CHAPTER 1: INTRODUCTION

This chapter introduces the background with the conceptual framework, the rationale of and the objectives of the study. It also discusses the limitations of the study and lastly the outline of how this study was presented.

1.1 Background

It is every child's right to be breast fed. Breastfeeding is widely known to benefit both the infant and the mother. The benefits of breastfeeding to the infant include nutritional by providing complete nutrition and non-nutritional benefits by reducing the risk of hospitalization for common childhood illnesses like lower respiratory infections (Chantry *et al.,* 2006), otitis media (Duffy, 1997) and non-specific gastrointestinal tract infections (Eidelman, 2012). Breastfeeding for the optimal duration of six months significantly protects infants from childhood leukaemia, type 1 and type 2 diabetes mellitus, obesity (Scholtens *et al.,* 2007), allergy and asthma (Eidelman, 2012 & Lawrence, 2010). In addition, breastfeeding has positive effects on neurodevelopment in preterm (Rozé *et al.,* 2012) as well as in term infants. Breastfed infants have been found to have higher intelligence than their formula fed counterpart after confounding factors have been adjusted (Walfisch, 2013). In mothers, it helps with family planning, enhances early return to normality following delivery and it has been associated with reduced risk of ovarian and breast cancer (Lawrence, 2010). It has also been proven to improve mental health (Mezzacappa & Katlin, 2002). Breast feeders have been reported to have less negative emotions. In fact they are calmer and better at coping with stress (Mezzacappa & Katlin, 2002). In addition, it positively affects maternal-infant bond (Jansen, de Weerth & Walraven, 2008). Breastfeeding is also economical and environment friendly (Eidelman, 2012). Thus, the World Health Organisation has

recommended exclusive breastfeeding for infants up to 6 months of age and continue breastfeeding up to 24 months (WHO, 2008).

The current breastfeeding scenario in Malaysia is not highly satisfactory. Despite the prevalence of breastfeeding among children less than 12 months old was 94.7% in Malaysia (National Health and Morbidity Survey NHMS II and III 2006), the prevalence of exclusive breastfeeding below 4 months was not high at 19.3%. Surprisingly, 45.8% of infants were not breastfed exclusively at 1 month of age. In comparison with other developing country in Asian region, prevalence of breastfeeding is slightly higher in Singapore. According to Singapore National Breastfeeding Survey 2011, the prevalence of breastfeeding initiation was 98.6%, while prevalence of exclusive breastfeeding at 6 months is 41.2%. In Indonesia, 32% of infants were exclusively breastfed in the first 6 months (Statistics Indonesia, 2008). However, the prevalence of exclusive breastfeeding is much lower in Taiwan and Saudi Arabia despite high initiation rate of breastfeeding. Only 16.7% of infants are exclusively breastfed at 3 months in Taiwan (Chien *et al.*, 2005) while in Saudi Arabia it is unexpectedly low, at only 1.7% (Al-Hreashy *et al.*, 2008). All these evidences indicated that the prevalence of exclusive breastfeeding is generally still below recommendation for countries in the Asian region. However, there is an increase in trend as seen in western countries over the past few years (Cai, Wardlaw, & Brown, 2012). Introduction of Baby-Friendly Hospital Initiative in Malaysia since 1992 and International Code of Marketing of Breast Milk substitutes showed a strong commitment from the government in promoting breastfeeding.

Therefore it is crucial to identify and understand the factors associated with non-exclusive breastfeeding which occurs as early as one month postpartum. In Malaysia, the factors included Indian ethnic mothers, working mothers, mothers from high household income, mothers who smoke, primiparous, infants not sharing the bed with

their mothers and infants born prematurely (Tan, 2011). The main highlight here in this study is the association of working outside the home and breastfeeding.

In this current modern society, more women are involved in the workforce. In Malaysia, according to the principal statistics of labour force by sex, Malaysia 2012, 49.5% of women participated in the labour force (Principal Statistic of Labour Force, Malaysia, 2012). The highest labour force participation rate is among the reproductive age group of 25-34 years old. It is ubiquitous to hear of women blaming the stress of working for lactation impairment. Full-time employment status is still a barrier to breastfeeding; it has been negatively correlated with breastfeeding initiation and duration, while part-time work promotes breastfeeding initiation and duration (Mandal *et al.,* 2010; Amin *et al.*, 2011; Cardenas & Major, 2005; Chuang *et al*., 2010).

It is shown that stress has a negative effect on lactation (Lau, 2001). The problem is more obvious when mothers resume working after 2-3 months of maternity leave. New mothers have to learn to adapt to the new role, handling an additional child, coping with housework and also dealing with work demands (daily stress perceived by mothers).

Stress can be divided into physical and psychological stress, both can inhibit lactation by affecting milk synthesis, milk ejection and maternal motivation to breastfeed (Lau, 2001). The interaction between stress and lactation is complex. Prolactin (PRL) and oxytocin are synthesized and released within the brain, therefore they can be influenced directly by corticotropin-releasing hormone (CRH) in the lactotrophic axis. Indirectly, lactation can be inhibited via the sympathoadrenomedullary system (Morales, 2001). On the contrary, some studies have shown the protective effect of breastfeeding by lowering the cortisol level in the presence of stress (Altemus, 1995). Although prolactin and oxytocin are involved in milk production and milk ejection respectively, they are also known to have a central

anxiolytic effect and they play an important role in maternal behaviour (Tu, Lupien & Walker, 2005). These hormones may be involved in mediating stress hyporesponsiveness during lactation (Tu *et al.*, 2005).

However, until now, to our knowledge, there is no study looking at the effects of working stress on the lactation process through the stress-neurohormones interaction. Thus, this study is focused on the association of working stress, neurohormonal changes and the performance of breastfeeding in apparently healthy Malaysian mothers.

1.2 Study objectives

This study aims to determine the effect of working stress on lactation. We hypothesized that working mothers are more stressful than non-working mothers thus have more profound effects. The correlation between perceived stress, hormonal levels (cortisol, prolactin and oxytocin) and volume of milk production would lead to greater understanding of the underlying causes of lactation failure.

Objectives of the study:

General Objective

- To determine the effects of perceived stress on lactating mothers.

Specific Objectives

- To determine perceived stress levels among working and non-working mothers and their effect on lactation.
- To determine serum prolactin and cortisol levels among working and non-working mothers during the postpartum period.
- To find out the association between perceived stress, serum cortisol and serum prolactin and lactation performance among working mothers during the postpartum period.
- To find out the association between stress, serum cortisol and serum prolactin and lactation performance among non-working mothers during the postpartum period.

1.3 Conceptual framework

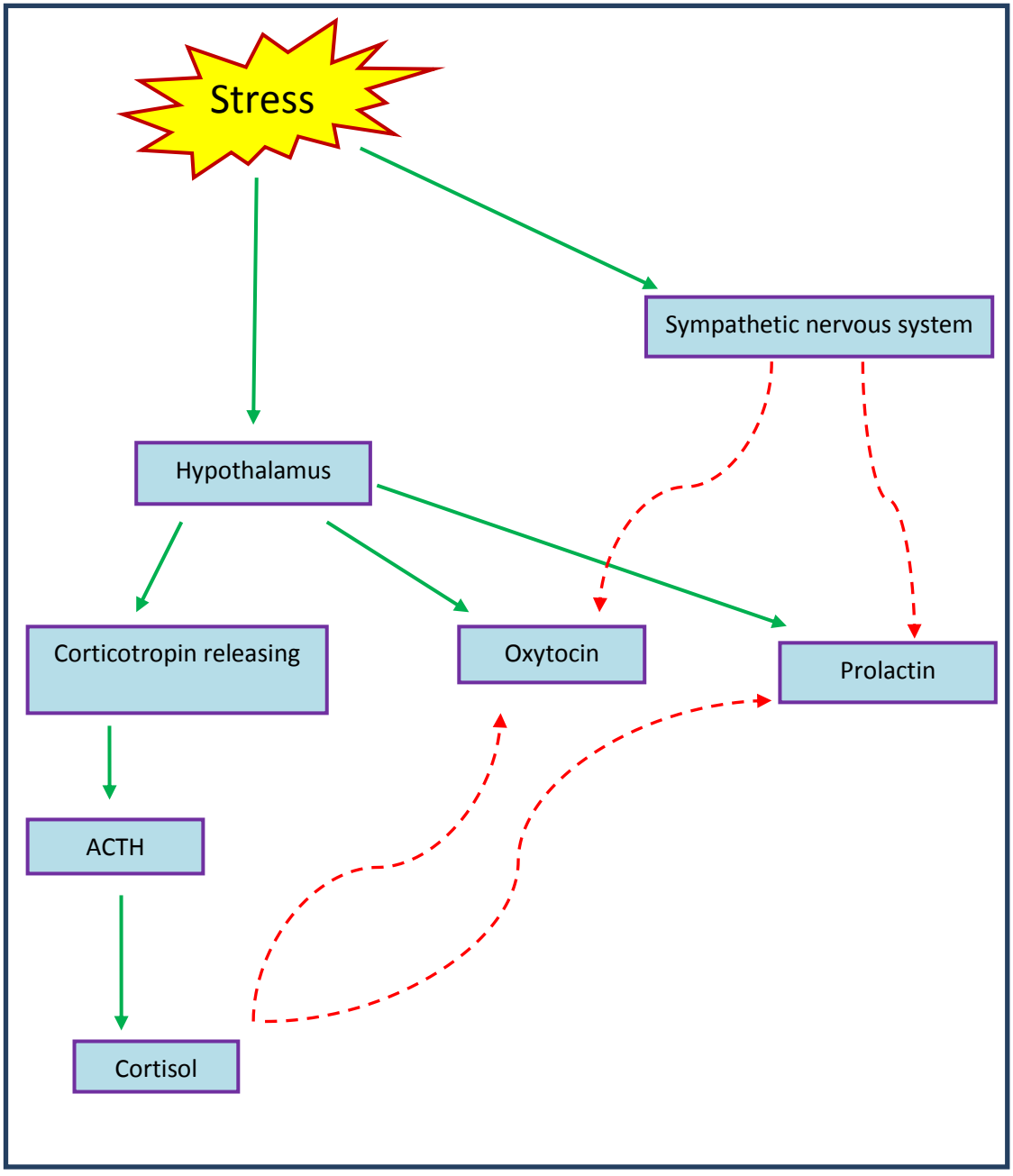

Figure 1.1: Interaction between stress and lactation and the postulated mechanism of action that stress inhibits prolactin and oxytocin

Note: ACTH = adrenocorticotropic hormone. Green arrow = stimulatory effect, red-dotted arrow = inhibitory effect

1.4 Rationale

The rationale of doing this study is to help in providing more data on the breastfeeding scenario in Malaysia. It can be used as evidence-based breastfeeding education to improve breastfeeding practices in all mothers. The study emphasizes the association between stress and breastfeeding in the context of working Malaysian women.

Postulated outcomes of the study are as follows:

1) Working mothers have more profound stress effects on lactation. Their additional workload, sleep disturbances and fatigue increase their stress levels therefore subsequently inhibit prolactin and oxytocin further.
2) Chronic stress hampers lactation performance by reducing prolactin and oxytocin level over time.
3) Lactogenic hormone (prolactin) may attenuate the hypothalamo-pituitary axis by lowering cortisol in the presence of stress.

1.5 The dissertation outline

This dissertation is presented in separate chapters as following:

Chapter 1 introduces the background and objectives of the study.

Chapter 2 discusses the literature review regarding the study topics and presents the findings of previous studies.

Chapter 3 outlines the methods used in this study with the flowchart showing the stages of study.

Chapter 4 presents the results and outcomes of the study in the forms of tables and graphs.

Chapter 5 elaborates the discussion on the study findings.

Chapter 6 gives the conclusion of the study and some suggestion for future work.

CHAPTER 2: LITERATURE REVIEW

Breastfeeding and stress have been a topic of intense debate. Many studies have been carried out on the relationship between breastfeeding and stress or stress response (Altemus *et al.*, 2001; Lau, 2001; Lau, Hurst, Smith, & Schanler, 2007; Mezzacappa, Kelsey, & Katkin, 2005). However, the effect of stress on breastfeeding and *vice versa* remains inconclusive. The postpartum period is regarded as a stressful period. Lactation can be considered to be one of nature's way of protecting the postpartum mothers from excessive stress reactivity (Heinrichs, Neumann, & Ehlert, 2002). This is evidenced by physiologic changes that occur at parturition and during lactation in which high cortisol levels were found to promote nurturing behaviour, maintaining milk production, enhancing immune function and decreasing catabolism (Groer, 2005). This chapter elaborates the relationship between stress and lactation and its association with employment status of the mothers.

2.1 Stress and cortisol

Hans Selye in 1930s defined stress as a "non-specific response of the body to any demand for change" (Szabo, Tache & Somogyi, 2012). It is further defined as a state of psychological and physiological imbalance resulting from the disparity between situational demand and the individual's ability and/or motivation to meet those demands. Activation of the stress system increases the ability to maintain homeostasis and increase chances for survival by behavioural and physiological changes (Tsigos & Chrousos, 2002). Stress generally activates the hypothalamo-pituitary-adrenal (HPA) axis resulting in increased basal cortisol level. Cortisol has multiple roles in our body and excessive level could lead to many pathologic conditions. It was well documented that chronic hyperactivity of stress system may cause metabolic syndrome, immunosuppression, osteoporosis and atherosclerosis (Tsigos & Chrousos, 2002). In

addition, the autonomic nervous system is also activated during stress response causing imbalance between the sympathetic and parasympathetic activities with predominant sympathetic activity. The resulted increased in catecholamine secretion was found to be stimulated by cortisol (Kudielka & Wust, 2010).

