1.00
	O2	12	59.38	40.32	11	75.20	47.98	-0.859	0.40
CA Low group	O1	12	69.99	35.46	12	59.90	30.83	0.744	0.47
	O2	12	55.48	32.49	12	52.88	27.28	0.212	0.83

Note. CA: Care factor of Parental Bonding Instrument. Independent *t*-test was conducted.

Table 32

Between Group Comparison of the Amount of Change for Experimental and Control CA High and CA Low Subgroups.

		Experimental group			Control group			<i>t</i>	<i>p-value</i>
		<i>n</i>	<i>Mean</i>	<i>SD</i>	<i>n</i>	<i>Mean</i>	<i>SD</i>		
CA High group		12	-16.29	28.05	11	-0.50	15.93	-1.638	0.12
CA Low group		12	-14.51	11.65	12	-7.02	23.28	-0.997	0.33

Note. CA: Care factor of Parental Bonding Instrument. Independent *t*-test was conducted.

Table 33

Within-group Comparison of Salivary Oxytocin Level before and after Intervention in the CA High and CA Low Subgroups. (pg/mL).

		<i>n</i>	<i>Mean</i>	<i>SD</i>	<i>MD</i>	<i>SD</i>	95%CI	<i>t</i>	<i>df</i>	<i>p-value</i>
PBI: CA High group										
Experimental group										
	O1	12	75.67	52.67	16.29	28.05	[-1.53 - 34.11]	2.012	11	0.07
	O2	12	59.38	40.32						
Control group										
	O1	11	75.70	59.70	0.50	15.93	[-10.20 - 11.2]	0.104	10	0.92
	O2	11	75.20	47.98						
PBI: CA Low group										
Experimental group										
	O1	12	69.99	35.46	14.51	11.65	[7.11 - 21.91]	4.314	11	0.00
	O2	12	55.48	32.49						
Control group										
	O1	12	59.90	30.83	7.02	23.28	[-7.77 - 21.8]	1.044	11	0.32
	O2	12	52.88	27.28						

Note. CA: Care factor of Parental Bonding Instrument. Paired *t*-test was conducted.

Salivary oxytocin level and PBI: Over-protection factor

For the comparison of salivary oxytocin level of O2 in the OP High experimental group and the OP High control group the salivary oxytocin level in the control group was higher than the experimental group, however there was not a significant difference between the groups ($t = -0.289, p = 0.78$) (**Table 34**). Change amount of salivary oxytocin level in the OP High sub-group was larger the experimental sub-group compared to the control sub-group, however there was no significant difference between two groups ($t = -0.662, p = 0.52$) (**Table 35**). To compare the differences of the oxytocin cortisol level at O1 and O2, a paired t -test was conducted in the OP High sub-group. For the comparison of the salivary oxytocin level at O1 and O2 in the experimental group, the O2 was significantly lower than O1 ($t = 2.400, p = 0.04$) (**Table 36**). Similarly, the paired t -tests at O1 and O2 in the control group showed that O2 was lower than O1 ($t = 0.859, p = 0.41$) (**Table 36**).

Comparisons of salivary oxytocin level of O2 in the experimental group and the control group who were classified as OP Low group showed that the salivary oxytocin level in the control group was higher than the experimental group, however there was not a significant difference between the groups ($t = -0.561, p = 0.58$) (**Table 34**). The change amount of salivary oxytocin level in the OP Low sub-group was significantly larger in the experimental sub-group compared to the control sub-group ($t = -1.908, p = 0.07$) (**Table 35**).

To compare the difference of the salivary oxytocin level at O1 and O2, a paired t -test was conducted in the OP Low sub-group. The comparison of the salivary oxytocin level at O2 was significantly lower than O1 ($t = 2.818, p = 0.02$) (**Table 36**). Similarly, the paired t -tests at O1 and O2 in the control group showed O2 was lower than O1 ($t = 0.541, p = 0.60$) (**Table 36**).

Table 34

Between Group Comparison of the Mean Level of Salivary Oxytocin Level in the Experimental Group and Control Group Classified PBI: OP (pg/mL).

Oxytocin level (pg/mL)		Experimental group			Control group			<i>t</i>	<i>p-value</i>
		<i>n</i>	<i>Mean</i>	<i>SD</i>	<i>n</i>	<i>Mean</i>	<i>SD</i>		
OP High group	O1	11	62.09	34.70	11	61.10	34.00	0.068	0.95
	O2	11	51.75	30.05	11	55.76	34.83	-0.289	0.78
OP Low group	O1	13	81.91	50.13	13	74.17	54.19	0.378	0.71
	O2	13	62.23	40.72	13	71.28	41.54	-0.561	0.58

Note. OP: Over-protection factor of Parental Bonding Instrument. Independent *t*-test was conducted.

Table 35

Between Group Comparison of the Amount of Change for Experimental and Control OP High and OP Low Subgroups.

	Experimental group			Control group			<i>t</i>	<i>p-value</i>
	<i>n</i>	<i>Mean</i>	<i>SD</i>	<i>n</i>	<i>Mean</i>	<i>SD</i>		
OP High group	11	-10.35	14.30	11	-5.34	20.61	-0.662	0.52
OP Low group	13	-19.68	25.17	13	-2.89	19.29	-1.908	0.07

Note. OP: Over-protection factor of Parental Bonding Instrument. Independent *t*-test was conducted.

Table 36

Within-group Comparisons of Salivary Oxytocin Level before and after Intervention of OP High and OP Low Subgroups. (pg/mL).

