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環境職業生殖危害（V）—液晶顯示器製造女性員工生殖內分泌研究

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計畫主持人：陳保中

計畫參與人員：黃耀輝、黃家昵、謝瑞豪

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摘要

在國際上已有許多的研究，利用偵測每天尿中荷爾蒙代謝物來評估女性生殖健康及生育能力。在先前的研究中，已利用偵測每天尿中荷爾蒙代謝物的方法証明職場上的壓力、抽菸、喝酒及職業方面對生殖系統上的影響。利用此評估方法，我們可以用來測量月經週期長度、濾泡期長度、黃體期長度、排卵狀況以及荷爾蒙濃度的差異，藉此我們可以評斷暴露物質危害標的器官。

本研究為一橫斷性研究，對國內液晶顯示器製造廠做一生殖內分泌的研究，評估液晶顯示器製造業女性員工職場暴露對生殖系統的影響。月經功能是以問卷調查來量測。生殖內分泌方面，除了問卷資料及日誌並利用每天收集的尿液，偵測尿中荷爾蒙代謝物來評估。在暴露評估方面，我們使用直讀式儀器針對無塵室三個區域做一個全面性的偵測，並利用揮發性有機化合物不鏽鋼筒被動採樣作 24 小時空氣採樣。並針對整個廠區，距離所有機台及設備 30 公分處作一電磁場強度的測量。

於 2003 年 10 月開始進行問卷調查，總共有 256 女性員工完成問卷我們利用相關資料篩選出合適的研究對象加入後續的生殖內分泌研究當中。除了已停經、懷孕、正哺餵母乳及，有 112 位女性員工同意加入生殖內分泌研究。另外，研究對象會收集每天起床後的第一泡尿液並收集日誌，為期約 1.5 個月經週期，於 2003 年 10 月至 2004 年 4 月間完成。利用偵測尿中荷爾蒙代謝物以評估其月經功能及生育力。促濾泡成熟激素（follicle-stimulating hormone, FSH）、雌激素（estrogen）及黃體激素（progesterone）為我們主要探討的荷爾蒙物質。尿中 FSH、estrogen 及 progesterone 之代謝物以酵素連結免疫吸附分析法（enzyme-linked immunosorbent assay, ELISA）來分析。之後利用分析日誌中月經來潮的資料及尿液中荷爾蒙代謝物質的濃度，評估月經週期中濾泡期及黃體期的長短、月經週期是否排卵及荷爾蒙濃度的差異。

結果顯示，模組區的女性員工於濾泡前期，尿中促濾泡成熟激素及雌激素的代謝物濃度比起其他暴露組來得高。於線性迴歸分析中，相對於面板區，模組區的女性員工於早期濾泡期，尿中促濾泡成熟激素及雌激素的代謝物濃度較高($\beta = 0.08$, 95% CI = 0.00, 0.16; $\beta = 2.38$, 95% CI = 0.25, 4.51)。由此結果推論，模組區女姓員工暴露於較高濃度多重化學物質，造成卵巢功能低落。因為卵巢功能低落，需要更多促濾泡成熟激素刺激濾泡成熟。而促濾泡成熟激素在濾泡前期主要刺激濾泡中細胞分泌雌激素，所以較高濃度的促濾泡成熟激素亦會帶動雌激素的上升。

關鍵詞：雌激素、促濾泡成熟激素、不孕症、液晶螢幕顯示器製造、黃體激素、月經

Abstract

Assays of prospectively collected daily urine samples for metabolites of reproductive endocrine such as follicle stimulating hormone (FSH), estrogens, and progesterone have

been used in much epidemiologic studies to assess ovulatory status, timing of ovulation and menstrual function. Previous epidemiologic studies have examined the effects of phychological stress in work place, smoking, and occupation on menstrual function by using daily urinary hormone metabolites. In this study, we used this tool to estimate the length of segment, ovulatory status, and hormone levels. By the assessment, we could determine the target organs of the occupational exposure.

The objective of this cross-sectional study was to assess the potential reproductive endocrine effects of occupational exposure in the LCD manufacturing. Menstrual function was estimated by questionnaire. Reproductive endocrine was evaluated by detecting the concentration of reproductive hormonal metabolites in urine, either questionnaire or daily diary. Furthermore, we used handheld volatile organic compound (VOC) monitor and 24 hours canister sampling to assess potential chemical exposure. Electric and magnetic field exposure data were collected from an EMDEX meter.

The study population consisted of female employees in a LCD plant in Taiwan. About 256 female workers have completed the questionnaire during the end of 2003. They were screened by a face-to-face interview to identify those who were eligible and willing to collect and freeze urine samples daily after waking for up to 1.5 menstrual cycles. Finally, urine samples of 96 subjects were included in urinary hormone analysis. Participants completed a detailed baseline questionnaire. We assayed FSH, E_1C (estrone conjugates) and PdG (pregnanediol-3-glucuronide) by ELISA. We also obtained reproductive and exposure information from baseline questionnaire and daily diary.