2.2 Lactation, prolactin and stress

Lactation is the physiologic completion of the reproductive cycle. Mammary glands begin to enlarge during early pregnancy in preparation for synthesis and secretion of milk. Mammogenesis is the process of proliferation of ductal tree to create multiple alveoli. The breast tissue becomes hypertrophied, subcutaneous veins become enlarged and the areolae become deeply pigmented (Jones & Spencer, 2007). Later in mid-pregnancy, there is increased in mRNA for many milk proteins and various enzymes (Jones & Spencer, 2007). This is important for milk formation and milk secretion. The switching of this process into secretory differentiation is called lactogenesis I. The fat droplets increase in size in secretory cells and become competent to secrete milk (Jones & Spencer, 2007). In addition, milk specific components like lactose, casein and α-lactalbumin and other milk constituents such as triglycerides, fatty acids, carbohydrates like glucose, galactose and oligosaccharides, minerals, electrolytes, vitamins, trace elements and water are produced (Kent, 2007). Lactogenesis II is signalled by an abrupt drop in progesterone in the presence of high prolactin level at parturition and it lasts 30-40 hours after delivery (Jones & Spencer, 2007).

Milk composition also changes during lactogenesis II, it was found that sodium and chloride concentrations were reduced while IgA, lactoferrin and oligosaccharides were increased (Jones & Spencer, 2007). During this period, lactation is controlled by lactogenic hormones and several other hormones like thyroid hormone and growth hormone. By day 3, milk removal is important for the successful continuation of

lactation. Galactopoiesis is the phase whereby the breast continues to produce milk indefinitely as long as milk removal continues (Jones & Spencer, 2007).

Milk removal is accomplished by the contraction of the myoepithelial cells. It involves the milk ejection reflex whereby the suckling effect of the infant initiate afferent impulses from the sensory receptors in the areola to travel to the central nervous system (Lawrence, 2010). This stimulates the posterior pituitary via the hypothalamus to release oxytocin. The oxytocin is circulated to the mammary glands where the hormones bind to oxytocin receptors located on the myoepithelial cells, thereupon, initiation of contraction occurs and milk is expelled from the alveoli into the ducts (Jones & Spencer, 2007). Listening to an infant cry or looking at an infant photo can stimulate the milk ejection reflex. The milk cannot be removed if this reflex is inhibited. During the initial phase of lactation, this reflex is unconditioned but once it is conditioned, it can be inhibited by physical stress such as pain and psychological stress like embarrassment or anxiety (Lawrence, 2010).

All these evidences point to the fact that the hormone prolactin plays an important role in lactation. Prolactin is a peptide hormone that has a structure similar to growth hormone. It consists of 199 amino acids. It has three disulphide bonds with a molecular weight of 22 kDa (Grattan & Kokay, 2008). It is secreted from the anterior pituitary with the main actions to stimulate growth and development of the mammary gland (mammogenesis), synthesis of milk (lactogenesis) and maintenance of lactation (galactopoiesis). Prolactin also plays a role in the regulation of maternal behaviour (Torner, Toschi, Nava, Clapp, & Neumann, 2002). Prolactin is involved in sleep-wake cycle and sexual behaviour by regulating gonadotrophin releasing hormone (GnRH) release (Torner & Neumann, 2002). The mechanism of action is through prolactin receptor (PRL-R) which is similar to the receptor for growth hormone. PRL-R is a single chain glycoprotein with a molecular weight of 75 kDa wherein upon binding with

prolactin, initiates secondary signalling pathway by dimerization and recruitment of tyrosine kinase (Torner *et al*., 2002). Prolactin secretion is under dominant negative control of dopamine. Due to this regulation, dopamine agonist pharmacologic agent such as bromocriptine is used for the cessation of breastfeeding. Metochlopromide which is the dopamine antagonist is prescribed to augment lactation (Lawrence, 2010). Physiologically, prolactin secretion is diurnal with its peak secretion during sleep and midmorning. The prolactin secretion is increased up to 10 fold during pregnancy, remains elevated during lactation and stimulated by suckling. Secretion rate declines during the later stage of lactation, noticeably after six weeks postpartum period (Hill, Chatterton & Aldag, 1999). Prolactin level is lower in preterm mothers who mostly had to mechanically or manually pump the milk (Lawrence, 2010). However prolactin is induced with skin to skin contact in this group of mother. This emphasizes the importance of maternal infant bonding and the stimulation of the nerve surrounding the nipple (Chatterton, Hill & Aldag, 2000).

Stress, either physical or psychological may impair lactation via physiological/hormonal responses by inhibiting milk synthesis, milk ejection and/or maternal behaviour (Lau *et al*., 2007). Women certainly experienced anxiety in their lifetime. Labour and evolving role of motherhood can be a stressful event. Therefore, strong family and social support is undoubtedly important. Maternal stress is correlated with lactation performance. There is a complex inter-relationship of maternal and infant factors that affect lactogenesis (Zanardo *et al*., 2011). Acute stress can affect the milk ejection almost immediately but, this is short-lived as the milk synthesis is not affected (Lau, 2001). It is postulated that stress hinders milk ejection and if this milk ejection is impaired often, the resulting incomplete emptying of the breast eventually will lead to reduced milk production. Chronic or prolonged stress would potentially affect the milk synthesis directly or as secondary to decrease milk ejection (Lau, 2001). In addition,

infant factors also contribute to lactation failure. Infants who undergo stressful delivery, prematurity and sickness also have feeding difficulties like poor suckling, difficulty in latching on and incoordination of the suck-swallow-breathe reflex (Dewey, 2001; Lau, 2001).

Contradictory to the above findings, it is observed that HPA responses to physical and psychological stress were blunted during postpartum period (Heinrichs *et al.*, 2002; Slattery & Neumann, 2008; Tu, Lupien, & Walker, 2005). Persistent low cortisol despite increased in stress level is seen in breastfeeding mothers and the low cortisol level in the body improves the overall maternal health (Altemus *et al.,* 2005). Nevertheless, alteration in this HPA activity is also associated with variable mood disorders including postpartum blues and postpartum depression. Suckling is a powerful neuroendocrine stimulus that can maintain the state of HPA axis hypo-responsiveness (Heinrichs, 2001; Heinrichs *et al*., 2002). Physiologically, suckling rapidly increases ACTH and cortisol secretion. This is important for metabolism action in favour of energy flow for milk production (Heinrichs, 2001). It is likely that HPA axis hypo-responsiveness in lactation is a result of reduced CRH production among others, contributed by activation of oxytocin and prolactin systems (Slattery & Neumann, 2008).

The physiological functions of prolactin as an attenuation of behavioural and neuroendocrine stress responses have been described during lactation (Torner & Neumann, 2002). This is due to the ability of the circulating prolactin from the anterior pituitary lactotrophs to enter the brain and up-regulating prolactin receptors in the choroid plexus during lactation (Torner & Neumann, 2002). Stress stimulates both prolactin release from the anterior pituitary lactotroph and extra-pituitary sources by increasing prolactin mRNA expression (Torner & Neumann, 2002). A study in rats

showed a decrease in ACTH secretory responses to stress when infused with prolactin (Brunton, Russell, & Douglas, 2008).

Therefore, this would be protective for mothers against overreacting to stress, conserving energy for nursing, improving immune system and counteracting the inhibition of lactation by stress (Heinrichs *et al*., 2002). However studies in human are scarce and the results vary. The neuroendocrine response is different depending on the type of the stressor (Altemus *et al*., 2001). In response to physical stress (treadmill exercise), they have found that plasma ACTH, cortisol, and glucose responses were significantly attenuated in lactating women. The result is consistent with animal studies (Altemus *et al*., 1995). In addition, using the similar study design, neuroendocrine response to psychological stress was carried out using Trier Social Stress Test as psychological stressor. The lactating women showed enhanced vagal control of cardiac activity while non-lactating women showed an increase in sympathetic activity and decreased parasympathetic tone (Altemus *et al.*, 2001). Lactating women also reported to perceive less life stress and negative moods than non-lactating women (Groer, 2005; Mezzacappa & Katkin, 2002). This could ultimately contribute to optimal maternal care to the offspring.

2.3 Work stress and lactation

In this fast moving world, more women with young children are in the workforce. This trend is negatively related to the rate of breastfeeding (Amin *et al*., 2011). Malaysia is of no exception but the prevalence of breastfeeding has increased after the introduction of the Malaysian Code of Ethics for Infants Formula Products in 1979. The prevalence of breastfeeding in working mothers is 25.4% as compared to 31.3% in non-working mothers. Meanwhile, the mean duration of breastfeeding among working mothers is 26 weeks postpartum is also lower as compared to 30 weeks postpartum among non-

working mothers. (Amin *et al*., 2011). This is well known to be attributed to length of maternity leave, feasibility for expression breast milk at work place which includes specific room, storage facility and time flexibility. Studies in Taiwan and Thailand showed that the length of maternity leave is positively related to initiation and duration of breastfeeding (Chuang *et al*., 2010). In Malaysia, Tan (2011) carried out a study in urban and rural population in Klang, Selangor and found higher prevalence of exclusive breastfeeding for infants aged between one and six months at 43.1% as compared to 19.3% in infants below 4 months of age by National Health and Morbidity Survey NHMS II and III 2006. This prevalence is highly associated with mothers who are non-working, Malay, rural residences, non-smoking, multiparous, term infants, mothers with supportive husband and practice bed-sharing (Tan, 2011). Generally all over the world, rate of initiation and duration of breastfeeding are lower among mothers who return to work after childbirth as opposed to stay-at-home mothers (Chuang *et al*., 2010; Mandal *et al*., 2010; Amin *et al*., 2011; Tan, 2011). Even, prior to returning to work, mothers who are expecting to return to full-time work have decreased breastfeeding initiation compared to mothers who are expecting to work part-time and not expecting to work (Mandal *et al.*, 2010). Hence, working is one of the major factors which hinder breastfeeding either psychologically or physiologically.

Work stress has been known to link with increased cortisol level because it affects autonomic nervous system neuroendocrine activity directly (Kunz-Ebrecht *et al.*, 2004). Among the factors that are important in influencing stress response are gender roles and psychological factors (Lundberg, 2005). In women, negative work character with high job demand in combination with low social support significantly contribute to stress level (Evolahti, Hultcrantz, & Collins, 2006). In sum, working stress could lead to lactation failure in women (Amin *et al.*, 2011; Cardenas & Major, 2005).

CHAPTER 3: MATERIALS AND METHODS

This chapter will explain the research design, sampling procedure and sample size, materials used in data collection, ethical considerations and statistical analysis.

3.1 Research design

This was a prospective non-interventional study involving postpartum Malaysian mothers with established breastfeeding. It measured the effects of stress on lactation by looking at serum prolactin and cortisol levels and lactation performance from infant weight gain.

3.2 Subjects

The study was carried out in two groups of postpartum Malaysian mothers with established breastfeeding, working and non-working. The subjects were recruited during their routine post-natal check-up at the selected *Klinik Kesihatan Ibu dan Anak* (KKIA) Taman Sri Rampai and KKIA Gombak over the duration of six months from 1st October 2011 until 1st April 2012. The two clinics were chosen due to accessibility to recruit the samples at the specified time and those clinics also have different designated post-natal clinic day which would increase the opportunity to recruit more subjects. In addition, the subjects from both clinics have similar socio-demographic background due to proximity of locations. Mothers in Malaysia are required to attend monthly post-natal check-up at government clinics from the first month of post-delivery up to one year. During these visits, the health of the mothers and infants are reviewed. Issues regarding contraception, post-delivery care, pre-existing medical illness or arising medical condition, infant feeding and immunization are discussed with the mothers.

The subjects were screened to fulfil the criteria below before recruitment in the study. Those women who did not fulfil the criteria were excluded from the study.

Inclusion criteria for subjects

1. Postpartum mothers between 22 and 40 years of age.
2. Mothers with established exclusive breastfeeding.
3. Mothers with full-time jobs.
4. Mothers who stayed at home.
5. Mothers who were willing to participate in the project

Exclusion criteria for subjects

1. Mothers who were unable to read and understand the DASS questionnaire.
2. Mothers with twin births.
3. Mothers with medical conditions that require chronic treatment e.g. hypertension, diabetes mellitus, hyper- or hypothyroidism or mental illness.
4. Mothers who gave supplemental formula feeding to infants even for one feeding per day
5. Mothers who had not given their consent.

3.3 Sample size and sampling method

The sample size required for each group (working and non-working mothers) of the study was 30. This figure was calculated *a priori* by using a sample size calculator for student t-Test with a setting of anticipated effect size (Cohen's d) of 0.65. This is for medium to large effect. The setting also included a desired statistical power level of 0.8 which is considered adequate and a probability level of less than 0.05 ($p<0.05$) to be deemed significant.

3.4 Data collection

Data were collected at four time-points each coinciding with the regular post-natal check-ups for both mothers and infants. At each visit, the following were performed: completing demographic data form (APPENDIX A) and the DASS questionnaire in English language (APPENDIX B) and in Malay language (APPENDIX C), measuring subjects' blood pressure and infants' weight, withdrawing blood sample for hormonal analysis and discussing about lactation performance.

Demographic data

A questionnaire designed to capture demographic information of subjects was constructed and subsequently translated into the Malay language. The information included age, marital status, education level, employment, ethnic background and household income. Other related information included family history and childcare as well as birth and breastfeeding histories.

Breastfeeding diary

The subjects were given breastfeeding diaries to determine the lactation performance. The participants were asked to document the amount of expressed breast milk daily (the first feeding in the morning). They were allowed to express the milk manually by hand or by using milk pumping device as long as they emptied both breasts.

Infants weight measurement

Infants' weights were recorded in the immunization card. All infants were weighed with no clothing. To minimize any confounding factor, similar weighing scales which had been previously calibrated were used at both clinics.

Assessment of perceived stress level

In order to assess the perceived naturalistic stress experienced by the mothers during the post-delivery period, the DASS questionnaire was used. This instrument is a 42-item questionnaire that consists of a set of self-reported scales to examine the degree of

severity of emotional experience encountered for the period for the three submodalities of depression, anxiety and stress (Lovibond & Lovibond, 1995). Subjects were asked to use 4-point severity/frequency scales to rate the extent to which they have experienced each emotional state over the past month. In this study, only scores for stress were calculated by summing the subscores for the stress (items 1, 6, 8, 11, 12, 14, 18, 22, 27, 29, 32, 33, 35 & 39) (APPENDIX B & C). The stress scale is sensitive to levels of chronic non-specific arousal. For those subjects who were most comfortable in the Malay language, a translated (from English) and validated DASS questionnaire was given to them (Ramli *et al*., 2012).