	<i>n</i>	<i>Mean</i>	<i>SD</i>	<i>MD</i>	<i>SD</i>	95%CI	<i>t</i>	<i>df</i>	<i>p-value</i>
PBI: OP High group									
Experimental group									
O1	11	62.09	34.70	10.35	14.30	[0.74 - 19.95]	2.400	10	0.04
O2	11	51.75	30.05						
Control group									
O1	11	61.10	34.00	5.34	20.61	[-8.51 - 19.19]	0.859	10	0.41
O2	11	55.76	34.83						
PBI: OP Low group									
Experimental group									
O1	13	81.91	50.13	19.68	25.17	[4.46 - 34.89]	2.818	12	0.02
O2	13	62.23	40.72						
Control group									
O1	13	74.17	54.19	2.89	19.29	[-8.77 - 14.55]	0.541	12	0.60
O2	13	71.28	41.54						

Note. OP: Over-protection factor of Parental Bonding Instrument. Paired *t*-test was conducted.

Secondary Outcomes: Approach Feeling and Avoidance Feeling.

Analyses by approach and avoidance groups.

Approach feeling was measured at two time-points: O1 and O2 in both groups. Approach feeling was measured for all participants in both groups at the point of O1. Similarly, the point of O2, the Approach feeling was measured in 38 women in the experimental group, and 40 women in the control group (missing rate: 4.76%). Therefore, statistical analysis was conducted on 38 women in the experimental group and 40 women in the control group.

Avoidance feeling was measured at two time-points: O1 and O2 in both groups. Avoidance feeling was measured for all participants in both groups in both points. Therefore, statistical analysis was conducted all participants in both groups.

Approach feeling analysis.

Shapiro-Wilk test for Approach feeling of O1 ($n=38$) and O2 ($n=38$) in the experimental group was conducted. The p -value of O1 was 0.02 and O2 was $p = 0.42$; the null hypothesis was only rejected at the point of O1.

In the control group, Shapiro-Wilk test for Approach feeling of O1 ($n=40$) and O2 ($n=40$) were conducted. The p -value of O1 was $p = 0.98$ and the p -value of O2 was $p = 0.31$. The null hypothesis was accepted.

Figure 15 and 16 depicts the histogram distribution of Approach feelings of O1 and O2 in both groups, which are similar to normal distributions.

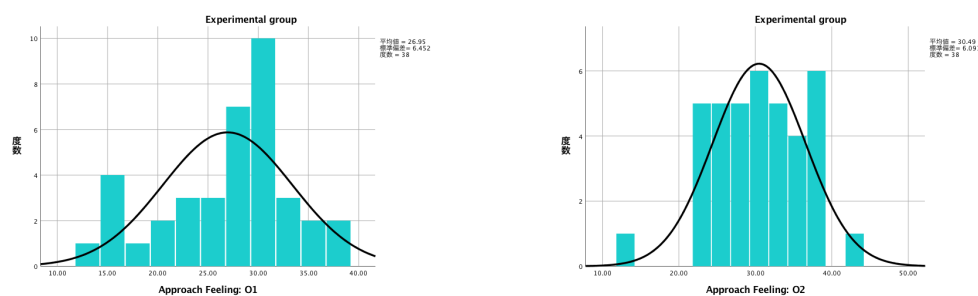


Figure 15 . The distribution of Approach Feeling in the experimental group.

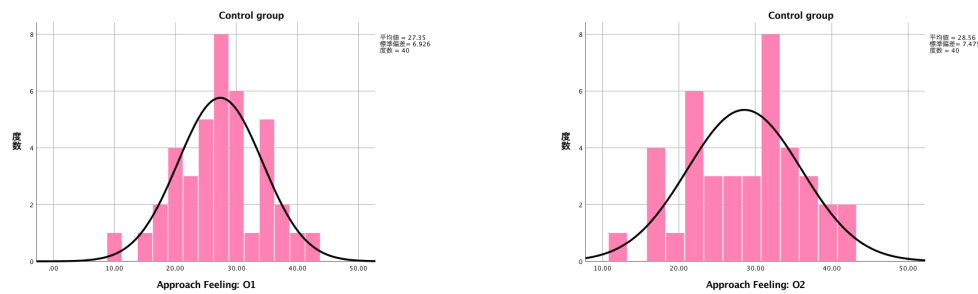


Figure 16. The distribution of Approach Feeling in the control group.

Comparison of Approach Feeling in Experimental group and Control group.

Table 37 shows the results of the between groups independent *t*-test conducted O2 between experimental group ($n=38$) and control group ($n=40$). The experimental group was 30.49 ± 6.09 and control group was 28.56 ± 7.47 . The score of experimental group was higher, however there was no significant difference for approach feeling of O2 between two groups ($t=1.243$, $p=0.22$) (**Table 37**).

To compare the amount of change defined as the mean Approach feeling score of O2 – the mean score of O1, the independent *t*-test was conducted between experimental group and control group. The amount of change in the experimental group was 3.54 ± 3.54 and control group was 1.21 ± 3.56 ; the amount of change in the experimental group was significantly larger than that of control group ($t=2.896$, $p=0.01$) (**Table 38**).

For each item of the approach feeling scale, the item change amount was calculated (change amount of item = score of item of O2 – score of item of O1) and compared between groups. As a result, there was a significant large change amount in the experimental group for the five items: joyful, refreshing, cheerful, present, and beautiful (**Table 39**).

Table 37

Between Group Comparison of Mean Scores of Approach Feeling in Experimental Group and Control Group.

Approach feeling	Experimental group			Control group			<i>t</i>	<i>p-value</i>
	<i>n</i>	<i>Mean</i>	<i>SD</i>	<i>n</i>	<i>Mean</i>	<i>SD</i>		
O1	38	26.95	6.45	40	27.35	6.93	-0.265	0.79
O2	38	30.49	6.09	40	28.56	7.47	1.243	0.22

Note. Independent *t*-test was conducted.