After adjusting effects of factors on hormonal excretion, E1C level of the female workers in module group still had a significantly increased (2.38 ng/mg Cr, 95% CI: 0.25, 4.51) compared with the female workers in panel group in early follicular phase. FSH level of the women in module group had a little significantly increased (0.08 mIU/mg Cr, 95% CI: 0.00, 0.16) adjusted by effects compared with the women in panel group in early follicular phase.

The possibility is that multiple chemical exposures may diminish ovarian oocyte reserve or induced ovarian failure. Furthermore, shorten follicular phase may lead to shorten menstrual cycle. It is consistent with the finding of our previous study.

Keywords: estrogens; follicle stimulating hormone; infertility, female; liquid crystal display manufacturing; luteinizing hormone; menstruation; progesterone

Introduction

In our previous study, we found an increased frequency of shorter menstrual cycles among the women working in module process. But, we have not found out the effects of occupational exposure. Further, using daily urine metabolites of sex steroid hormones may allow us to obtain detailed menstrual function data and assess the potential reproductive effect of occupational exposure in LCD manufacturing.

The length and regularity of menstrual cycles reflect changes in ovarian steroid production. In addition, ovarian hormone excretion is critically important to several aspects of women's health. In general, shorter cycle have been linked to earlier menopause and to high risk of breast cancer. However, self-reported bleeding patterns cannot distinguish ovulatory and anovulatory cycles or timing of ovulation in ovulatory cycles. Menstrual cycle length may be misclassified if self-reported information is used alone.[1] Ovulatory and anovulatory menstrual intervals and different phases of ovulatory cycles[2] may relate to risk factors in environment or occupation. Thus, assays of prospectively collected daily urine samples for metabolites of FSH, estrogen and progesterone have been use in epidemiology studies to assess ovulatory status and timing of ovulation.

Previous epidemiologic studies have examined the effects on menstrual function by using daily urinary hormone metabolites. By use of urinary hormone metabolites as proxy markers of circulating gonadotropin and sex steroid hormones, it was possible to determine the window of fertility in a women's cycle, as well as to described the effects of psychological stress in the workplace,[3] smoking,[4] caffeine consumption,[5] and occupation[6] on hormone patterns. Because of instability of urinary metabolite of LH, we didn't choose urinary metabolites of LH to evaluate female fertility in this study.

In this study, we have focused on the effects of some important exposure examined from air sampling on the female reproductive endocrine system using a cross-sectional study of women ages 19-45 from the LCD manufacturing in Taiwan. The purpose of this study was to assess the potential effects of occupational exposures on menstrual cycle function. We monitored daily urinary specific endocrine end points that are predictive of menstrual cycles as subclinical markers of female reproductive dysfunction to identify early, subtle reproductive effects of low-dose exposures.

Material and Methods

Research design and population

This study was a cross-sectional study. This study used urinary metabolites of sex steroids to explore the associations between menstrual function and several environmental and

occupational exposures of a LCD manufacturing female employees. We examined the relation of these hormone patterns to more easily obtained characteristics of the menstrual cycle. The study provided the opportunity to identify predictors of ovarian steroid levels that may be related to adverse health outcomes.

The study population consisted of a LCD manufacturing female employees worked in fabs. During the end of 2003, they were screened by a face-to-face interview and a detailed baseline questionnaire to identify those who were eligible and willing to collect and freeze urine samples daily after waking for up to 1.5 menstrual cycles. Eligibility criteria include who were not premenopause or menopause and requirements that the subjects had not used steroid hormones (which interfere with urinary hormone assays), oral contraceptives or hormone replacement for three months; had not been pregnant or breast-feeding for three months; had no history of female uro-genital diseases.[7] 0f 297 women, 41 didn't complete face-to-face interview and the detailed baseline questionnaire, and 175 agreed to participate. Of nonparticipants, 11 were pregnant or lactating, and 47 were premenopause or menopause. They provided informed consent and participated by maintaining daily diaries and collecting daily urine samples. Daily diary was used to obtain critical daily information that might change over time. It contained very few questions, usually focusing on menstruation lifestyle, and work pattern. Of participants, 63 dropped out during urine collection, leaving 112 women who completed urine collection. Of these women, 11 subjects were over 45 years old, 2 subjects had use hormone therapy in the duration, and 3 subjects didn't recorded the menses in the diary. They were not included.