Collection of blood samples

Blood samples were collected between 8.00 to 10.30am for every mother on each visit. This time-limitation was necessary due to the diurnal nature of secretion of cortisol and prolactin. Ten (10 ml) of blood were withdrawn from the antecubital vein of the mothers. Blood withdrawal was done using a 25G needle which was attached to a 10 ml syringe. The blood samples were then transferred to plain vacutainers and were placed in ice box for transportation to the laboratory in the Department of Physiology, University of Malaya. At the laboratory, the blood samples were centrifuged at 3000 rpm for 15 minutes to separate the sera. The sera were transferred into Eppendorf tubes and coded according to the participant's number and visit number. The sera were kept frozen at -80^{o}C until further analyses. When sample collection was completed, the tubes were sent to the Clinical Diagnosis Laboratory (CDL), University Malaya Medical Centre for hormone analyses. Serum cortisol and prolactin were measured by using ADVIA Centaur Assay (Siemens Medical Solutions Diagnostics, USA). The ADVIA Centaur Assay is a competitive immunoassay using direct chemiluminescent technology.

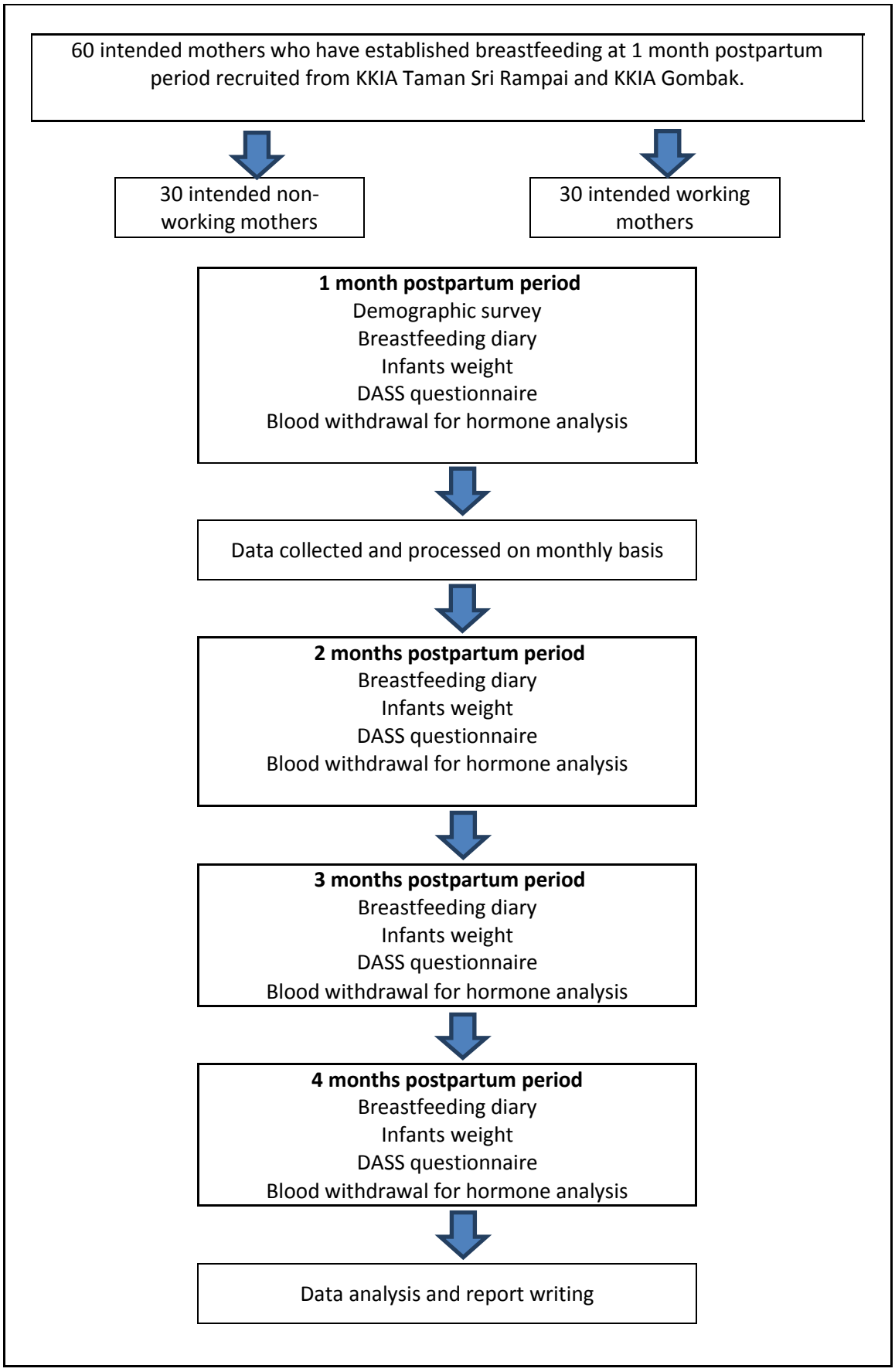

Figure 3.1: Proposed flowchart outlining the process of data collection

3.5 Ethical considerations

This study was approved by the Medical Ethics Committee, University Malaya Medical centre and National Ethic Committee (NMRR ID: 8804). All subjects gave written informed consent before taking part in the study (APPENDIX D & E). The information about the study was given in the patient information sheet (APPENDIX F & G). All the information from the questionnaires was kept confidential. The subjects were allowed to withdraw from the study at any stage of the study by merely informing the researcher.

3.6 Statistical Analyses

The collected data were explored for normal distribution by using Shapiro-Wilk test (normality test for small samples < 50 samples). The serum cortisol and prolactin were normally distributed. The relationships between the parameters were done using Pearson correlation test. The comparison between the groups was done using the student t-test. For data that were not normally distributed, the Mann-Whitney test. Significance level for all analyses was set at probability value of 0.05 or less ($p \leq 0.05$).

CHAPTER 4: RESULTS

This chapter presents the findings from the study. For comparison between non-working mothers versus working mothers in different time-points, results were taken from sixteen mother-infants pairs who successfully completed the study. The results are presented in descriptive statistics with tables and graphs to describe the data.

4.1 Subjects' study compliance

A total of 59 subjects were recruited during their first month postpartum from both KKIA Gombak and KKIA Taman Sri Rampai. However, there were many non-compliance cases due to several factors which is discussed in the next section. About 44.1% of recruited mothers participated at the second visit whereas only 27.1% of them completed data collection for four visits as required in the study (Figure 4.1). As we were interested in looking at the longitudinal effect of stress on lactation over the four months span, only mothers who had completed the four visits were included in the data analyses.

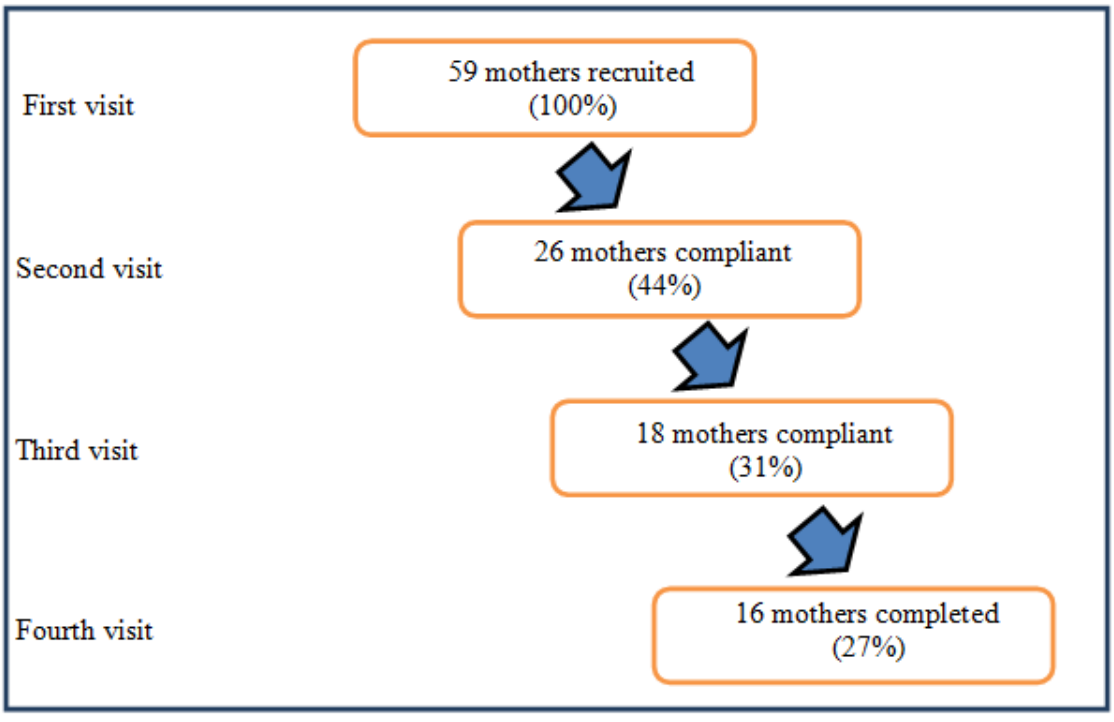

Figure 4.1: Subjects attrition rate across the four visits

4.2 Socio-demographic data

The socio-demographic characteristics of the sixteen mothers who completed the required four visits are shown in Table 4.1. There are nine working mothers and seven non-working mothers. Mean age for working mothers is 31.67 ± 2.10 years old while 30.71 ± 2.42 years old for the non-working mothers. All mothers are married and come from the Malay race. About 56% of the working mothers had tertiary education level while 71% of non-working mothers had below diploma education level. The working mothers have higher combined household income with mean of RM5136.22 ± 594.41 SEM per month while non-working mothers have lower household income with mean of RM2642.86 ± 684.47 SEM per month obviously due to the single source of income. The difference between the household income is significant with $p < 0.05$ (p=0.02). Sixty seven percent of working mothers are multiparous while 57% of non-working mothers are primiparous. About 75% of working and non-working mothers gave birth by normal vaginal delivery. All infants are born at term gestation with birth weight more than 2500g. About 55% of working mothers express their breast milk regularly while non-working mothers do not express breast milk as they are not separated from their infants at all time.

Table 4.1: The socio-demographic data of subjects (n = 16)

	Total (n = 16)	**Working mothers (n = 9)**	**Non-working mothers (n = 7)**
Maternal age (mean ± SEM)	31.75±1.59	31.67±2.10	30.71±2.42
Marital status			
Married	16	9	7
Educational level			
Primary	1	1	0
Secondary	6	1	5
Diploma	4	2	2
Tertiary	5	5	0
Ethnic group			
Malay	16	9	7
Household Income (mean ± SEM)	4045.37 ± 538.50	5136.22 ± 594.41*	2642.86 ± 683.47*
Type of delivery			
EMLSCS	2	0	2
ELLSCS	2	2	0
SVD	12	7	5
Assisted SVD		0	0

Note: EMLSCS emergency lower section caesarean section, ELLSCS elective lower section caesarean section, SVD spontaneous vaginal delivery.
*$p < 0.05$

4.3 Lactation performance

Lactation performance is defined from the measurement of milk volume and infant weight gain. However, the mothers did not complete the breastfeeding diary accordingly and most non-working mothers did not express their breast milk. The input was variable and could not be properly analysed. Therefore, in this study, infant weights recorded at each visit were used as an indicator for lactation performance. The increase in the infants' weights was following the WHO Growth Chart for breastfed infants as shown in Figure 4.3. All the mothers indeed did not require supplementation with formula milk.

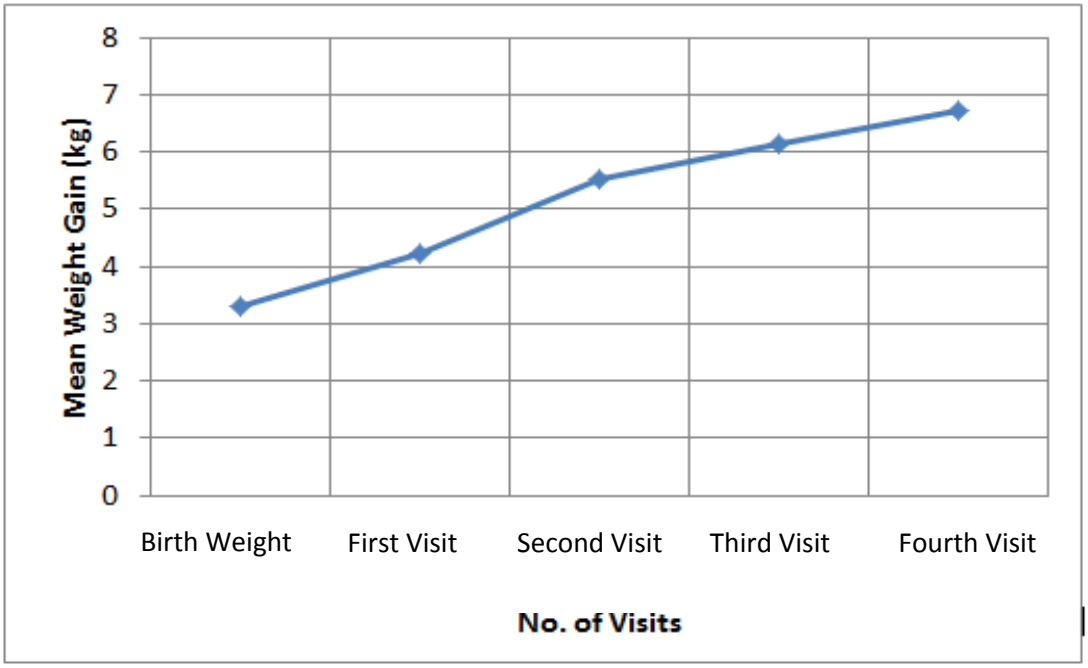

Figure 4.2: Mean weight gain in infants (n=16)