Table 38

Between Group Comparison of the Amount of Change for Experimental Group and Control Group.

Change amount of score	Experimental group			Control group			<i>t</i>	<i>p-value</i>
	<i>n</i>	<i>Mean</i>	<i>SD</i>	<i>n</i>	<i>Mean</i>	<i>SD</i>		
	38	3.54	3.54	40	1.21	3.56	2.896	0.01

Note. Independent *t*-test was conducted.

Table 39

Between Group Comparison of the Amount of Change of each Item.

Change amount of score	Experimental group			Control group			<i>t</i>	<i>p-value</i>
	<i>n</i>	<i>Mean</i>	<i>SD</i>	<i>n</i>	<i>Mean</i>	<i>SD</i>		
warm	38	0.66	0.47	40	0.25	0.36	0.434	0.67
joyful	38	0.32	0.61	40	0.05	0.65	1.864	0.07
refreshing	38	0.71	0.70	40	0.26	0.72	2.768	0.01
lovable	38	-0.03	0.72	40	0.14	0.81	-0.945	0.35
white	38	0.21	0.81	40	0.08	0.74	0.772	0.44
charming	38	0.05	0.34	40	0.05	0.41	0.031	0.98
innocent	38	0.00	0.52	40	0.18	0.71	-1.244	0.22
cheerful	38	0.46	0.64	40	0.01	0.77	2.781	0.01
sweet	38	0.24	0.81	40	0.19	0.70	0.289	0.77
pleasant	38	0.20	0.52	40	-0.23	0.62	3.268	0.00
fresh	38	0.22	0.88	40	0.08	0.67	0.847	0.40
gentle	38	0.43	0.74	40	0.25	0.71	1.127	0.26
beautiful	38	0.55	0.72	40	0.15	0.70	2.497	0.02
amazing	38	0.10	0.45	40	-0.01	0.74	0.844	0.40

Within-group Comparison of Approach Feeling before and after Intervention.

Comparing Approach feeling of O1 and O2 in the experimental group ($n=38$) by paired t -test of O1 was 26.95 ± 6.45 and O2 was 30.49 ± 6.09 . Therefore, O2 was significantly higher than O1 ($t = -6.170, p=0.00$) (Table 40).

Similarly, the paired t -test for O1 in the control group ($n=40$) was 27.35 ± 6.93 and O2 was 28.56 ± 7.47 ; O2 was significantly higher than O1 ($t = -2.156, p = 0.04$) (Table 40).

Table 40

Within-groups Comparison of Approach Feeling before and after Intervention.

	<i>n</i>	<i>Mean</i>	<i>SD</i>	<i>MD</i>	<i>SD</i>	<i>95%CI</i>	<i>t</i>	<i>df</i>	<i>p-value</i>
Experimental group									
O1	38	26.95	6.45						
O2	38	30.49	6.09	3.54	3.54	[-4.70 - -2.38]	-6.170	37	0.00
Control group									
O1	40	27.35	6.93						
O2	40	28.56	7.47	1.21	3.56	[-2.35 - -0.07]	-2.156	39	0.04

Note. Paired t -test was conducted.

Avoidance Feeling.

Shapiro-Wilk test for avoidance feeling of O1 ($n=38$) and O2 ($n=38$) in the experimental group was conducted. The p -value of before intervention was $p = 0.02$ and the p -value of after intervention was $p = 0.22$, therefore the null hypothesis was only rejected at the point of O1.

In control group, the Shapiro-Wilk test for approach feeling of O1 ($n=42$) and O2 ($n=42$) was conducted. The p -value of O1 was $p = 0.26$ and O2 was $p = 0.01$, therefore the null hypothesis was only rejected at the point of O2.

The distribution of avoidance feeling O1 and O2 in both groups are shown in the histograms **Figures 17 and 18**, which are similar to normal distribution curves.

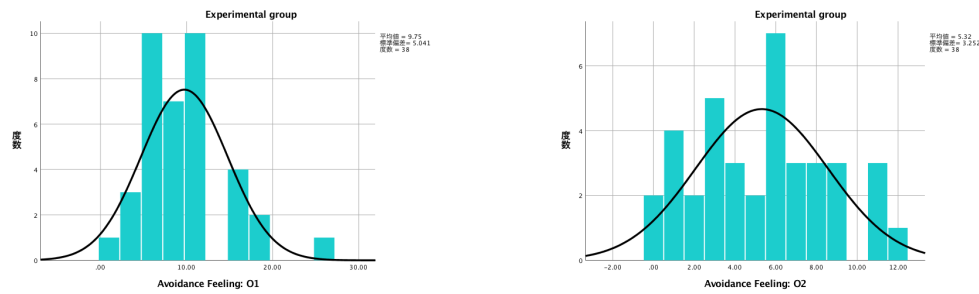


Figure 17. The distribution of Avoidance Feeling in the experimental group.

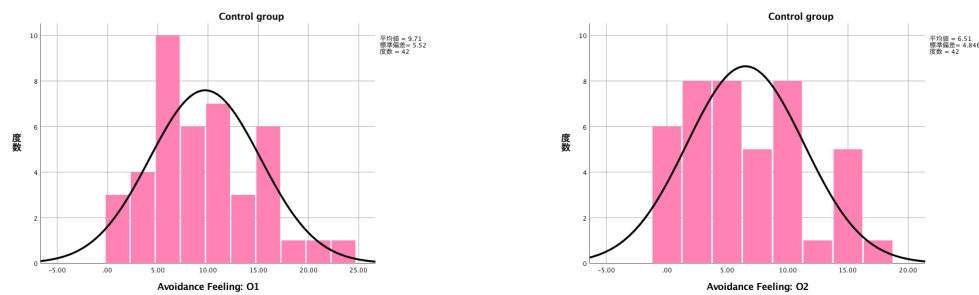


Figure 18. The distribution of Avoidance Feeling in the control group.