Initial participant interview and questionnaire

During the initial interview, we explained the study procedures, eligibility criteria, and we obtained informed consent. Next, we administered the baseline questionnaire to collect information including age, working history, income group, marital status, education, lifestyle factors, an estimation of the usual sleep duration, the number of previous pregnancies, and reproductive and menstrual histories. Lifestyles are such as occupational activity, sports activity, smoking, alcohol and caffeine consumption and special diet. We also collected information of subjective symptoms about visional, hearing, respiratory, cardiovascular, urinary, reproductive, digestive, blood, nervous, dermal, muscle and skeletal systems. Furthermore, we used the Chinese Health Questionnaire for screening psychosocial stress. According to the validation studies of the Chinese Health Questionnaire, a score greater than or equal to four indicated some overt psychiatric symptoms, and so we will categorize males into low (0), middle (1-3), and high ($\geq$4) stress level.[8]

Exposures Assessment

We previously demonstrated that relatively low doses of solvents in this manufacturing could be measured with greater sensitivity from environment than in blood or urine. Therefore, we estimated exposures by 8-hour personal air sampling for PGME, propylene glycol PGMEA, MEA, NMP, and isopropanol. The measurements were personal samples placed on the shoulder of the study subjects at the start of the work shift. Measurements were taken for at least eight hours. The active sampling equipment for PGME, PGMEA, NMP, and isopropanol consisted of a charcoal tube (SKC Lot 120, part. No. 226-01) connected to a pump via a plastic tube with airflow through the charcoal tube which is in acceptance with the NIOSH protocol (NIOSH, 1994). And then desorption was via carbon disulfide (CS_2) and analyzed by gas chromatography. MEA was collected on silica tubes, desorption via methanol, analyzed by gas chromatography, FID. Sampling pumps calibrated to a flow rate of 150ml/min, depending on the anticipated concentration of chemicals and shift length. The pumps were pre and post calibrated every time. Field blanks were collected throughout the course of the exposure evaluation for quality control purpose. We evaluated every working area. There were 10 areas among three processes. Etching, chemical vapor deposition (CVD), and photolithography were in array process. Polarizer attachment, rubbing, diffusion and LC injection were in panel process. Testing, COG process and cell assembly were in module process. Moreover, we used handheld volatile organic compound (VOC) monitor, photoionisation detector (PID) with a dynamic detecting range between a few ppb and 200 ppm, to measure the concentration of total VOC in fab. We also examined VOCs by canister sampling. Canister sampling followed by condensation by liquid nitrogen and GC-MS analysis was used as a standard method for Hazardous air pollutants.

Intensity of electric and magnetic field were characterized on the same days using EMDEX II meters. We measured every kind of machine to evaluate the intensity of electric and magnetic field. There was 30 centimeters at a distance from the machine.

Due to the difference floor of the three processes, we divided all the subjects into three groups by their work area. Because women were usually exposed to multiple chemical agents, chemicals were also examined in functionally similar work area.

Urine samples collection

We asked subjects to begin maintaining their diaries and collecting urine samples from the day 16 of their latest menstrual cycle through the first day of their second post-interview menstrual period. All subjects provided with a toilet-type urine catch kit with a box containing forty-nine 15 ml plastic relabeled tubes and instructions for collection and cool storage of urine. Tubes were placed by the subject in monthly boxes with a calendar in the

bottom, to ensure that the tubes were placed correctly in box. They collected urine at the first urine void after waking each day for a period of 1.5 menstrual cycles and store the samples without preservatives in home freezers (at about -20℃) until the end of the collection. All urine samples amassed in freezer packs at the plant and then were delivered to the laboratory. We stored samples at -20℃ until assay.

Concurrent with urine collection, the date and the time of sample collection were collected. The subjects recorded the amount of vaginal bleeding (number of pads or tampons used per day) and other information during the menses in the diary. The diary was also included menstrual and sample collection information, alcoholic beverage consumption, coffee and caffeine consumption, second-hand smoke exposure, hours worked, shift time, hours slept, sexual intercourse, physical activity at home and work.

Determination of menstrual endpoints and phase characteristics

Subjects were instructed to mark on the label of the urine sample collection after the beginning of what they think was a menstrual period, and this was generally considered to be the first day of a menstrual cycle. Since a woman may begin bleeding after collection the day's urine sample, cycle start dates were adjusted 1 day earlier if a woman reports bleeding on her diary (>1 pad/day) on the before the flagged sample. Retrospectively reported menses was accepted up to 14 days after data collection for participants with missing diary menstrual bleeding entries.