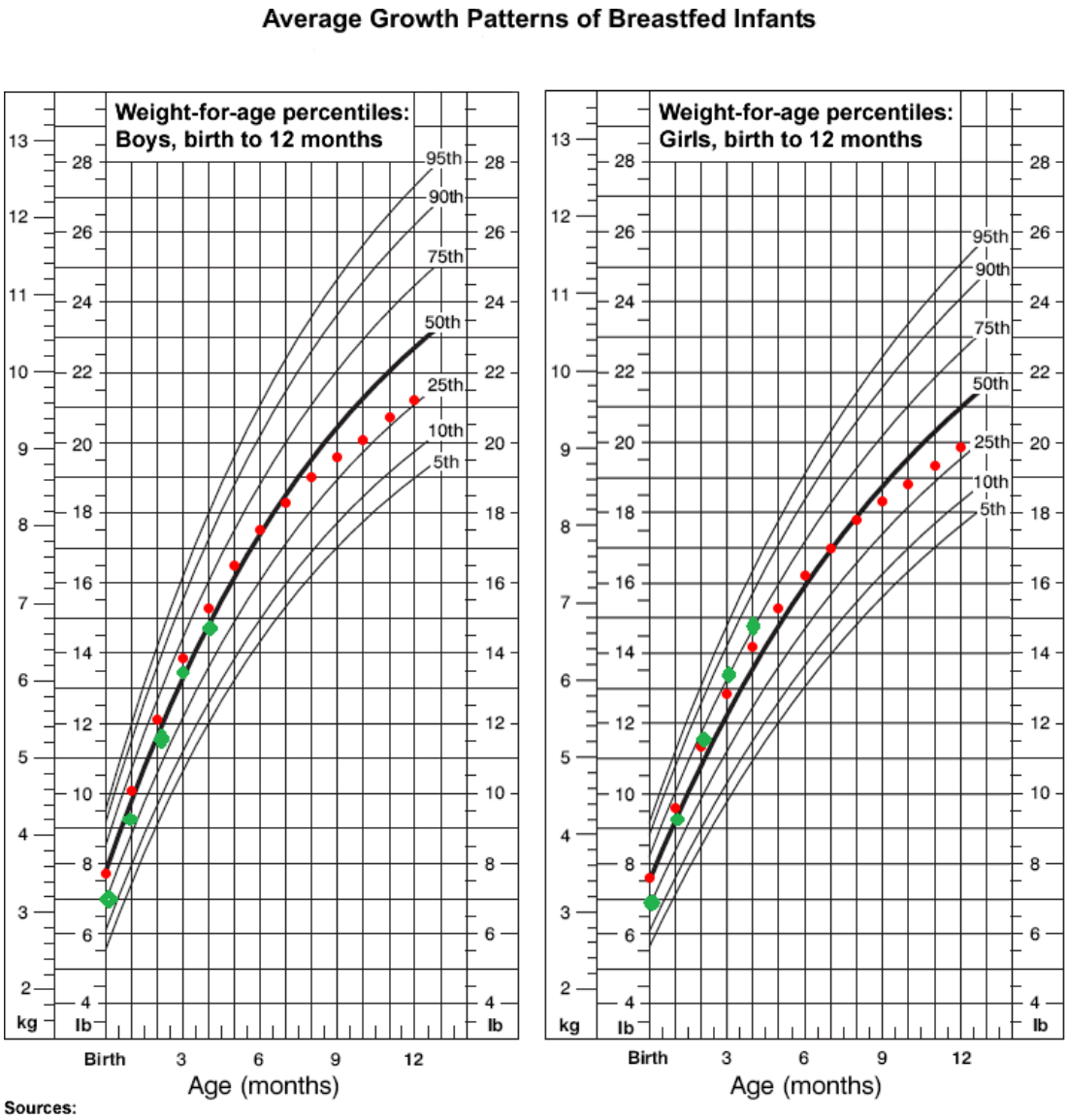

Figure 4.3: Infant weight gain and WHO Growth Chart for infants and children from 0 to 2 years of age

Notes :

-The red points plotted on the WHO Growth Chart represent the average weight-for-age for a small set of infants boys and girls who were breastfed for at least 12 months.

- The green points plotted on the WHO Growth Chart represent the average weight gain in infants who were breastfed in the study up for the first four months of age.

4.4 Assessment of perceived stress level

Stress score ranging from 0-14 is regarded as normal according to the DASS manual. The mean stress score for naturalistic perceived stress by the subjects from working and non-working groups across the four visits is tabulated in Table 4.2 and shown in Figure 4.4. The stress score was not normally distributed, therefore the statistical difference was measured by Mann-Whitney test. There was a significant difference (p=0.04) during the second visit between working and non-working mothers. Apparently the non-working mothers experienced more stress at two months postpartum period. No significant difference was seen at first, third and fourth visit. In a larger sample size wherein all the participated mothers from both groups were analysed, no significant difference was seen in mean stress score at each monthly visit (APPENDIX H). Again when the mean stress scores for each visit from both groups were compared to the first month postpartum, there were no significant difference seen (APPENDIX I).

Table 4.2: Mean stress score of working and non-working mothers across the four postpartum visits

	Stress Score		
Visits	Working mothers (n=9)	Non-working mothers (n=7)	*P* value
First Month	5.89 ± 1.09	7.86 ± 1.68	NS
Second Month	7.00 ± 1.59	9.57 ± 2.64	0.04*
Third Month	6.78 ± 1.98	9.86 ± 1.98	NS
Fourth Month	7.22 ± 1.66	10.86 ± 4.07	NS

Note: Values are mean ± SEM, NS = not significant, * = $P \leq 0.05$

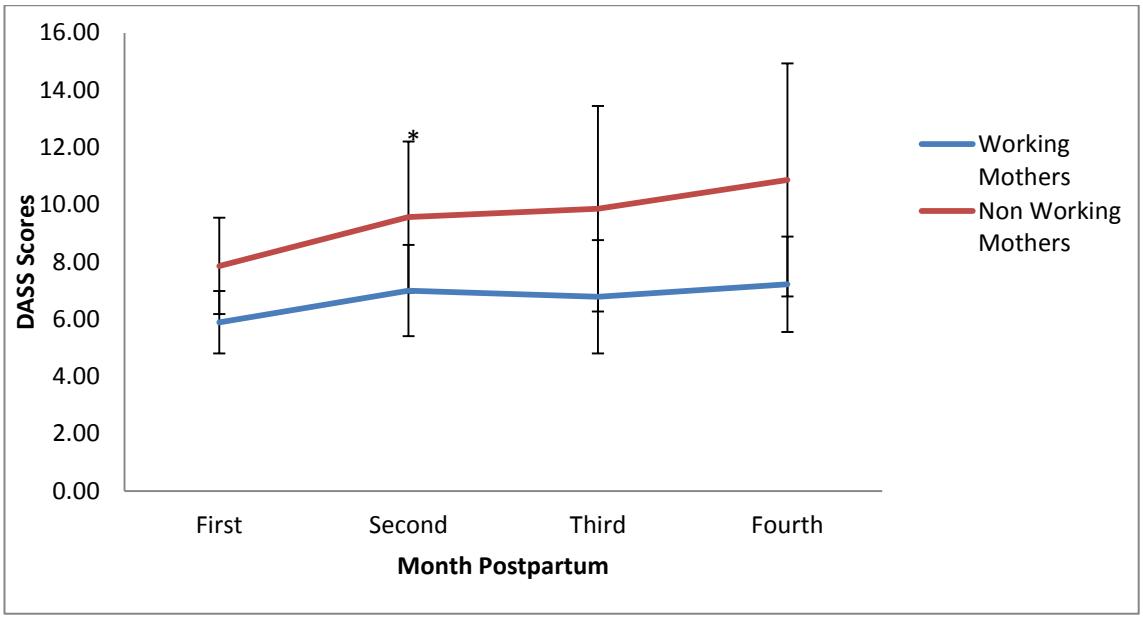

Figure 4.4: Comparison of mean ± S.E.M perceived stress score between working and non-working mothers across the four months of postpartum period. * = $P \leq 0.05$

4.5 Serum cortisol

The non-working mothers group showed significant increase in serum cortisol level at the second and third month postpartum (p=0.02 and 0.01 respectively) as tabulated in Table 4.3 and the comparison graph is demonstrated in Figure 4.5. This correlated with higher stress score seen at the same time-point. There was no significant difference between the two groups in the first month and fourth month visits. Similarly, the serum cortisol level is significantly higher in all participated non-working mothers at second and third months (APPENDIX J). However, when the mean serum cortisol for each visit were compared to the first month postpartum, the working mothers significantly less cortisol level on second and third months postpartum while the non-working mothers highest cortisol level on second month postpartum (APPENDIX K).

Table 4.3: Mean serum cortisol level of working and non-working mothers across the four postpartum visits

	Serum Cortisol nmol/L		
Visits	Working mothers (n=9)	Non-working mothers (n=7)	*P* value
First Month	267.67 ± 24.43	333.71 ± 49.77	NS
Second Month	249.44 ± 33.22	378.00 ± 34.48	0.02*
Third Month	190.33 ± 19.91	326.57 ± 47.53	0.01*
Fourth Month	259.33 ± 34.37	343.00 ± 46.78	NS

Note: Values are mean ± SEM, NS = not significant, * = $P \leq 0.05$

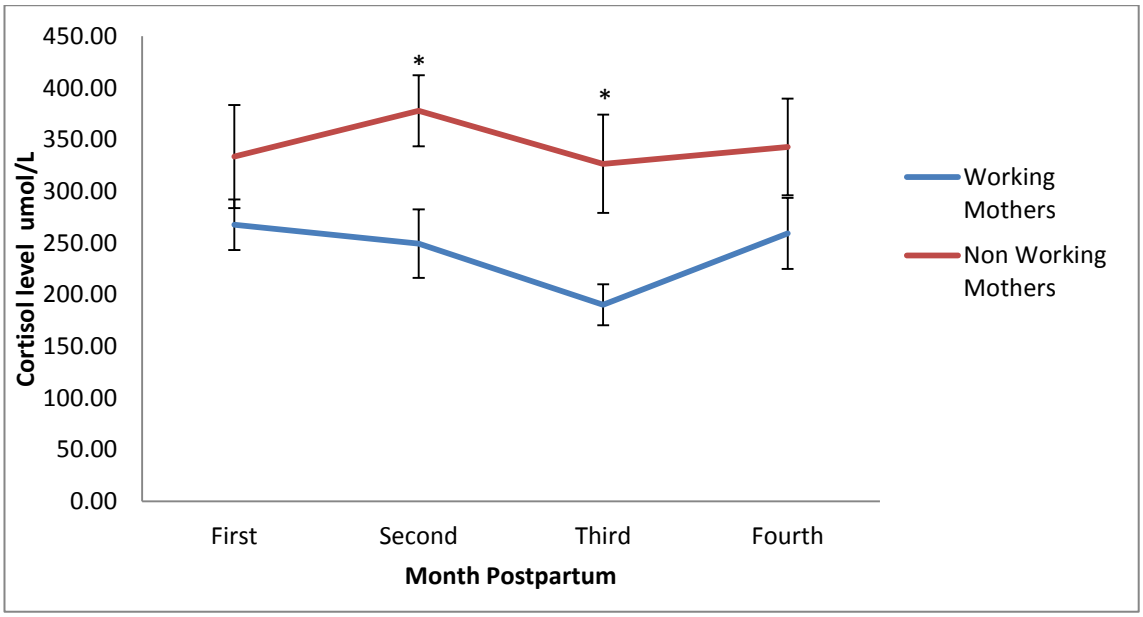

Figure 4.5 Comparison of mean ± S.E.M serum cortisol level between working and non-working mothers across the four months of postpartum period. * = $P \leq 0.05$

4.6 Serum prolactin

There was no significant difference between the mean serum prolactin level of working and non-working mothers at each postpartum visit. Interestingly, data of all participated mothers from both groups showed non-working mothers had significantly lower serum prolactin level at first month postpartum (APPENDIX L). Physiologically, serum prolactin level declines gradually after six weeks postpartum. It was apparently seen in both groups as shown in Table 4.4 and diagrammatically in Figure 4.6. From 3273.00 ± 569.78 uIu/mL at the first month in working mothers, to 2406.11 ± 452.94 uIu/mL, 1579.89 ± 271.04 uIu/mL and 1438.78 ± 279.33 uIu/mL at second, third and fourth month respectively. While in non-working mothers, serum prolactin gradually declined from 2633.86 ± 1001.14 uIu/mL and 1976.14 ± 594.44 uIu/mL at first and second month to 1779.00 ± 430.91 uIu/mL and 1280.14 ± 313.49 uIu/mL at third and fourth month postpartum. Moreover, the decline in serum prolactin level was significant in third and fourth month postpartum in all participated working mothers when compared to the level in the first month postpartum, the significant reduction was not seen in non-working mothers (APPENDIX M).