Comparison of Avoidance Feeling in Experimental Group and Control Group.

An independent *t*-test was conducted of O2 between the experimental group ($n=38$) and control group ($n=42$). The experimental group was 5.32 ± 3.25 and control group was 6.51 ± 4.85 . The avoidance feeling of the experimental group was lower than the control group, however there was no significant difference between groups ($t = -1.282, p = 0.20$) (**Table 41**).

An independent *t*-test was conducted between the experimental group and control group to compare the amount of change defined as the mean avoidance feeling score of O2 – the mean score of O1. The amount of change in the experimental group was -4.43 ± 3.75 and control group was -3.20 ± 3.45 ; the amount of change in the experimental group was larger than that of control group, however there was no significant difference between groups ($t = -1.530, p = 0.13$) (**Table 42**).

For each item of the avoidance feeling score, the item change amount was calculated (change amount of item = score of item of O2 – score of item of O1) and

compared between groups. As a result, there was a significantly large change amount in the experimental group for the one item, weak (**Table 43**).

Table 41

Between Group Comparison of Mean Scores of Avoidance Feeling in Experimental Group and Control Group.

Avoidance feeling	Experimental group			Control group			<i>t</i>	<i>p-value</i>
	<i>n</i>	<i>Mean</i>	<i>SD</i>	<i>n</i>	<i>Mean</i>	<i>SD</i>		
O1	38	9.75	5.04	42	9.71	5.52	0.030	0.98
O2	38	5.32	3.25	42	6.51	4.85	-1.282	0.20

Note. Independent *t*-test was conducted.

Table 42

Between Group Comparison of the Amount of Change for Experimental Group and Control Group.

Change amount of score	Experimental group			Control group			<i>t</i>	<i>p-value</i>
	<i>n</i>	<i>Mean</i>	<i>SD</i>	<i>n</i>	<i>Mean</i>	<i>SD</i>		
	38	-4.43	3.75	42	-3.20	3.45	-1.530	0.13

Note. Independent *t*-test was conducted.

Table 43***Between Group Comparison of the Amount of Change of each Item.***

Change amount of score	Experimental group			Control group			<i>t</i>	<i>p-value</i>
	<i>n</i>	<i>Mean</i>	<i>SD</i>	<i>n</i>	<i>Mean</i>	<i>SD</i>		
weak	38	-0.82	0.74	42	-0.38	0.88	-2.388	0.02
shy	38	0.20	0.59	42	0.02	0.65	1.245	0.22
painful	38	-0.12	0.49	42	-0.14	0.57	0.206	0.84
noisy	38	-0.80	0.78	42	-0.65	0.82	-0.821	0.41
impudent	38	-0.09	0.40	42	0.01	0.32	-1.286	0.20
difficult	38	-1.09	0.87	42	-0.75	1.01	-1.618	0.11
embarrassing	38	0.14	0.96	42	-0.04	0.45	1.09	0.28
over-familiar	38	-0.08	0.43	42	0.02	0.78	-0.719	0.47
troublesome	38	-0.38	0.67	42	-0.30	0.58	-0.597	0.55
scary	38	-0.64	0.85	42	-0.52	0.76	-0.672	0.50
complicated	38	-0.22	0.64	42	-0.07	0.60	-1.094	0.28
annoying	38	-0.17	0.66	42	-0.19	0.45	0.154	0.88
frustrating	38	-0.26	0.59	42	-0.14	0.67	-0.845	0.40
reproachful	38	-0.09	0.36	42	-0.07	0.34	-0.262	0.79

Within-group Comparisons of Avoidance Feeling before and after Intervention.

Table 44 displays the results of the paired *t*-test within-group comparison of avoidance feeling means of O1 and O2 in the experimental group ($n=38$), O1 was 9.75 ± 5.07 and O2 was 5.32 ± 3.25 . Therefore, O2 was significantly lower than O1 ($t = 7.280$, $p = 0.00$).

Similarly, the paired *t*-test between O1 and O2 in the control group ($n=42$) was 9.71 ± 5.52 and O2 was 6.51 ± 4.85 ; O2 was significantly lower than O1 ($t = 6.020$, $p = 0.00$) (**Table 44**).

Table 44***Within-group Comparisons of Avoidance Feeling before and after Intervention.***

	<i>n</i>	<i>Mean</i>	<i>SD</i>	<i>MD</i>	<i>SD</i>	<i>95%CI</i>	<i>t</i>	<i>df</i>	<i>p-value</i>
Experimental group									
O1	38	9.75	5.04						
O2	38	5.32	3.25	4.43	3.75	[3.20 - 5.67]	7.280	37	0.00
Control group									
O1	42	9.71	5.52						
O2	42	6.51	4.85	3.20	3.45	[2.13 - 4.28]	6.020	41	0.00

Note. Paired *t*-test was conducted.

Score of Antagonism

Score for antagonism was calculated (avoidance feeling/ approach feeling $\times 100$).

Table 45 shows the results of the between groups independent *t*-test conducted O2 between experimental group ($n=38$) and control group ($n=40$). The experimental group was 18.42 ± 12.36 and control group was 24.13 ± 20.62 . The score of control group was higher, however there was no significant difference for antagonism at O2 between groups ($t=-1.491$, $p=0.14$) (**Table 45**).