We used urinary endocrine measurement and menses dates to derive the end points using established algorithms. We estimated the day of ovulation for each cycle (designed luteal day 0) by the urinary FSH peak and by the concurrence of the day of or the day after the midcycle E1C peak.[9] The menstrual cycle length was defined as the number of days from day 1 of menses to the day before day 1 of the next menses. The follicular phase length was defined as the numbers of days from day 1 of menses up to and including the day of the FSH surge. The luteal phase length was defined as the difference between the cycle length and the follicular phase. We defined short cycles as 23 days or less, and long cycles as 36 days or more. Long follicular phase length was defined as 24 days or more. Short luteal phase length was defined as 10 days or less. Long menses length was defined as 8 days or more.[10,11]

We reassayed all cycles that are not ovulatory or cycles with an "abnormal" day of ovulation (the day of ovulation is day ≥20 of the cycle). In reassayed cycles with discordant results, the more "normal" assays will be used.

Endocrine measurements

We analyzed urine samples for the estradiol metabolites, estrone sulfate and estrone glucuronide (estrone conjugates [E1C]), and the progesterone metabolite, pregnanediol glucuronide (PdG), and total urinary FSH beta subunit. Such assays have been developed for the urinary metabolites of estradiol (E_1Conj) and progesterone (PdG),[12] and FSH.[13,14] It was under protocols approved by the laboratory of B.L. Lasley, University of California, Davis, CA. We assayed the major urinary metabolites of estrogen and progesterone using competitive enzymeimmunoassay (EIA). We assayed urinary FSH using triple-antibody enzyme-linked immunosorbent assay (ELISA).

E1C and PdG both were indexed to creatinine excretion in the same sample to control for variations in urine volume. E1C and PdG are expressed as nanograms and micrograms per mg Cr, respectively. FSH was also indexed to Cr excretion in the same sample to control for variations in urine volume. Urine samples in which the Cr level is less than 0.2 mg/mL was considered too dilute to yield accurate measurements; levels for these measurements was treated as missing values.

Monoclonal Anti-beta FSH (FS2-4A10-G10) as primary antibody will be purchased from Scantibodies Laboratory (Santee, CA). Polyclonal Rabbit Anti-hFSH-β Sera as Second Antibody was purchased from the laboratory of B.L. Lasley, University of California, Davis, CA. Biotinylated Goat Anti-Rabbit IgG (H+L) Human IgG will be purchased from Bio-RAD (Cat # 170-6401). Lyophilized beta FSH subunit standard will be provided by the National Hormone and Pituitary Program, National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). E1C antibody (R522) and PdG antibody (R13904 PAB) will be obtained from the laboratory of B.L. Lasley, University of California, Davis, CA. E1C and PdG standards will be purchased from Sigma Chemical Company, St. Louis, MO. In FSH assay, each assay plate will consist of seven standards (in duplicate), three quality control (QC) samples (high, medium and low, in duplicate) and up to 24 samples (in duplicate). In E1C assay, there will be six standards (in duplicate), two quality control (QC) samples (high and low, in tetraplicate) and up to 24 samples (in duplicate). In Pd3G assay, there will be eight standards (in duplicate), two quality control (QC) samples (high and low, in duplicate) and up to 24 samples (in duplicate).

Statistical analysis

Using those questionnaire data of eligible women, we first evaluated whether demographic, working or lifestyle factors were associated with menstrual outcomes of interest using univariate analyses. Variables included age, body mass index, education, smoking, alcohol, and caffeine consumption, parity, shift work, and psychosocial stress score. Our base model

included age, body mass index (BMI; [kg] per height squares [m^2]), education and CHQ12. Age and education were divided into two groups and BMI was divided into three groups. Because of rare sample in high level of CHQ12 score, we combined the high level with the middle level. Thus, CHQ12 was two levels such as low and middle or high.

FSH, E1C and PdG excretions were assessed during three different periods; 1) early follicular phase, defined as days 2-4 with day 1 as the first day of menstrual bleeding; 2) periovulatory , defined as ovulation ±1; and 3) luteal phase, defined as the day +5, +7, and +9 after the FSH surge to the day before the next menses.

To take into account the repeated feature of the hormonal measures and the fact that hormonal levels were nested within cycles and cycles within women, we performed mixed linear regression analyses instead of analyses of variance for repeated measures. The analyses were performed using the procedure 'PROC MIXED' of the SAS statistical package (SAS Institute, Cary, NC). All tests were two-sided, and the differences were considered statistically significant at P <0.05. The linear mixed model for continuous measures to account for the intrawomen correlation of outcomes, are appropriate statistical tools to analyze reproductive endocrine data for each participant. Using the linear mixed model, we estimated the effect of risk factors on hormone. Age, education, BMI, smoking, alcohol and caffeine consumption, and psychosocial stress (CHQ12) were considered potential determinants of outcomes and were analyzed as categorical variables. In the literature, BMI[15] and stress[3] were consistently shown to be association with menstrual function. Education and caffeine consumption were neither important in present data nor consistently demonstrated to be important in literature. Thus, age, BMI, smoking, alcohol consumption, and CHQ12 were included in the final models.