Table 4.4: Mean serum prolactin in working and non-working mothers across the four postpartum visits

	Serum Prolactin uIu/mL		
Visits	Working mothers (n=9)	Non-working mothers (n=7)	*P* value
First Month	3273.00 ± 569.78	2633.86 ± 1001.14	NS
Second Month	2406.11 ± 452.94	1976.14 ± 594.44	NS
Third Month	1579.89 ± 271.04	1779.00 ± 430.91	NS
Fourth Month	1438.78 ± 279.33	1280.14 ± 313.49	NS

Note: Values are mean ± SEM, NS = not significant

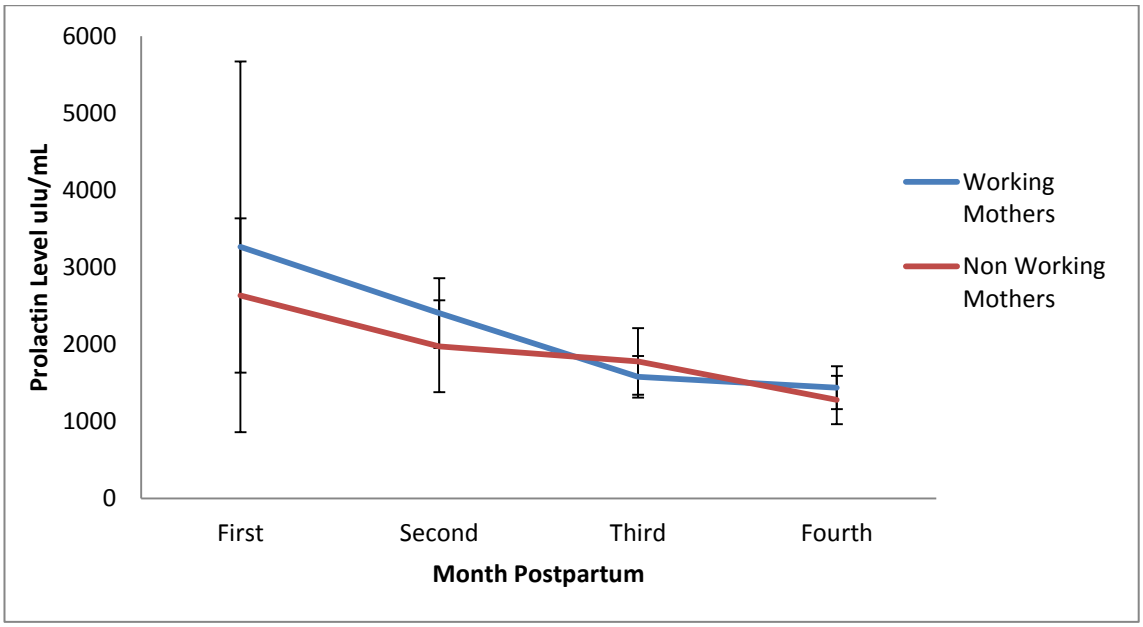

Figure 4.6 Comparison of mean ± S.E.M serum prolactin level between working and non-working mothers across the four months of postpartum period. * = $P \leq 0.05$

4.7 Correlation between perceived stress level, serum cortisol and serum prolactin

The overall perceived stress level from both groups was positively correlated with serum cortisol (p=0.02) across the four visits. But the perceived stress level has no correlation with serum prolactin across the same period. Similarly, there was no relationship between serum cortisol and serum prolactin for the measured timeline, both in working and non-working mothers.

CHAPTER 5: DISCUSSION

In this chapter, findings of the study are discussed and possible explanations offered whenever necessary. Like any other studies, experimental limitations are expected and they will be highlighted as well as recommendations for clinical practice and future studies.

At the start of the project, it was anticipated that at least 80% of lactating mothers would complete the study. However, in the end, only 16 mothers manage to successfully complete the four visits required of data collection. This represented only 27% of initial recruitment of 59 mothers. But the results from this smaller group are comparatively similar to the results from all the participated mothers at each month postpartum (APPENDIX H - M). Several factors are implicated for the high non-compliance rate and they include mothers who were unable to spend more time at the clinic, mothers who chose to continue infants immunization at private clinics after the first few months, mothers who were given subsequent appointments in the afternoon making blood sampling inconsistent and mothers who were not exclusively breastfeeding within the four months for various reasons. Nevertheless, the data collected are still valid for analysis and the results do highlight several important issues. Moreover, the eventual sample size used is found to be comparable to other studies on lactation (Altemus *et al.*, 2001, Cox, Owens & Hartmann, 1996).

In this study, lactation performance was measured by infant weight gain as it directly reflected adequate milk production by the mothers (Lawrence, 2010, Eidelman, 2012). Since the infant weight gain measured (Figure 4.2) was comparable to the normal reference curve of the WHO Growth Chart (Figure 4.3) of breastfed infants, it was deemed that the lactating mothers in the study were producing adequate milk throughout the four months postpartum period despite a gradual decline in serum

prolactin (Figure 4.6 and APPENDIX M) similar to the observations of Cox *et al.,* 1996 but in contrast to that of Torner *et al.,* 2002.

Nevertheless, adequate milk production with decreasing PRL was possible probably because PRL is important for initiating lactation (Torner *et al.,* 2002), but not necessary once milk production has been established as this phase is regulated by an autocrine control mechanism (Hill, Chatterton & Aldag, 1999) and PRL is seen to be imparting a permissive action to the process (Torner *et al.,* 2002). Furthermore, the normal stress level seen in this cohort (Figure 4.4) may have helped towards the maintenance of lactation since stress is known to be one of the factors that inhibit lactation (Lau, 2001).

Naturalistic stress perceived by the mothers was determined by the stress score component of DASS which assesses the severity of core symptoms of depression, anxiety and stress. The value of DASS is to identify the locus of emotional disturbances. For the stress component, the measures to be taken into consideration include difficulty in relaxing, nervous arousal, agitation, irritability and impatience. As in other studies, subjects who have positive attitudes towards a particular study would be the ones most likely to participate willingly. Therefore, we could have missed the mothers who were really stressed out and that could possibly have different outcomes and implications.

The data obtained showed that the stress scores have a positive association with serum cortisol. This observation is supported by previous studies that found serum cortisol level being high during stress (Evolathi *et al.,* 2006, Meinlschmidt *et al.,* 2010). When faced with physical or emotional stress, the body will undergo adaptive responses to achieve homeostasis. Although these adaptive responses are specific to the stressor to which they are exposed, they tend to be lost as the severity of stressor increases (Tsigos & Chrousos, 2002). The brain components that initiate and maintain the stress

response are located in the hypothalamus and brain stem, and include the parvocellular corticotropin-releasing hormone (CRH) and argininevasopressin (AVP) neurons of the paraventricular nuclei (PVN) of the hypothalamus and the locus ceruleus (LC)-norepinephrine system (central sympathetic system) (Tsigos & Chrousos, 2002). Pulsations of CRH and AVP increase during acute stress resulting in increases in the secretion of ACTH and cortisol (Tsigos & Chrousos, 2002).

Results also showed that there is no relationship between serum cortisol and PRL contrary to the observations of Heinrichs in 2001. Similarly there is no direct correlation between perceived stress level and serum PRL. This could possibly be explained by the finding that both the perceived stress score and serum cortisol levels were not significantly elevated.

This study postulated that working mothers would have higher perceived stress levels due to the increased work load at the place of employment in addition to the household chores and sustained separation from their infants. It is interesting to note that the converse was true with the finding that non-working mothers actually experienced a statistically significant perceived stress level at the two month postpartum period (Figure 4.4). There were, however, no differences at first-, third- and fourth-month postpartum period. A possible explanation for this observation could be that the second month postpartum represents a transition period between confinement and returning to daily routine of housework. In Malaysia, most postpartum mothers and new-borns would undergo a confinement period of two months post-delivery. During this period, they are taken care by a family member, very often, the mother or mother-in-law or more commonly by a hired confinement lady. The postpartum mothers would have time to rest and recuperate while the daily chores are taken care of by the helper. In this study, all mothers were well looked after by close family members and friends during the confinement period. After the confinement period, the group of non-working

mothers would have difficulty in adjusting initially to the caring of the infants as well as dealing with household chores. Moreover, the non-working mothers in this cohort have significantly lower household incomes as compared to the working mothers as shown in Table 4.1. It is known that socioeconomic status can influence the biological and psychological stress response in humans (Kunz-Ebrecht, Kirscbaum, & Steptoe, 2004).

In contrast, the working group has other people to do the childcare and this would give them time off from nursing and caring for their children. Other considerations that made working mothers in this study less stressed include support from friends and colleagues at work and sharing of problems or concerns (Hirani & Karmaliani, 2013). In addition, going out to work may give them more space to explore and to inculcate a positive work culture than the stay-at-home mothers with the fixed routine.

Even though employment is associated with lactation impairment by reduction of PRL levels (Tan, 2011) due to prolong separation from infants and lack of facilities to express breast milk to maintain PRL level (Amin *et al*., 2011), lactation performance was not affected as discussed earlier. This could be due to the strong determination of the mothers to ensure exclusive breastfeeding and to the highly motivated working mothers, in particular, to frequently express their breast milk at work. Through brief interviews with them, they apparently encountered minimal problems with expressing breast milk at work even though proper places for expressing and storage of breast milk were not adequately provided in some instances.

Recent findings suggest yet another explanation for the low stress scores and adequate lactation seen in this group. In 2002, Heinrichs, Neumann & Ehlert found that the act of breastfeeding itself could confer a protective effect on the maternal stress response. The mechanism of attenuation of the stress response in lactation is thought to be related to hormones of lactation. Suckling alone involving oxytocin and PRL via the

neuroendocrine pathways appears to exert short term suppressions of cortisol secretion at the HPA level (Heinrichs, 2001). Moreover, breastfeeding is associated with reduced perceived stress and negative mood swings (Mezzacappa & Katkin, 2002). However, amelioration of the HPA stress response by breastfeeding appears to be quite selective in that the suppression of the HPA reflex is significantly lower in physical than in psychological stress (Altemus *et al.*, 2001).

This study has several limitations. First, the recruitment of subjects may not include those with severe stress. Time and financial constraints also limit further data collection. The measurement for lactation performance was not directly on milk volume which could lead to overestimation of correlation between stress, lactation performance and PRL. The cofounding factor like household income would be better eliminated if the financial status was analysed instead of looking at the difference between the actual income figures. Since this study was concentrated on mothers who were exclusively breastfeeding, this precluded comparisons with mothers who were not breastfeeding. Therefore, it is recommended for future studies to include non-lactating mothers as well in order to elicit whether the protective effect of lactation is indeed regulated by PRL.

CHAPTER 6: CONCLUSION

In conclusion, perceived stress level is positively correlated with serum cortisol level. There is no relationship found between perceived stress level and serum PRL as well as serum cortisol and serum PRL. Mothers in this cohort did not experience significant stress, therefore the effects of stress on lactation was not elucidated. Most probable underlying reason for this observation is maternal hypo-responsiveness to stress during lactation. However, overall the objectives of the study were met.

Contrary to expectation, this study also highlighted that employment outside the home did not adversely increase the stress level in postpartum mothers. Indeed, the non-working mothers were found to have significantly higher stress scores towards the end of confinement period. Other intangible factors that appear to be just as important in maintaining lactation include good family and social support and financial stability. While the factors from this study may contain implications for public health education, further research is required to explore the differences in the stress response in non-lactating mothers who belong to either the stay-at-home or work-outside-home group. This information may be helpful in policy making, guideline formulation and the promotion of breastfeeding being beneficial to mother and child.

APPENDIX A

PERSONAL INFORMATION

Code Number: ☐☐☐

SECTION A

Mother's information / *Maklumat Ibu*

1. Name /*Nama*:……
2. IC Number / *Nombor KP :* .....
3. Age / *Umur*: ☐☐

   Ethnic group / *Kumpulan etnik* : ☐

   (1=Malay/*Melayu*, 2=Chinese/*Cina*, 3=Indian/*India*, 4=Others/*Lain-lain*)
4. Marital status / *Taraf perkahwinan* : ☐

   (1=Married/*Berkahwin*, 2=Divorced/*Bercerai,* 3=Unmarried/*tidak berkahwin*)
5. Address / *Alamat* : ……

   ……..
6. Education level */ Tahap pendidikan* : ☐

   (1=No formal education/*Tidak Bersekolah*, 2=Primary/*Sekolah rendah*,

   3=Secondary/*Sekolah menengah*, 4=Diploma/*Diploma*, 5=Tertiary/*Universiti*)
7. Occupation / *Pekerjaan* : ……
8. Working hours / *Jumlah jam bekerja* : ……..
9. Income / *Pendapatan bulanan* : …….
10. Smoking / *Merokok*: ☐

    (0=Never/*Tidak pernah*, 1=Occasional/*Jarang-jarang*, 2=Frequent/*kerap*,

    3=Regular/*Selalu*)
11. Alcohol drinking / *Pengambilan minuman beralkohol :* ☐

    (0=Never/*Tidakpernah*, 1=Occasional/*Jarang-jarang*, 2=Frequent/*kerap*,

    3=Regular/*Selalu*)

Father's information / *Maklumat bapa*

1. Name / *Nama*: ……
2. Age */ Umur* : ☐☐
3. Education level / *Tahap Pendidikan* : ☐

   (1=No formal education/*Tidak Bersekolah*, 2=Primary/*Sekolah rendah*,

   3=Secondary/*Sekolah menengah*, 4=Diploma/*Diploma*, 5=Tertiary/*Universiti*)
4. Occupation / *Pekerjaan :*
5. Income / *Pedapatan bulanan* :
6. Smoking / *Merokok* : ☐

   (0=Never/*Tidak pernah*, 1=Occasional/*Jarang-jarang*, 2=Frequent/*kerap,*

   3=Regular/*Selalu*)
7. Alcohol drinking / Pengambilan minuman beralkohol : ☐

   (0=Never/*Tidakpernah*, 1=Occasional/*Jarang-jarang*, 2=Frequent/*kerap*,

   3=Regular/*Selalu)*

Family history / *MaklumatKeluarga*

8. Number of children / *Bilangan anak* :

Name */ Nama*	Gender / *Jantina*	Age / *Umur*

9. Childcare / *Penjagaan anak :* ☐

   (1=Home/*Rumah*, 2=Babysitter/*Penjaga*, 3=Nursery/*Taska*, 4=Family's house/*Rumah ahli keluarga*)
10. Any helper at home / *Mempunyai pembantu rumah* : ☐

    (0=No/*Tidak ada*, 1=Yes/*Ada)*

SECTION B

Birth and breastfeeding history

11. Mode of delivery / *Cara kelahiran* : ☐

    (1=Emergency Caesarean/*Pembedahan kecemasan*, 2=Elective Caesarean/*Pembedahan elektif,* 3=Normal vaginal delivery/*Kelahiran normal*, 4=Instrumental delivery/*Kelahiran bantuan alatan)*

12. First day of lactation */ Hari pertama penyusuan* :

13. Expressed breast milk / *Perahan susu ibu* : ☐ (0=No/*Tidak*, 1=Yes/*Ya)*

    If Yes/ *JikaYa :*

    Frequency / *Kekerapan :*

    Volume expressed per session / *Jumlah perahan pada satu sesi* :

14. Supplement to increase lactation / *Kaedah penambahan penyusuan* :

    ……..