Comparing the antagonism score at O1 and O2 in the experimental group ($n=38$) by paired *t*-test, at O1 was 33.13 ± 22.58 and O2 was 18.42 ± 12.36 . Therefore, O2 was significantly lower than O1 ($t=3.980$, $p=0.00$) (**Table 46**).

Similarly, the paired *t*-test for O1 in the control group ($n=40$) was 28.10 ± 33.21 and O2 was 24.13 ± 20.62 was not significantly different between O1 and O2 ($t=0.687$, $p=0.50$) (**Table 46**).

Table 45***Between Group Comparison of Mean Scores of Antagonism in Experimental Group and Control Group.***

Score of Antagonism	Experimental group			Control group			<i>t</i>	<i>p-value</i>
	<i>n</i>	<i>Mean</i>	<i>SD</i>	<i>n</i>	<i>Mean</i>	<i>SD</i>		
O1	38	33.13	22.58	40	28.10	33.21	0.779	0.44
O2	38	18.42	12.36	40	24.13	20.62	-1.491	0.14

Note. Independent *t*-test was conducted.

Table 46***Within-group Comparisons of score of Antagonism before and after Intervention.***

	<i>n</i>	<i>Mean</i>	<i>SD</i>	<i>MD</i>	<i>SD</i>	<i>95%CI</i>	<i>t</i>	<i>df</i>	<i>p-value</i>
Experimental group									
O1	38	33.13	22.58						
O2	38	18.42	12.36	14.71	22.79	[7.22 - 22.20]	3.980	37	0.00
Control group									
O1	40	28.10	33.21						
O2	40	24.13	20.62	3.97	36.59	[-7.73 - 15.67]	0.687	39	0.50

Note. Paired *t*-test was conducted.

Process Evaluation

Statistical analysis was conducted on 38 participants in the experimental group and 41 participants in the control group who had answered all items (**Table 47**).

Changed image of infant.

After the intervention, there were 15 participants (39.5%) in the experimental group and six participants (14.6%) in the control group with a changed image of infant. The change was significantly more in the experimental group the control group ($\chi^2=6.235, p=0.01$).

Change for anxiety about living with infant.

After the intervention, there were 29 participants (76.3%) in the experimental group and 14 participants (34.1%) in the control group with changed anxiety about living with infant. The change for anxiety about living with infant were decrease all participants in both groups and there were significantly more in the experimental group than that of in the control group ($\chi^2=14.139, p=0.00$).

Change for anxiety about delivery.

After the intervention, there were 28 participants (73.7%) in the experimental group and 13 participants (31.7%) in the control group with changed anxiety the delivery. The change in anxiety about delivery decreased for all participants in both

groups and there were significantly more in the experimental group than the control group ($\chi^2=13.920$, $p=0.00$).

Change for anxiety about family relationship.

After the intervention, there were five participants (13.2%) in the experimental group and six participants (14.6%) in the control group with changed anxiety about family relationship. All participants in the experimental group decreased their anxiety about family relationship, and five participants decreased anxiety and one participant increased anxiety about family relationship in the control group. There was not a significant difference about the change for anxiety about family relationship between groups ($\chi^2=0.946$, $p=0.623$).

Change for anxiety about economic situation.

After the intervention, there was one participant (2.6%) in the experimental group and one participant (2.4%) in the control group with changed the anxiety economic situation. The anxiety about economic situation decreased for each participant in both groups. There was not a significant difference between groups ($\chi^2=0.003$, $p=0.957$).

Burden.

Two patients in the experiment group (5.3%) and three in the control group (7.3%) responded that they felt burdened after the intervention. About the burden, one of the two in the experimental group replied that, “to store saliva in the mouth” and another was, “did not know what to do when the infant cried”. One of the two in the control group responded that, “I felt sleepy (watching the DVD image of infant)”, another one replied “long time for saliva collection and was difficult”.

Satisfaction.

After the intervention, the researcher asked, “Do you want to join the program again?” and 37 participants (97.4%) answered “Yes” in the experimental group and 36 participants (87.8%) answered “Yes” in the control group.

The reason for one who answered “No” in the experimental group was unspecified, however in the control group the reasons were obtained from three out of five who answered “No”. One of the three was “I do not feel anything even if I see the image of infant not crying or laughing”, one another “I had many opportunities to see infant”, the other one “I do not think that I do not want to participate, but I also do not think that I would like to participate again”.

Table 47

Between Group Comparison of Process Evaluation in Experimental Group and Control Group.

		Experimental group		Control group		χ^2	<i>p</i>
		<i>n</i>	%	<i>n</i>	%		
Change of image	Yes	15	39.5	6	14.6	6.235	0.01
	No	23	60.5	35	85.4		
Anxiety about child care	No change	9	23.7	27	65.9	14.139	0.00
	decrease	29	76.3	14	34.1		
	increase	0	0	0	0		
Anxiety about delivery	No change	10	26.3	28	68.3	13.92	0.00
	decrease	28	73.7	13	31.7		
	increase	0	0	0	0		
Anxiety about family relationship	No change	33	86.8	35	85.4	0.946	0.62
	decrease	5	13.2	5	12.2		
	increase	0	0	1	2.4		
Anxiety about economic conditions	No change	37	97.4	40	97.6	0.003	0.96
	decrease	1	2.6	1	2.4		
	increase	0	0	0	0		
Burden	Yes	2	5.3	3	7.3	0.14	0.71
	No	36	94.7	38	92.7		
Satisfaction	Yes	37	97.4	36	87.8	2.570	0.11
	No	1	2.6	5	12.2		

Description data for interaction with infant.