Results

The results showed that the average magnetic flux densities for the machines in three processes were between 0.20 mill-Gauss (mG) and 42.00 mG, except that the assembly machine in module process was about 850 mG. The average magnetic flux densities for the main power areas were about 100 mG. Allowed TWA for routine exposure for whole body are 600G. From the measurement of handheld VOC monitor, we found that the total VOC concentrations in some subarea of the module area were very high up to 20 ppm. For investigating the volatile organic compounds besides those compounds used on the processes in fabrication, we used canister sampling to measure the indoor airborne. The sampling was analyzed by Industrial Technology Research Institute of Taiwan. Chemicals or sets of chemicals were selected for examination of potential reproductive effects showed in Table 1. By literature review, we found some studies showed that ethanol, acetone,

toluene, xylene, benzene, and styrene, affect on female fertility or menstrual disorder. Although many chemicals (some highly toxic) were used in array area, airborne concentrations were low or undetectable by standard industrial hygiene methods. From the results, we found that ethanol and acetone were major compounds in airborne in module area. That formed the high concentration of total VOC in module area.

Of the 117 fab women selected for participation, 11 subjects were over 45 years old, 2 subjects had use hormone therapy in the duration, and 3 subjects didn't recorded the menses in the diary. They were not included. A total of 96 women contributed completed cycle of urine samples even though some of them had some missing samples.

Of the female in three groups, the ages of the workers in module groups were older than those in other work areas. Education level in array groups was higher than others. There was no difference in other variance. The work pattern in three groups was almost 12-hours day or night shift. The greater part of those subjects had low psychosocial stress. (Table 2)

Menstrual characteristics showed in Table 3. The average length of cycle, follicular phase, and luteal phase show no difference between three groups. There was no difference in frequencies of short menstrual cycle, long follicular phase and short luteal phase.

The mean (and SD) of endocrine outcomes and working areas between three phases are compared in Table 4. In early follicular phase, we found that E1C level in module group was higher than other groups. And in this group, FSH level was higher slightly than others. In periovulatory phase, PdG level in module group was the highest. In luteal phase, E1C level in panel group was lower than other groups.

Table 5, 6, and 7 present the crude and adjusted association between work groups, confounder and urinary FSH, E1C, and PdG levels. When the linear mixed model used, we found that E1C level of the female workers in module group had a significantly increased (2.81 ng/mg Cr, 95% CI: 0.81, 4.81) compared with the female workers in panel group in early follicular phase. PdG level of the female workers in module group had a little significantly increased (0.11 μg/mg Cr, 95% CI: 0.00, 0.22) compared with the female workers in panel group in periovulatory phase. Compared with the female workers in panel groups, E1C level of the women in array and module groups had a significantly increased (5.24 ng/mg Cr, 95% CI: 0.07, 10.41; 4.35 ng/mg Cr, 95% CI: 0.81, 7.89) in luteal phase. After adjusting effects of factors on hormonal excretion, E1C level of the female workers in module group still had a significantly increased (2.38 ng/mg Cr, 95% CI: 0.25, 4.51) compared with the female workers in panel group in early follicular phase. FSH level of the

women in module group had a little significantly increased (0.08 mIU/mg Cr, 95% CI: 0.00, 0.16) adjusted by effects compared with the women in panel group in early follicular phase. Compared with the female workers in panel groups, E1C level of the women in array and module groups still had a significantly increased (6.70 ng/mg Cr, 95% CI: 1.45, 11.94; 4.35 ng/mg Cr, 95% CI: 0.72, 7.79) adjusted by effects in luteal phase

Adjusted effects of factors on hormonal excretion in early follicular phase and luteal phase are shown in Tables 6 and 7. FSH concentration of women with middle or high level of psychosocial stress had a significantly increased (0.17 mIU/mg Cr, 95% CI: 0.04, 0.31) compared with low level in early follicular phase. In luteal phase, FSH concentration of the women with BMI less than 19 kg/m^2 had a significantly increased (0.05 mIU/mg Cr, 95% CI: 0.01, 0.10) and of the women with BMI over than 24 kg/m^2 had a significantly decreased (-0.03 mIU/mg Cr, 95% CI: -0.13, 0.00). FSH, E1C, and PdG concentration did not vary significantly by age, smoking, and alcohol consumption in three phases.

Discussion

In our previous report, we found that women working in module group were likely to have a short menstrual cycle. In this study, working in module area was found to be associated with a higher urinary FSH and E1C levels in early follicular phase and higher E1C level in luteal phase. There must be potential adverse reproductive health effects in module area. By the air sampling data, adverse reproductive effects of multiple chemicals such as ethanol, acetone, toluene, xylene, benzene, and styrene may alter endocrine excretion and lead to irregular menstrual cycle.