    ……..

15. Lactation support / *Sokongan penyusuan* : ☐

    (1=Home visit nurse/*Jururawat melawat ke rumah*, 2=Support group/*Kumpulan sokongan*, 3=Family/*Keluarga*, 4=Family doctor/*Doktor keluarga*)

16. Intended duration for breastfeeding / *Tempoh penyusuan yang diinginkan :*

17. Do you breastfed your previous children ?*Adakah anda memberi penyusuan susu ibu pada anak-anak anda sebelum ini?*

☐ (0=No/*Tidak,* 1=Yes/*Ya)*

If Yes*, JikaYa:*

Duration of breastfeeding / *Tempoh penyusuan susu ibu* :

18. Do you feed your baby by a schedule? *Adakah anda menyusukan anak anda mengikut jadual*?

☐ (0=No/*Tidak,* 1=Yes/*Ya*)

19. Does your baby have a pacifier ?*Adakah bayi anda mempunyai puting tiruan?*

☐ (0=No/*Tidak,* 1=Yes/*Ya*)

20. Do you give your baby night feeding? *Adakah anda memberi penyusuan susu ibu pada waktu malam?* ☐ (0=No/*Tidak*, 1=Yes/*Ya*)

21. Do you co-sleeping with your baby ? *Adakah bayi anda tidur bersama anda di waktu malam?*

☐ (0=No/*Tidak,* 1=Yes/*Ya*)

22. Does your workplace provide facilities for breastfeeding or expressing breatmilk? *Adakah tempat anda bekerja menyediakan kemudahan untuk penyusuan susu ibu atau pemerahan dan penyimpanan susu ibu?*

☐ (0=No/*Tidak*, 1=Yes/*Ya)*

23. Why do you choose to breastfeed your child? *Kenapa anda memilih untuk menyusu badan anak anda?*

……

……

……

……

24. Where did you learn about benefits of breastfeeding? *Di mana anda mendapat pengetahuan mengenai kebaikan susu ibu?*

……

……

……

THANK YOU */ TERIMA KASIH*

APPENDIX B

DASS

Name: *Date:*

Please read each statement and circle a number 0, 1, 2 or 3 which indicates how much the statement applied to you *over the past week*. There are no right or wrong answers. Do not spend too much time on any statement.

1	I found myself getting upset by quite trivial things	0	1	2	3
2	I was aware of dryness of my mouth	0	1	2	3
3	I couldn't seem to experience any positive feeling at all	0	1	2	3
4	I experienced breathing difficulty (eg, excessively rapid breathing, breathlessness in the absence of physical exertion)	0	1	2	3
5	I just couldn't seem to get going	0	1	2	3
6	I tended to over-react to situations	0	1	2	3
7	I had a feeling of shakiness (eg, legs going to give way)	0	1	2	3
8	I found it difficult to relax	0	1	2	3
9	I found myself in situations that made me so anxious I was most relieved when they ended	0	1	2	3
10	I felt that I had nothing to look forward to	0	1	2	3
11	I found myself getting upset rather easily	0	1	2	3
12	I felt that I was using a lot of nervous energy	0	1	2	3
13	I felt sad and depressed	0	1	2	3
14	I found myself getting impatient when I was delayed in any way (eg, lifts, traffic lights, being kept waiting)	0	1	2	3
15	I had a feeling of faintness	0	1	2	3
16	I felt that I had lost interest in just about everything	0	1	2	3
17	I felt I wasn't worth much as a person	0	1	2	3
18	I felt that I was rather touchy	0	1	2	3
19	I perspired noticeably (eg, hands sweaty) in the absence of high temperatures or physical exertion	0	1	2	3
20	I felt scared without any good reason	0	1	2	3
21	I felt that life wasn't worthwhile	0	1	2	3

Please turn the page ☞

Reminder of rating scale:

0 Did not apply to me at all

1 Applied to me to some degree, or some of the time

2 Applied to me to a considerable degree, or a good part of time

3 Applied to me very much, or most of the time

22	I found it hard to wind down	0	1	2	3
23	I had difficulty in swallowing	0	1	2	3
24	I couldn't seem to get any enjoyment out of the things I did	0	1	2	3
25	I was aware of the action of my heart in the absence of physical exertion (eg, sense of heart rate increase, heart missing a beat)	0	1	2	3
26	I felt down-hearted and blue	0	1	2	3
27	I found that I was very irritable	0	1	2	3
28	I felt I was close to panic	0	1	2	3
29	I found it hard to calm down after something upset me	0	1	2	3
30	I feared that I would be "thrown" by some trivial but unfamiliar task	0	1	2	3
31	I was unable to become enthusiastic about anything	0	1	2	3
32	I found it difficult to tolerate interruptions to what I was doing	0	1	2	3
33	I was in a state of nervous tension	0	1	2	3
34	I felt I was pretty worthless	0	1	2	3
35	I was intolerant of anything that kept me from getting on with what I was doing	0	1	2	3
36	I felt terrified	0	1	2	3
37	I could see nothing in the future to be hopeful about	0	1	2	3
38	I felt that life was meaningless	0	1	2	3
39	I found myself getting agitated	0	1	2	3
40	I was worried about situations in which I might panic and make a fool of myself	0	1	2	3
41	I experienced trembling (eg, in the hands)	0	1	2	3
42	I found it difficult to work up the initiative to do things	0	1	2	3

APPENDIX C

DASS

Nama: Tarikh:

Sila baca setiap kenyataan dibawah dan bulatkan pada nombor 0,1,2 atau 3 bagi menggambarkan keadaan anda sepanjang minggu yang lalu. Tiada jawapan yang betul atau salah. Jangan mengambil masa yang terlalu lama untuk menjawab mana-mana kenyataan.

Skala pemarkahan adalah seperti berikut:

0 Tidak langsung menggambarkan keadaan saya
1 Sedikit atau jarang-jarang menggambarkan keadaan saya.
2 Banyak atau kerap kali menggambarkan keadaan saya.
3 Sangat banyak atau sangat kerap menggambarkan keadaan saya

1	Saya dapati diri saya menjadi kesal/marah disebabkan perkara-perkara yang kecil.	0	1	2	3
2	Saya sedar mulut saya terasa kering	0	1	2	3
3	Saya tidak dapat mengalami perasaan positif sama sekali	0	1	2	3
4	Saya mengalami kesukaran bernafas (contohnya pernafasan yang laju, tercungap-Cungap walaupun tidak melakukan senaman fizikal)	0	1	2	3
5	Saya rasa diri saya tidak bergerak ke mana-mana	0	1	2	3
6	Saya cenderung untuk bertindak keterlaluan dalam sesuatu keadaan	0	1	2	3
7	Saya mempunyai perasaan gementar (seperti kaki menjadi lemah)	0	1	2	3
8	Saya rasa sukar untuk relaks	0	1	2	3
9	Saya dapati diri saya di dalam keadaan yang menjadikansaya amat risau dan menjadi tenang semula selepas ianya berakhir	0	1	2	3
10	Saya rasa saya tidak mempunyai apa-apa untuk diharapkan	0	1	2	3
11	Saya dapati diri saya mudah merasa kesal	0	1	2	3
12	Saya rasa saya menggunakan banyak tenaga dalam keadaan cemas	0	1	2	3
13	Saya rasa sedih dan murung	0	1	2	3
14	Saya dapati diri saya hilang kesabaran sekiranya saya dilambatkan oleh sesuatu (seperti lif, lampu trafik, terpaksa lama menunggu)	0	1	2	3
15	Saya rasa macam nak pengsan	0	1	2	3
16	Saya rasa saya hilang minat dalam segala hal	0	1	2	3
17	Saya tidak begitu berhargasebagai seorang individu	0	1	2	3
18	Saya rasa yang saya mudah tersentuh	0	1	2	3
19	Saya banyak berpeluh (contohnya pada tangan) walaupun bukan pada suhu tinggi atau tiada pergerakan fizikal.	0	1	2	3
20	Saya berasa takut tanpa sebab yang munasabah	0	1	2	3
21	Saya rasa hidup ini sudah tidak bermakna lagi	0	1	2	3

Ingatan skala permarkahan:

0 Tidak langsung menggambarkan keadaan saya
1 Sedikit atau jarang-jarang menggambarkan keadaan saya.
2 Banyak atau kerapkali menggambarkan keadaan saya.
3 Sangat banyak atau sangat kerap menggambarkan keadaan saya

22	Saya dapati diri saya sukar ditenteramkan	0	1	2	3
23	Saya rasa sukar menelan	0	1	2	3
24	Saya tidak dapat merasakan keseronokan dalam apa yang saya lakukan	0	1	2	3
25	Saya sedar tindakbalas jantung saya walaupun tidak melakukan aktiviti fizikal (contohnya kadar denyutan jantung bertambah, atau denyutan jantung berkurangan)	0	1	2	3
26	Saya rasa duka dan tidak keruan	0	1	2	3
27	Saya dapati diri saya mudah marah	0	1	2	3
28	Saya rasa hampir-hampir menjadi panik/cemas	0	1	2	3
29	Saya dapati sukar untuk bertenang setelah sesuatu membuatkan saya kesal	0	1	2	3
30	Saya risau saya akan 'dihambat' oleh tugas yang remeh dan tidak biasa dilakukan	0	1	2	3
31	Saya tidak bersemangat dengan apa jua yang saya lakukan	0	1	2	3
32	Saya sukar bersabar pada gangguan terhadap perkara yang sedang saya lakukan	0	1	2	3
33	Saya di dalam keadaan yang terlalu gementar	0	1	2	3
34	Saya rasa diri saya langsung tidak berharga	0	1	2	3
35	Saya hilang pertimbangan pada perkara yang menghalang saya meneruskan apa yang saya lakukan	0	1	2	3
36	Saya rasa amat takut	0	1	2	3
37	Saya melihat tiada masa depan untuk saya menaruh harapan	0	1	2	3
38	Saya rasa hidup ini tidak bermakna	0	1	2	3
39	Saya dapati diri saya semakin gelisah	0	1	2	3
40	Saya bimbang keadaan di mana saya mungkin menjadi panik dan melakukan perkara yang membodohkan diri sendiri	0	1	2	3
41	Saya rasa menggeletar (contohnya pada tangan)	0	1	2	3
42	Saya sukar untuk mendapatkan semangat bagi melakukan sesuatu perkara	0	1	2	3

APPENDIX D

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CONSENT FORM

I, _____ Identity Card No _____

(Name)

of _____

(Address)

hereby agree to participate in the clinical research (clinical study/questionnaire study/drug trial) specified below:-

The effect of stress on lactation among working and non–working Malaysian mothers.

the nature and purpose of which has been explained to me by Dr Nik Raihan Hj Mohamed and interpreted by

_____

(Name & Designation of Interpreter)

to the best of his/her ability in ……..…… language/dialect.

I have been informed of the nature of this clinical research in terms of procedure, possible adverse effects and complications (as per patient information sheet). I understand the possible advantages and disadvantages of participating in this research. I voluntarily give my consent for my relative to participate in this research specified above.

I understand that I can withdraw my relative from this clinical research at any time without assigning any reason whatsoever and in such situation, my relative shall not be denied the benefits of usual treatment by the attending doctors. Should my relative regains his/her ability to consent, he/she will have the right to remain in this research or may choose to withdraw.

Date: Signature:

IN THE PRESENCE OF

Name ……..…..

Identity Card No. ……. Signature

(Witness)

Designation ……

I confirm that I have explained to the patient's relative the nature and purpose of the above-mentioned clinical research.

Date ……. Signature

(Dr Nik Raihan Hj Mohamed)

APPENDIX E

KEIZINAN OLEH PESAKIT UNTUK PENYELIDIKAN KLINIKAL

Saya _____ No. Kad Pengenalan _____

(*Nama pesakit*)

beralamat _____

(*Alamat*)

dengan ini bersetuju menyertai dalam penyelidikan klinikal disebut berikut

Kesan tekanan terhadap penyusuan susu ibu di kalangan ibu–ibu yang bekerja dan tidak bekerja di Malaysia.

yang mana sifat dan tujuannya telah diterangkan kepada saya oleh Dr Nik Raihan Hj Mohamed mengikut terjemahan _____

(Nama & jawatan penterjemah)

yang telah menterjemahkan kepada saya dengan sepenuh kemampuan dan kebolehannya di dalam bahasa/loghat _____

Saya telah diberitahu dasar penyelidikan klinikal dalam keadaan metodologi, risiko dan komplikasi (mengikut kertas maklumat pesakit). Selepas mengetahui dan memahami semua kemungkinan kebaikan dan keburukan penyelidikan klinikal ini, saya merelakan/mengizinkan sendiri menyertai penyelidikan klinikal tersebut di atas.

Saya faham bahawa saya boleh menarikdiri dari penyelidikan klinikal ini pada bila-bila masa tanpa memberi sebarang alasan dalam situasi ini dan tidak akan dikecualikan dari kemudahan rawatan dari doktor yang merawat.

Tarikh : Tandatangan :

DI HADAPAN

Nama :

No. K.P : Tandatangan :

Jawatan : (*Saksi untuk tandatangan pesakit*)

Saya sahkan bahawa saya telah menerangkan kepada pesakit sifat dan tujuan penyelidikan klinikal tersebut di atas.

Tarikh : Tandatangan :

(*Dr Nik Raihan Hj Mohamed*)

APPENDIX F

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PATIENT INFORMATION SHEET

Please read the following information carefully, do not hesitate to discuss any questions you may have with your Doctor.