Table 48 shows the description data for ‘interact with infant’. The description data was classified into six categories.

Category of “could have concrete image the life with my baby” consisted of four code: <can imagine the life after delivery>, < what I am going to do with my baby >, < I'd like to play with my baby like the mother taught me > and < get the image of the

baby six months after birth >. First-time pregnant women noted, “could have concrete image of the life with my baby” though the interaction with infant < can imagine the life after delivery >, < what I am going to do with my baby >, < I'd like to play with my baby like the mother taught me > and < get the image of the baby six months after birth >. Category of “have confidence” consisted of one code: < I feel like I can do >. First-pregnant women could “have confidence” thorough the interaction < I feel like I can do >. Category of “anxiety relieved by listening to mother’s story” consisted of two codes: < life with may baby looks like fun > and < the advice from the mother was a relief for me >. “Anxiety relieves by listening to mother’s story” because they felt < life with may baby looks like fun > and < the advice from the mother relieved me >. Category of “looking forward to seeing my child” consisted of one code: < want to see my baby >. First-pregnant women were “looking forward to seeing my child” because of they could feel through interaction < want to see may baby >. Category of “could feel the power of baby” was consisted of two codes: < feel the vitality of the baby > and < baby can communicate with mother without words >. First-pregnant women “could feel the power of baby” because of they could feel through the interaction < feel the vitality of the baby > and < baby can communicate with mother without words >. Category of “awareness of myself as a mother” consisted of four codes: < do my best for my baby >, < have a firm feeling so as not to worry about my baby >, < baby needs mother > and < grew together with my baby >. First-pregnant women could have “awareness of myself as a mother” through the interaction and felt < do my best for my baby >, < have a firm feeling so as not to worry about my baby >, < baby needs mother > and < grew together with my baby >.

Table 48***Descriptive data for interactions with infant.***

Category	Code
Could have concretely image the life with my baby	« can image the life after derively » « what I am going to do with my baby » « I'd like to play with may baby what the mother taught me » « get the image of the baby 6 months after birth »
Have confidence	«I feel like I can do»
Anxiety relieves by listening to mother's story	« life with may baby looks like fun » « the advice from the mother relief me »
Looking forward to seeing my child	« want to see may baby »
Could feel the power of baby	« feel the vaitality of the baby » « baby can communicate with mother without words »
Awareness of myself as a mother	« do my best for my baby » « have a firm feeling so as not to worry about my baby » « baby needs mother » « grew together with my baby »

Discussion

Primary Outcome: Salivary Cortisol Level.

In this study, there was no difference in salivary cortisol levels between the experimental group and the control group after the intervention. However, salivary cortisol level significantly decreased in both the experimental group and the control group before and after the intervention.

Cortisol is a biomarker that is reflected in saliva about 20 minutes after stress stimulations (Clements, 2013). As a result, in this study, salivary cortisol levels represented biological changes in first-time pregnant women in the late pregnancy due to the intervention of the experimental group's interaction with infants, and the control group watching DVD images of infants. These interventions were not designed to be a stress stimulus for first-time pregnant women.

Researchers have also found that cortisol levels decreases due to stress relief during pregnancy (Urizar Jr. et al., 2004; Field. et al., 2013). Likewise in this study, salivary cortisol levels significantly decreased in both groups after intervention. Therefore, results from both the experimental group and the control group suggested the interventions have the possibility of biological changes associated with stress relief.

However, there was no differences in the salivary cortisol level after the interventions of the experimental group compared to the control group; the effect on relaxation of stress caused by first-time pregnant women interact with infants could not be clarified. The reason for the interventions of the experimental group and the control group were similar. Severi, et al. (2005) researched the change of salivary cortisol level of 12 pregnant women between 30 to 32 weeks gestation after using Fetouch system: 3D images using ultrasound and a system of interaction with fetus using 3D images and haptic devices. As a result, the salivary cortisol level after the intervention decreased with 11 of the pregnant women. In a preliminary study (Sonoda, et al., 2018) we compared an intervention group that interacted with infants and a control group that

only watched a DVD image of landscape. In the intervention group the salivary cortisol level 30 minutes after the intervention was significantly decreased, whereas no significant decreased was observed in the control group. These results suggest that the decrease in salivary cortisol level in the control group was related to the stimulation from images of infants. Therefore, in comparison with the experimental group of this study and the control group, the effect of interacting with infants was not clear. One could also assert that salivary cortisol levels will decrease with either interaction: with an infant or by viewing infants on a DVD.

Similarly, the PHQ-9: minor and moderate levels and the PBI: CA High group, CA Low group, OP High group and OP Low group, showed no differences between the experimental and control subgroups. However, salivary cortisol level significantly decreased in all subgroups except the CA High experimental subgroup before and after the intervention. For the PHQ-9 minor and moderate groups and the PBI CA High group, CA Low group, OP High group and OP Low group, there were no differences between the experimental and control groups. In other words, there was no differences in the salivary cortisol level depending on the depression level if they were raised lovingly or raised to excessive interference.

It is said that the depression level and PBI scores respond to cortisol secretion (Dedovic, et al., 2009; Engert, et al., 2011; Kudielka, et al., 2009; Vogetline, 2013), however in this study there was no significant difference; all subgroup salivary cortisol levels decreased before and after intervention. It may be that the cortisol levels in these low-risk first-time pregnant women in this study were not affected by depression or the PBI score:

From the results of this study, it was suggested that the first-time pregnant women interactions with infants was not a stress stimulus, and that stress could be reduced by interacting with infants in person or by image. However, in this study, fluctuations of salivary cortisol level as an index of stress reduction did not appear. Therefore, for future research, it is necessary to consider the appropriate contents for the control group.