Some studies demonstrated that increasing levels of FSH in the early follicular phase is a characteristic of reproductive ageing or for the determination of diminished ovarian oocyte reserve and ovarian failure.[16,17] There are several studies proposing a relationship between increased FSH as well as reduced follicular phase length.[18] Throughout the reproductive life, there is a steady reduction in the number of newly recruited ovarian follicles. Because these follicles produce inhibin B, a dimeric glycoprotein that inhibits FSH secretion by the anterior pituitary, a diminution in the number of recruited follicles results in raised FSH.[19] During the follicular phase, there is a process of follicle recruitment, growth, and selection as well as synthesis of estrogens. Because FSH plays a central role in this process by stimulating the granulose cells,[20] a rise in FSH might affect follicular phase length, possibly by accelerating follicular development. Some researchers have found a different explanation that earlier start of follicular growth may reduce follicular phase.[21] In summary, these results support the concept that the follicular phase of the menstrual cycle shortens concomitantly with increasing FSH levels. Because, in addition to follicular growth, FSH

stimulates estrogen production in granulose cells, there are changes in estrogen production in relation to increased FSH. One study comparing between women 40-45 and 20-25 years old found higher serum FSH, shorter follicular phase length, and elevated estrodiol.[18] One such study reported a substantial increase in urinary estrone during the follicular phase in older women, who also had higher FSH.[22]

However, in our study, the majority is older than 34 years old in module group. We still found that FSH and E1C levels of women aged 34 years or younger were much higher in the module group in the follicular phase. Thus, we can obviate the effect of age. The possibility is that multiple chemical exposures may diminish ovarian oocyte reserve or induced ovarian failure. Furthermore, shorten follicular phase may lead to shorten menstrual cycle. It is consistent with the finding of our previous study. However, there was no difference in the average of cycle length and in frequencies of short menstrual cycles between three groups. The possible reason is this study not a comprehensive study. We asked for the volunteer not all the eligible female workers to participate. Thus, we might miss the subjects who had irregular menstrual cycle.

One study demonstrated that FSH levels were significantly higher but were high during all three phases of the cycle (early follicular, periovulatory, and luteal phase) in women with low BMI than in those with high BMI.[23] In our study, FSH levels and BMI were associated during the luteal phase. FSH, E1C, and PdG concentration did not vary significantly by smoking, and alcohol consumption in three phases. Cigarette smoking had been suggested to have anti-estrogen effects.[24] MacMahon et al reported lower urinary levels of oestrone, oestradiol and oestriol during the luteal phase of the cycle among the premenopausal smokers compared to non-smokers and ex-smokers and suggested that smoking might reduce luteal estrogen production.[25] Westhoff et al further reported that cigarette smoking was associated with decreased midcycle and luteal phase estradiol levels.[26] In our study, we didn't find the effects of smoking on endocrine excretion. Data from a dietary intervention study showed that alcohol significantly increased plasma and urinary E_2 levels, especially around the time of ovulation.[27] However, there is no association between alcohol consumption and urinary E1C level in our study. We conjecture that subjects might conceal their smoking or alcohol consumption. The possible explanations for these inconformity included inadequate sample size, population differences.

In the early follicular phase, we found that FSH level of the women with middle or high level of psychological stress was higher than the women with low level. Some studies showed that women in stressful jobs had a more than doubled risk for short cycle length (<= 24 days) compared with women not working in stressful jobs.[3] These results support by the

concept that the follicular phase of the menstrual cycle shortens concomitantly with increasing FSH levels. It is consistent with our results. Nevertheless, we didn't found the increasing level of E1C among the women with middle- or high-level psychological stress in our population.

Our study has some important limitation. We restricted our analysis to women who had completed questionnaires and met a strict set of reproductive criteria, which raises concerns about possible biases. Participants are self-selected volunteers, not a random sample of women. A few women who otherwise met the age criteria for inclusion were taking oral contraceptives. However, because oral contraceptives are often prescribed for women who are having menstrual irregularities, our data may underestimate the prevalence of irregular cycles in our population. Selection bias could also have resulted if women chose to participate both because they had menstrual problems and related reproductive outcomes such as infertility and because they experienced the exposure of interest. Although we omitted from analysis women who were currently breast feeding, pregnant, or ended a pregnancy, and they may have had cycles that did not reflect their typical patterns. But, we can ascertain they were pregnable.

Urine collection was a limitation in this study. In previous studies, subjects were either in some kinds of program or gave compensation for time and inconvenience. They were asked to collect daily urine sample at least two menstrual cycles. The method is administered first time in Taiwan. Because of manners, storing urine samples in home freezer is not acceptable readily. The subjects in our study only collected one full menstrual cycle. Therefore, we couldn't compare menstrual and hormonal data from within-cycle. Moreover, the recognition of each subjects of the first day of the menstrual cycle (the onset of menses) may be a bias in this study.