Study Title

The effects of stress on lactation among working and non-working Malaysian mothers

Introduction

Breastfeeding is well known for its benefits to infants and to mothers. World Health Organization has recommended exclusive breastfeeding for the first 6 months of life. However, many working mothers especially have problem to continue breastfeeding once they resume working, thus both the infants and mothers do not get the benefits of exclusive breastfeeding. This problem also occurs among non-working mothers. There are many factors for a successful lactation. Among the factors are maternal experience, parity, mode of delivery, shape of the breast/nipple, medical illness, stress, motivation, social support, infant's behaviour and suckling activity. Stress has been shown to inhibit lactation. On the other hands, lactation also can promote good feelings in mothers and helps the mother to cope with stress better.

What is the purpose of this study?

The purpose of this study is to determine the effect of perceived stress on lactation and correlate this with hormones such as prolactin, oxytocin and cortisol. We want to see whether perceived stress reduces the hormones that are important to maintain successful lactation and leads to lactation failure. On the other hand, we also want to see whether these hormones will help mothers in coping with stress better.

What are the procedures to be followed?

Mothers will be recruited during their postnatal check-up and baby's immunization schedule. Mothers do not have to change her infant's feeding pattern, mothers have to give breastfeeding as usual. No restriction in diet or activity. On the day of visit which is monthly from 1st month until 4th month, mothers will be interviewed, will be asked to complete DASS questionnaire (42-item questionnaire of three self-report scales to measure negative emotion) and blood will be withdrawn for hormonal assay. Mothers also have document their amount of expressed breast milk of first morning feeding daily in a breastfeeding diary. And baby's weight is noted from the routine check-up.

Who should not enter the study?

- Mother who has not consented
- Mother who is illiterate
- Mother with twin infants
- Mother with medical condition which require prolong treatment. E.g. Hypertension, Diabetes Mellitus, Hyper-or Hypothyroid, mental illness etc.
- Mother who give supplement formula feeding to infant even for one feeding per day

What will be benefits of the study:

(a) to you as the subject?

- get free breastfeeding support and counselling should any problem arise
- get to find the factors of lactation failure should it happen
- get to help future mothers and infants with new knowledge from the study

(b) to the investigator?

-get the data and evidence to help medical professionals in lactation counselling

-help to promote breastfeeding among mothers

-for publication and presentation

What are the possible drawbacks?

There is no drug administration or any changes in lifestyle or daily activity. Only 10mls of blood is required from the mothers on each visit and it is relatively no negative effect to the mother and infant health.

Can I refuse to take part in the study?

Mothers who refused should inform the investigator earlier and to give reasonable explanation.

Who should I contact if I have additional questions during the course of the study?

Doctor's Name: Dr Nik Raihan Hj Mohamed Tel: 0122710688

APPENDIX G

LAMPIRAN MAKLUMAT PESAKIT

Sila baca maklumat di bawah dengan teliti, sebarang persoalan dialu-alukan untuk berbincang dengan doktor anda.

Tajuk Projek

Kesan tekanan terhadap penyusuan susu ibu di kalangan ibu-ibu yang bekerja dan tidak bekerja di Malaysia.

Pengenalan

Penyusuan susu ibu banyak memberi kebaikan kepada ibu dan anak. Organisasi Kesihatan Dunia (WHO) telah menyarankan penyusuan susu ibu secara eksklusif selama 6 bulan pertama. Walaubagaimanapun, kebanyakan ibu-ibu bekerja mengalami masalah untuk meneruskan penyusuan susu ibu setelah kembali bekerja, maka ibu dan anak tidak mendapat kebaikan susu ibu secara eksklusif. Masalah ini juga dapat dilihat dikalangan ibu-ibu yang tidak bekerja (suri-rumah sepenuh masa). Terdapat banyak faktor untuk menjayakan penyusuan susu ibu. Antara faktor-faktor tersebut adalah pengalaman ibu, bilangan anak, cara kelahiran, bentuk buah dada/puting, penyakit ibu atau anak, tekanan, motivasi, sokongan sosial dan cara penyusuan anak. Tekanan boleh mengurangkan penyusuan susu ibu dan menyebabkan kegagalan untuk menyusukan bayi bagi tempoh yang lama. Selain daripada itu, penyusuan susu ibu juga boleh mempromosikan perasaan positif kepada para ibu dan membantu ibu-ibu untuk menangani tekanan dengan lebih baik.

Apakah tujuan kajian ini dilakukan?

Kajian ini dilakukan bertujuan untuk mengkaji kesan-kesan tekanan terhadap penyusuan susu ibu dan mengaitkan kesan ini dengan hormon-hormon seperti prolaktin, oksitosin dan kortisol. Kami ingin melihat sama ada tekanan yang dialami boleh menyebabkan kekurangan penghasilan hormon-hormon yang

penting untuk menjayakan penyususan susu ibu. Pada masa yang sama, kami juga ingin melihat sama ada hormon-hormon ini membantu para ibu dalam menangani tekanan dengan lebih baik.

Apakah yang diperlukan/dilakukan dalam projek ini?

Kajian ini melibatkan para ibu yang dating ke klinik kesihatan untuk pemeriksaan selepas bersalin dan untuk pengambilan suntikan imunisasi. Para ibu tidak perlu menukar cara penyusuan anak, ibu hendaklah member susu ibu seperti biasa. Tidak ada perubahan diet atau aktiviti yang perlu dibuat. Pada hari lawatan ke klinik iaitu setiap bulan bermula bulan pertama sehingga bulan ke-empat, para ibu akan ditemuduga, perlu mengisi borang soal-jawab DASS (42 soalan skala untuk mengukur emosi negatif) dan darah akan diambil sebanyak 10 ml untuk ujian hormon. Para ibu juga dikehendaki untuk memerah susu pertama pada setiap pagi dan mencatatkan jumlah perahan susu di dalam diari susu ibu. Peningkatan berat badan bayi juga dicatatkan daripada rutin pemeriksaan kesihatan.

Siapa yang tidak perlu mengambil bahagian dalam kajian ini?

- Ibu yang tidak member keizinan untuk menyertai kajian
- Ibu yang tidak tahu membaca
- Ibu yang mempunyai anak kembar
- Ibu yang mempunyai masalah kesihatan yang memerlukan rawatan berpanjangan seperti darah tinggi, kencing manis, penyakit tiroid, masalah mental dan sebagainya.
- Ibu yang member susu formula kepada bayi (bukan penyusuan secara eksklusif)

Apakah kelebihan kajian ini:

(a) kepada para ibu sebagai subjek?

- mendapat sokongan penyusuan susu ibu dan kaunseling
- mengetahui faktor-faktor yang menyebabkan kegagalan penyusuan susu ibu
-dapat membantu para ibu dan bayi dengan ilmu baru yang dapat diperolehi daripada kajian ini

(b) kepada pengkaji?

- mendapatkan data untuk membantu pakar perubatan dalam member kaunseling penyusuan susu ibu
- membantu mempromosikan penyusuan susu ibu dikalangan ibu-ibu di Malaysia
- untuk pembentangan dan penerbitan

Apakah risikonya?

Tiada. Kajian yang dijalankan tidak melibatkan pengambilan ubat-ubatan atau perubahan gaya hidup / aktiviti harian. Kajian hanya memerlukan pengambilan darah sebanyak 10ml daripada ibu pada setiap lawatan dan tiada kesan negative terhadap kesihatan ibu dan anak.

Adakah saya perlu mengambil bahagian?

Penyertaan adalah secara sukarela. Anda tidak perlu memberikan alas an jika anda tidak mahu menyertai kajian ini. Anda juga boleh menarik diri pada bila-bila masa dan perlu memberitahu pengkaji.

Jika ada sebarang pertanyaan bolehlah menghubungi :

Dr Nik Raihan Hj Mohamed

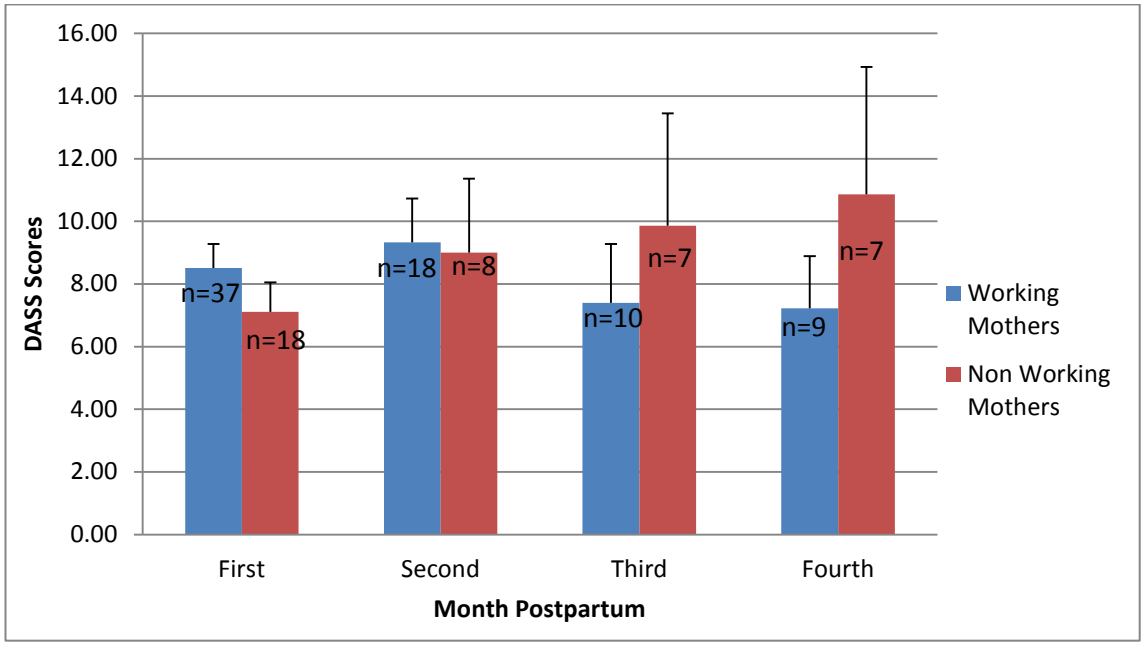
Jabatan Fisiologi

Fakulti Perubatan

Universiti Malaya

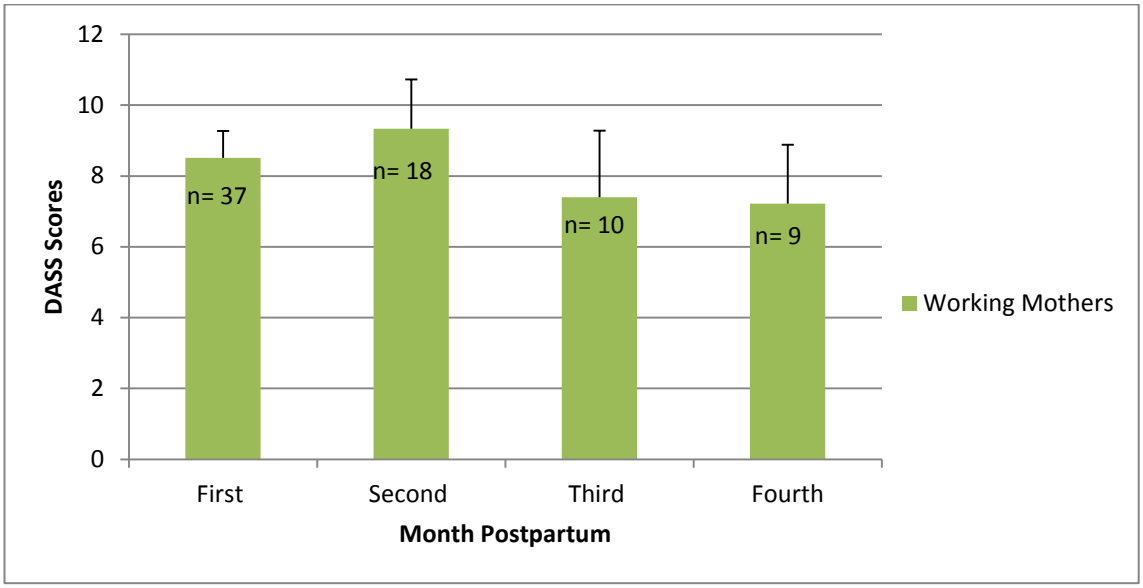
Tel: 0122710688

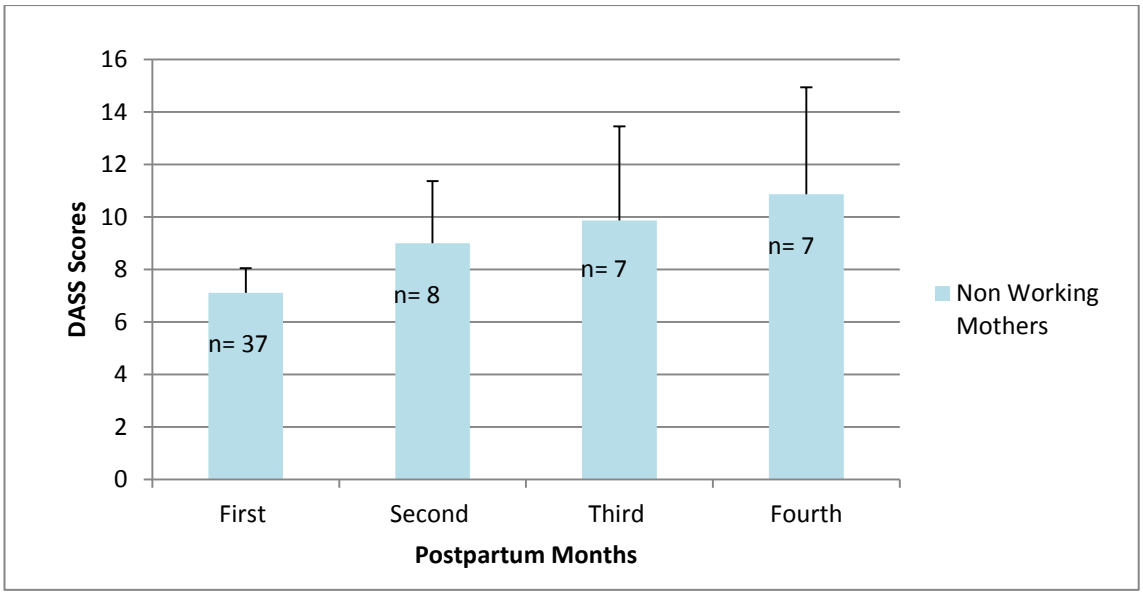
APPENDIX H



Comparison of mean ± S.E.M stress score (DASS) between working and non-working mothers for four monthly visits