Secondary Outcome: State Anxiety Score.

In this study, comparison of state anxiety scores after intervention showed that the experimental group was significantly lower. However, since the state anxiety score before intervention was significantly lower in the experimental group, the change amount of each participant was calculated and compared between the two groups. As a result, there was no difference in the amount of change between the two groups. In comparison of before and after intervention, the state anxiety score was significantly lowered in both groups after intervention.

The state anxiety score of low-risk pregnant women in late pregnancy was reported as 41.4 [*SD*: 8.3] (Okumura, et al., 2015). In this study, the state anxiety scores after intervention were 29.51 in the experimental group and 34.39 in the control group. Those scores were lower than the ones found by Okumura, et al. (2015). It is possible that both the intervention of interacting with an infant and watching a DVD image of infant reduced the participants' state anxiety score. Women in pregnancy have higher levels of anxiety compared with non-pregnant women (Nakajima, et al., 1998), which was a result of being pregnant (Hanazawa, 1992). In this study, more than half women had anxiety related to pregnancy (see **Table 1**). It is said that receiving knowledge of childcare, growth and development of child reduced anxiety of childcare (Hagiwara, et al., 2017). In this study, it may be that the interaction with an infant and watching a DVD image of an infant gave some knowledge of childcare for first-time pregnant women, and it caused a reduced state anxiety score. Although it did not appear in the score of difference, in the experimental group anxiety of child-care and of delivery were significant decreased compared to the control group after intervention (see **Table 60**). From this result, it may be that the experimental group obtained more knowledge from the interaction with infant, and it caused a reduced anxiety of child-care and delivery. However in this study contents, there was no mention of delivery. In the future research, anxiety about delivery should be assessed to avoid its potential confounding effect.

Secondary Outcome: Salivary Oxytocin Level.

In this study, there was no significant between-group salivary oxytocin level differences after the intervention. Salivary oxytocin level in both groups decreased after intervention, and the salivary oxytocin level significantly lowered in the experimental group. The results of this study were totally different from the hypothesis. In this study, the rise in salivary oxytocin level induced by interacting with an infant was not observed but rather it decreased. The following reasons are considered as possibilities for salivary oxytocin level decrease in the experimental group.

There was the possibility that the implementation protocol did not provide appropriate stimulus for oxytocin secretion. It was well known that oxytocin level increases with maternal-infant interactions (Feldman, et al., 2010; Markova, 2018). Feldman, et al. (2010) analyzed salivary oxytocin level before and after a 15-minute "play-and-touch" interaction for 71 mothers, 41 fathers with 4-6 month-old infants. Mothers who used more gentle stroking and affectionate touch had salivary oxytocin levels significantly increased before and after 15-minutes "play-and-touch" interaction compared with the few mothers who did not use the gentle touch. Participants in this study were primipara and it was the first time to interact with an infant. Interactions with infants were carried out under the instruction from the infant's mother; however, there was a possibility that the interaction was difficult for first-time pregnant women. **Figure 5** shows holding the infant. It seems that the way of holding infant was far from a gentle stroking and affectionate touch. Researchers found that the frequency of "Motherese" was positively related to oxytocin levels during the pregnancy period (Feldman, et al., 2007). In this study, the definition of "Motherese" was ambiguous, so it may be that the way of "Motherese" was not appropriate stimulation for oxytocin release.

Oxytocin secretion is generally increased when the person feels relaxed (Moberg, 2014): such as massage. It is said that C-tactile afferents are present in tactile receptors such as skin and that the activation of C-tactile afferents triggers oxytocin release (Walker, et al., 2017). The one way for stimulation to activate C-tactile afferents is the

sense of softness (Sakamoto, et al., 2018). In this study, salivary oxytocin level did not rise because of it may be difficult for participants to feel the softness of the infant.

Future research, should reconsider the protocol and clarify the definition of “Motherese”. Furthermore a revised protocol should include the use of appropriate stimulation as well as provide more support for first-time pregnant women to achieve tactile stimulation that would increase the areas touched to activate the C- tactile afferents such as skin-to-skin contact. Furthermore, increasing the frequency of interactions may also be taken into consideration. Markova (2018) measured salivary oxytocin levels before and after natural interactions for 43 pairs of mothers and infants. As a result, maternal salivary oxytocin level was increased before and after the natural interactions. Natural interactions meant they were done every day, so the result may be related to frequency of interaction. In the future, research is needed to observe the change of salivary oxytocin level by the interaction frequency.

In this study, salivary oxytocin level in all subgroups decreased. It may be possible that there were background factors not related to salivary oxytocin level in this study, however the protocol had limitations and the sample size was not large enough to clarify the relationship. It will be possible that these background factors affected the oxytocin release. In future research, these background factors must be identified.

Secondary Outcome: Approach Feeling and Avoidance Feeling.

In this study, the change amount of approach feeling was significantly larger in the experimental group compared to the control group. Especially, in the experimental group, the change amount of these items: “joyful”, “cheerful”, “present” and “beautiful” were significantly larger compared the control group.

Approach feeling is known to be a positive emotion and accepting for the infant (Hanazawa, 1992), and interaction with an infant affected the approach feeling (Hanazawa, 1992; Kadomori, et al., 2012). In this study, the amount of change of approach feeling in the experimental group was 3.54 ± 3.54 and the control group was 1.30 ± 3.63 ; the amount of change in the experimental group was significantly larger

than that of control group ($t= 2.722, p= 0.01$). That was one of the big differences between the two groups. Kadomori, et al. (2012) reported that there was no difference of approach feeling in the group of having the experience of interaction with an infant during pregnancy ($n=19$) and the group of having no experience of interaction with an infant ($n=29$); interaction group was 30.95 ± 5.6 and non-interaction group was 27.6 ± 8.7 . From this study, the well-considered and planned ‘tactile stimulation’ that happens when the woman touched a real infant stimulated the reward system and gave rise to the approach feeling for first-time pregnant women.