In conclusion, we have a novel finding in this study. Although hundreds of chemicals are used in array area, we didn't find out the potential reproductive effects on the female worker working in this area. On the contrary, the module area considered to be low chemical exposure in general was full of hazards. Further, using daily urine metabolites of sex steroid hormones allowed us to obtain detailed menstrual function data and assess the potential reproductive effect of occupational exposure in this study site. And developing of less onerous methods of sample collection and storage may increase participation rates in further studies.

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Table 1. Air sampling levels (ppb) of volatile organic compound with potential reproductive hazards by three areas in fabrication.

Compound [a]	Array	Panel	Module
Ethanol	281.6	2256.9	1974.9
Acetone	58.9	592.2	2283.2
Toluene	20.8	7.5	11.1
Isopropyl Alcohol	23.8	4.3	81.0
m/p-Xylene	6.9	1.7	1.1
2-Butanone	4.5	2.9	3.5
Ethyl Acetate	2.6	1.8	2.0
Ethylbenzene	4.6	0.6	0.7
Methylene Chloride	0.9	1.5	3.5
Benzene	1.1	0.5	0.7
Acetaldehyde		0.6	1.0
Styrene	0.7		0.1
1-Propanol, 2-methyl-			46.5
1-Butanol	30.1		10.7
Hexane			2.9
Pentane, 3-methyl-			2.1
Pentane, 2-methyl-	1.8		1.8
Methyl Isobutyl Ketone	0.3		
1-Methoxy-2-propyl acetate	130.8		
2-Propanol, 1-methoxy-	36.9		

[a] Analyzed by GC-MS from 24-hours canister sampling.

Table 2. Distributions of demographic and life style characteristics in female workers between three work areas

Characteristics	Array	Panel	Module
Number of workers	23	52	21
Age (years)			
<35	14	34	8
35+	9	18	13
Mean (S.D.)	29.8 (7.0)	31.1 (7.5)	36.3 (7.0)
Education			
College/university	6	4	1
High school	17	48	20
BMI (kg/m^2)			
<19	7	10	4
19-24	10	26	8
>24	6	16	9
Smoking			
Current	2	6	3
Alcohol consumption			
Current	1	2	2
Coffee			
Current	14	18	10
Tea			
Current	11	16	8
Parity			
0	12	23	7
1	8	14	7
2+	3	13	6
Working pattern			
Regular day work	0	2	5
12-h shift work	23	50	16
CHQ12			
Low	17	44	20
Middle and high	6	8	1

Table 3. Distributions of menstrual cycle characteristics in female workers between three work areas

Menstrual cycle characteristics	Array	Panel	Module
Number of workers	23	52	21
Menstrual cycle length			
Mean (S.D.)	31.8 (5.4)	29.9 (4.6)	29.6 (3.7)
<24 days	1	4	2
24-35 days	18	45	17
>35 days	4	3	2
Follicular phase length			
Mean (S.D.)	17.0 (3.2)	16.7 (4.0)	16.1 (4.1)
>23days	1	3	1
Luteal phase length			
Mean (S.D.)	14.7 (3.3)	13.8 (3.2)	14.1 (3.2)
<11days	2	6	4
Anovulation	1	7	2

Table 4. Unadjusted endocrine outcomes: means by three groups and three phases

Endocrine outcome	Array	Panel	Module
Early follicular phase[a]			
Number of data	37	134	78
FSH (mIU/mg Cr)	0.55 (0.49)	0.58 (0.59)	0.72 (0.52)
E1C (ng/mg Cr)	14.87 (10.28)	12.04 (8.87)	22.52 (19.73)
PdG (μg/mg Cr)	0.49 (0.44)	0.55 (0.61)	0.76 (0.98)
Periovulatory phase[b]			
Number of data	49	121	74
FSH (mIU/mg Cr)	0.83 (0.69)	0.83 (0.79)	0.80 (0.72)
E1C (ng/mg Cr)	47.43 (30.55)	54.06 (84.44)	56.07 (36.19)
PdG (μg/mg Cr)	0.68 (0.65)	0.71 (0.70)	1.03 (0.99)
Luteal phase[c]			
Number of data	48	115	70
FSH (mIU/mg Cr)	0.28 (0.22)	0.25 (0.16)	0.28 (0.23)
E1C (ng/mg Cr)	40.04 (21.48)	29.57 (16.52)	47.03 (38.49)
PdG (μg/mg Cr)	3.01 (2.32)	2.95 (2.11)	3.66 (2.82)
Peak FSH[d]	1.42 (0.80)	1.47 (0.96)	1.46 (0.89)
Number of data	16	41	25

[a] Mean hormonal concentration at cycle day2~4.
[b] Mean hormonal concentration at day of ovulation±1.
[c] Mean hormonal concentration at day of ovulation+5,+7,+9.
[d] At the day of ovulation.