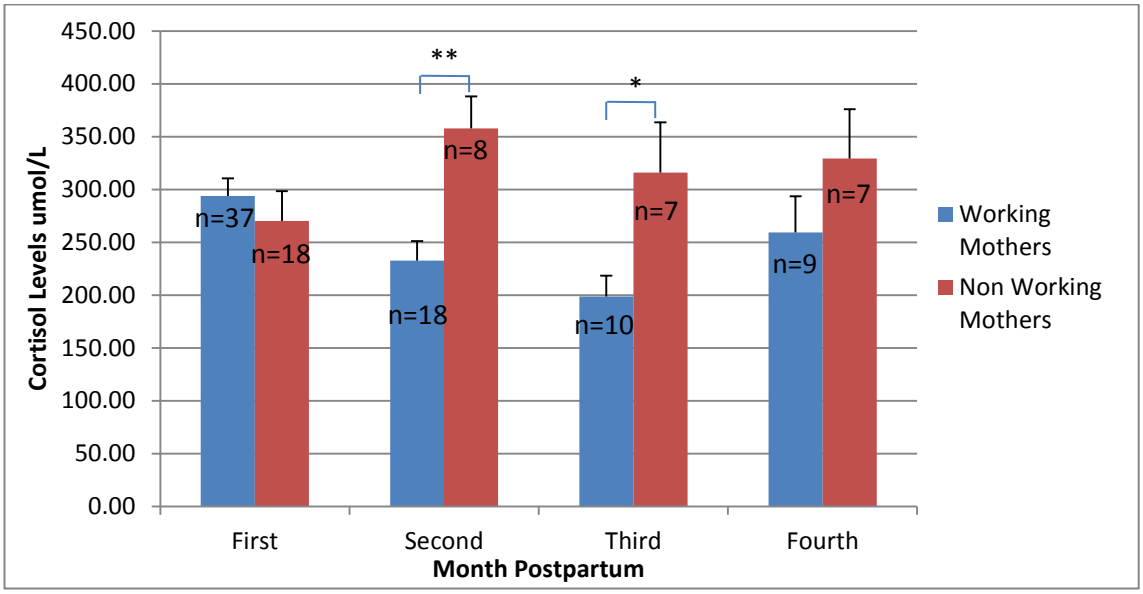
APPENDIX I





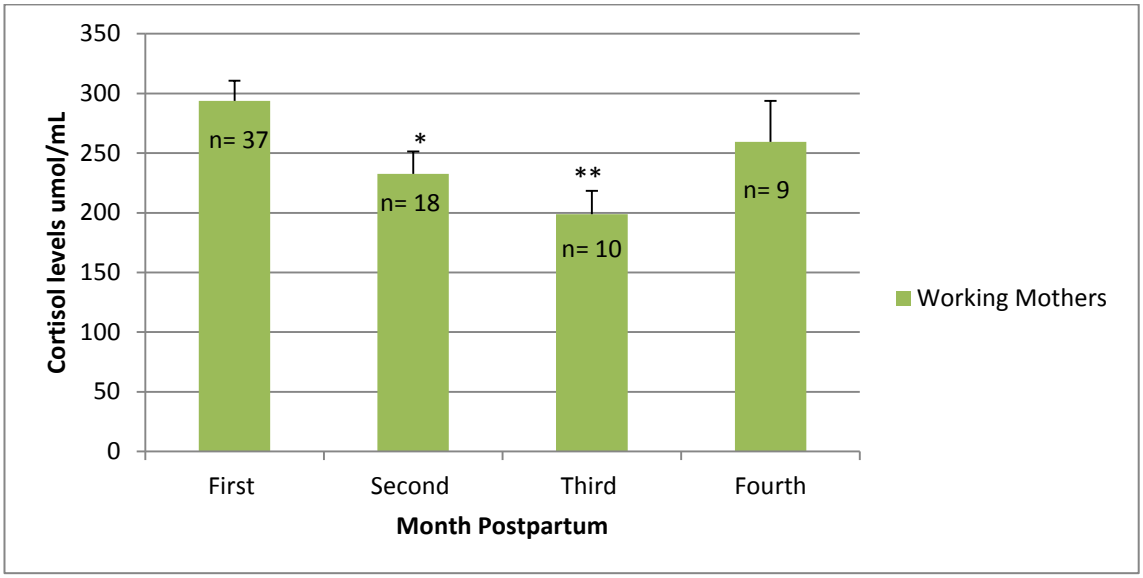
Mean stress scores in working and non-working mothers from first to fourth months of postpartum

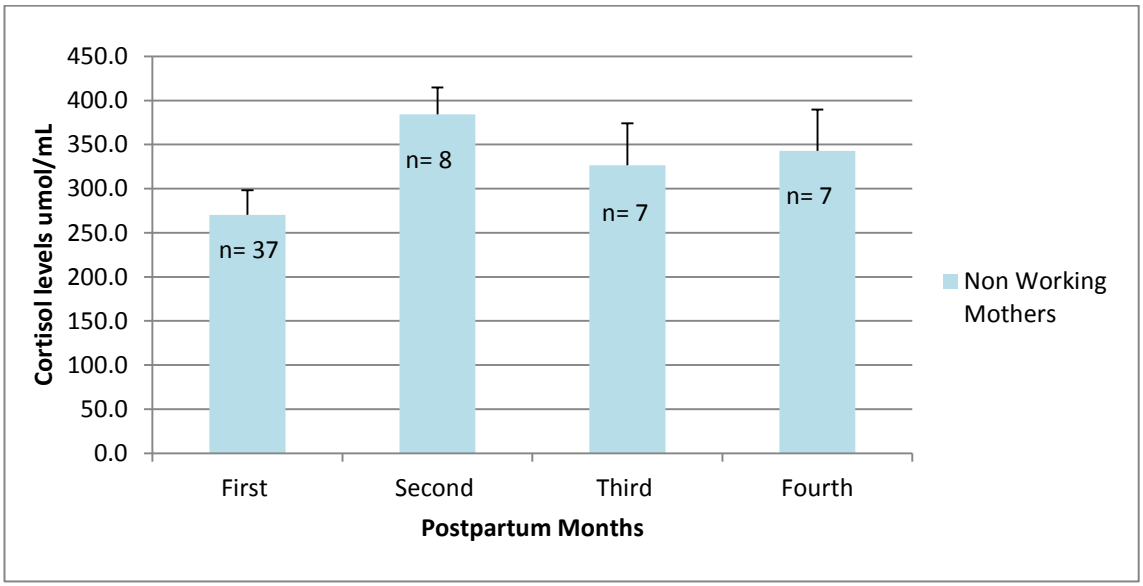
APPENDIX J



Comparison of mean ± S.E.M serum cortisol between working and non-working mothers for four monthly visits * = $P \leq 0.05$, ** = $P \leq 0.01$

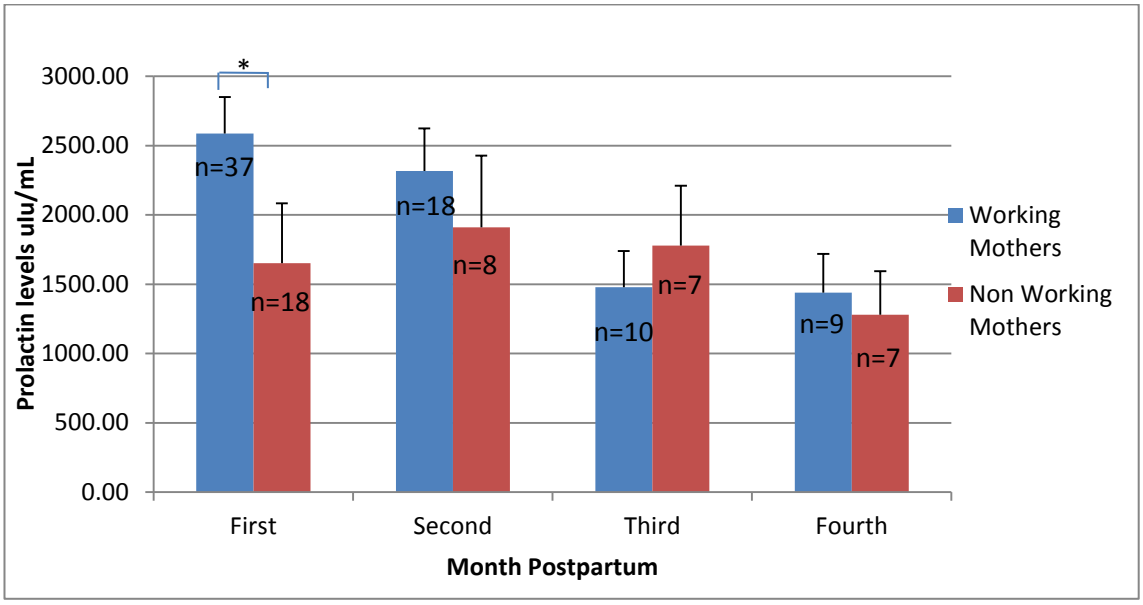
APPENDIX K





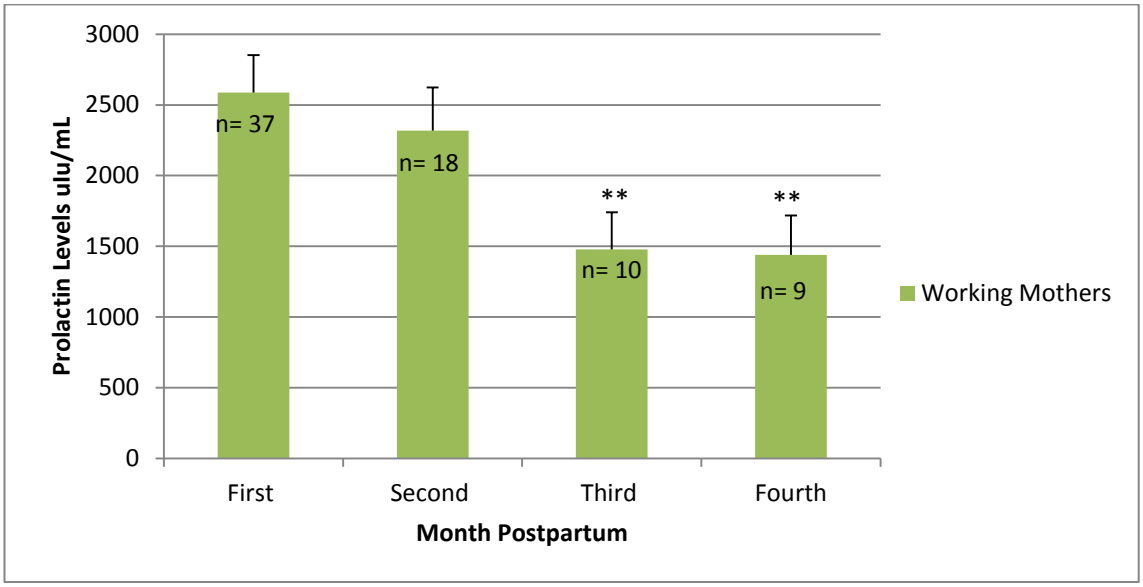
Mean serum cortisol in working and non-working mothers from first to fourth months of postpartum * = $P \leq 0.05$, ** = $P \leq 0.01$ compared to first month postpartum

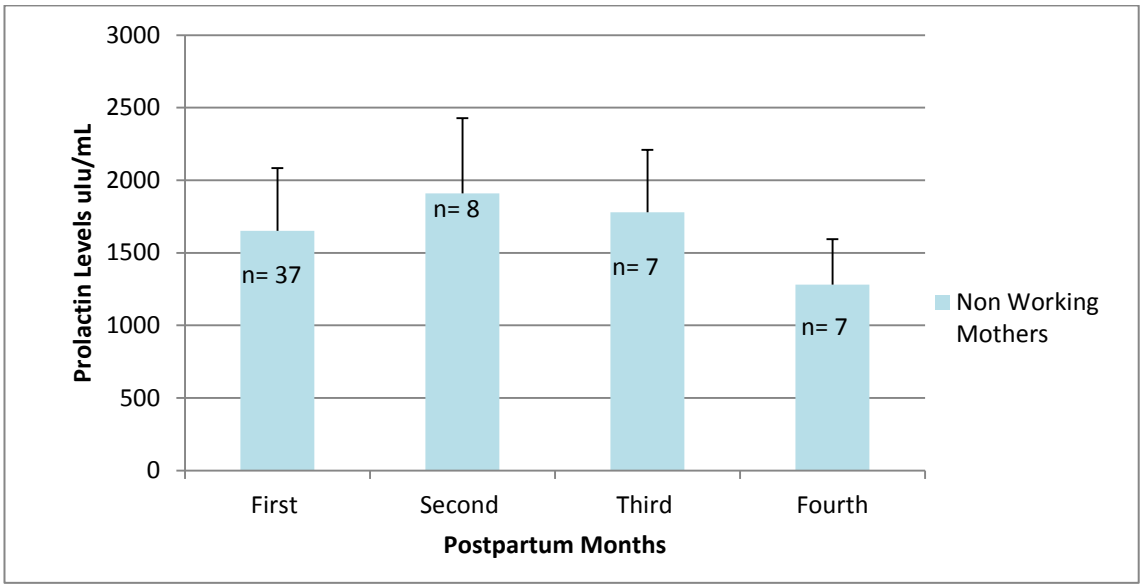
APPENDIX L



Comparison of mean ± S.E.M serum prolactin between working and non-working mothers for four monthly visits * = $P \leq 0.05$

APPENDIX M





Mean serum prolactin in working and non-working mothers from first to fourth months of postpartum. ** = $P \leq 0.01$ compared to first month postpartum

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