It has been reported that interactions (e.g. daily interactions, massage for infant, and programs facilitating interaction with infants) increase the attachment emotion and attachment behavior for infants (Sung, et al., 2005; Shoghi, et al., 2018). From the results of the study, interacting with infants increased the feelings of “joyful”, “cheerful”, “present” and “beautiful” all which may facilitate attachment feelings and behaviors.

Second, in this study, avoidance feelings before and after the intervention were significantly decrease after the intervention in both groups. Especially, in the experimental group, the change amount of these item: “weak” was significantly larger compared with the control group. According to the date of protocol evaluation (see **Table 60**), first-pregnant women “could feel the power of baby” though the interaction with infant. It may be possible that first-pregnant women felt the power of baby, and decreased the item of “weak”.

Avoidance feelings are known to be negative emotions associated with denying and rejecting the infant (Hanazawa, 1992). Avoidance feelings of first-time pregnant women were reported as 8.0 [SD : 4.8] (Moriyama, et al., 2008) and 8.0 [SD : 4.9] (Suzuki, et al., 2013). Avoidance feeling of this study before the intervention were 9.85 [SD : 5.07] in the experimental group and 9.71 [SD : 5.52] in the control group, and after intervention were 5.39 [SD : 3.26] in the experimental group and 6.62 [SD : 4.85] in the control group. These scores after the intervention were lower than those reported by Moriyama, et al. (2008) and Suzuki, et al. (2013). From this study, there is a possibility of both interaction with infant and watching a DVD image of infant could

reduce the avoidance feeling. Furthermore, the contents of DVD image of infant were the infant spend only calmly. It may be possible that the Avoidance feeling was affected the DVD contents.

Process evaluation.

From the result of the process evaluation, it is suggested that interactions with infants could change the image of infant and decreased the anxiety of child-care and delivery compared to the control group. From the descriptive data for interact with infant, first-time pregnant women changed the image of infant and their life with infant. This change was a big difference compared to the control group. Omori, et al. (2005) and Watanabe, et al. (2013) suggested the change of image for infant interacted with infant; these results of the process evaluation supported those results.

Another big difference of the experimental group compared to the control group was a significant decrease in the anxiety of child-care and delivery in the experimental group. First-pregnant women feel anxiety over something they have not yet experienced (Nakashima, et al., 1998). By experiencing an interaction with an infant, may have contributed to decreasing the anxiety of child-care. However, the contents of interaction did not include the feelings about delivery, so it may be the experience of interaction was indirectly affected. It is possible that the change of image of an infant decreased anxiety. The interaction with an infant may have decreased anxiety of delivery. It is suggested that interaction with infant was effective for changing the image of an infant and decreasing anxiety.

Only one participant felt a burden because of the infant's cry. First-pregnant women feel anxiety over what they have never experienced (Nakashima, et al., 1998). Based on this feature of first-time pregnant women, it was possible that this participant felt burden because of infant's cry. More support for first-time pregnant women may be needed when interacting for the first time with infants.

Almost all participants in the experimental group felt satisfaction interacting with an infant. It is necessary to improve support for first-time pregnant women based on the understanding that the novel situation of interacting with an infant for the first time

raises anxiety. There is a possibility of changing the image of infant and reducing anxiety, which can be said to be necessary for care in modern times of declining birthrate and nuclear families.

Limitations and Future Studies.

A limitation of this study was the control group protocol of viewing the DVD image of infant. Even though the DVD did not stimulate either the tactile sense or olfactory sense it was similar to the intervention of experimental group, so there was possibility of no difference in cortisol level as a primary outcome. Another limitation was that the problem of implementation of protocol and lack of more support for first-time pregnant women interact with the infant.

For future research, it is necessary to reconsider for the contents of the control group to classified salivary cortisol level, that is content not to related to an infant. Additionally, consideration for a protocol that requires the appropriate stimulation, to clarify the definition of “Motherese” and provide more support for first-time pregnant women for tactile stimulation. Furthermore, increasing the frequency of interactions and observations of the change of salivary oxytocin level by the interaction of frequency should also be taken into consideration.

Conclusion

This randomized, controlled, two-armed trial was to verify the change in the experimental group ($n=38$) after 30 minutes' of interaction with infants compared to the control group ($n=42$) after 30 minutes watching a DVD of infants.

Both the experimental group and control group had decreased: salivary cortisol levels, state anxiety scores and avoidance feelings after the intervention. Both interacting with an infant and watching a DVD image of infant decreased the stress and anxiety of first-pregnant women. Furthermore, interacting with an infant changed the first-time pregnant women's image of an infant, and decreased anxiety about child-care and delivery.

The experimental group's approach feeling change level was significantly higher than that of the control group. Furthermore, the change of the image of the infant, the change in anxiety about living with an infant and change in anxiety about delivery were more in the experimental group. These were large differences between the groups. In this study, the change of salivary oxytocin level could not be clarified for either group because of the limitations of the protocol.

For future research, it is necessary to consider the contents of the intervention that is not to related infants for the control group to clarify salivary cortisol level. Additionally, researchers need to reconsider the protocol to get appropriate stimulation; to clarify the definition of "Motherese" and provide more support for first-time pregnant women to improve tactile stimulation. Furthermore, increasing the frequency of interactions may also be taken into consideration and then observe the change of salivary oxytocin level by the interaction frequency.