Table 5. Associations between work groups, confounders and urinary FSH, E1C, and PdG levels in early follicular phase.

	FSH		E1C		PdG	
	Crude	Adjusted	Crude	Adjusted	Crude	Adjusted
	β (95%CI)	β (95%CI)	β (95%CI)	β (95%CI)	β (95%CI)	β (95%CI)
Area						
Array vs. panel	-0.02 (-0.15, 0.11)	-0.06 (-0.18, 0.07)	1.75 (-1.54, 5.03)	2.10 (-1.29, 5.48)	-0.03 (-0.21, 0.14)	-0.05 (-0.23, 0.13)
Module vs. panel	0.05 (-0.02, 0.13)	0.08 (0.00, 0.16)	2.81 (0.81, 4.81)	2.38 (0.25, 4.51)	0.07 (-0.04, 0.18)	0.07 (-0.04, 0.19)
Age (years)						
35+ vs. <35	0.05 (-0.04, 0.15)	0.05 (-0.05, 0.15)	1.91 (-0.70, 4.51)	1.94 (-0.75, 4.64)	0.07 (-0.06, 0.20)	0.07 (-0.08, 0.22)
BMI (kg/m^2)						
<19.0 vs. 19.0-24.0	-0.04 (-0.17, 0.09)	-0.03 (-0.16, 0.11)	2.04 (-1.69, 5.77)	2.03 (-1.62, 5.67)	0.04 (-0.15, 0.23)	0.05 (-0.14, 0.25)
>24.0 vs. 19.0-24.0	-0.05 (-0.12, 0.02)	-0.05 (-0.13, 0.02)	-0.87 (-2.79, 1.05)	-1.40 (-3.36, 0.55)	-0.05 (-0.15, 0.05)	-0.05 (-0.15, 0.06)
Smoking						
Current vs. none	-0.05 (-0.20, 0.10)	-0.04 (-0.19, 0.11)	0.76 (-3.38, 4.90)	1.72 (-2.32, 5.76)	-0.12 (-0.33, 0.08)	-0.10 (-0.32, 0.12)
Alcohol consumption						
Current vs. none	-0.001 (-0.23, 0.23)	-0.01 (-0.24, 0.21)	-3.31 (-9.53, 2.90)	-3.47 (-9.56, 2.62)	-0.15 (-0.46, 0.16)	-0.15 (-0.47, 0.18)
CHQ12						
Middle and high vs. low	0.15 (0.02, 0.28)	0.17 (0.04, 0.31)	-2.64 (-6.19, 0.92)	-2.22 (-5.77, 1.34)	-0.02 (-0.20, 0.16)	0.02 (-0.17, 0.21)

Table 6. Associations between work groups, confounders and urinary FSH, and E1C levels in periovulatory phase.

	FSH		E1C		PdG	
	Crude	Adjusted	Crude	Adjusted	Crude	Adjusted
	β (95%CI)	β (95%CI)	β (95%CI)	β (95%CI)	β (95%CI)	β (95%CI)
Area						
Array vs. panel	0.002 (-0.11, 0.11)	-0.01 (-0.12, 0.10)	-3.04 (-15.21, 9.13)	-2.39 (-15.04, 10.26)	-0.02 (-0.18, 0.15)	-0.01 (-0.18, 0.16)
Module vs. panel	-0.01 (-0.08, 0.06)	-0.001 (-0.08, 0.07)	1.82 (-6.45, 10.10)	0.95 (-7.74, 9.64)	0.11 (0.00, 0.22)	0.09 (-0.02, 0.20)
Age (years)						
35+ vs. <35	-0.01 (-0.10, 0.08)	0.01 (-0.09, 0.11)	5.78 (-04.39, 15.95)	5.95 (-5.23, 17.14)	0.04 (-0.10, 0.18)	0.06 (-0.09, 0.21)
BMI (kg/m^2)						
<19.0 vs. 19.0-24.0	0.003 (-0.11, 0.12)	0.02 (-0.10, 0.14)	-10.39 (-23.69, 2.90)	-8.15 (-22.20, 5.89)	0.09 (-0.09, 0.27)	0.09 (-0.09, 0.28)
>24.0 vs. 19.0-24.0	-0.01 (-0.08, 0.05)	-0.01 (-0.08, 0.06)	2.40 (-4.79, 9.60)	0.83 (-6.95, 8.60)	-0.05 (-0.15, 0.05)	-0.08 (-0.19, 0.02)
Smoking						
Current vs. none	0.06 (-0.08, 0.21)	0.08 (-0.07, 0.23)	10.44 (-5.31, 26.19)	11.45 (-5.28, 28.18)	0.16 (-0.05, 0.38)	0.19 (-0.03, 0.42)
Alcohol consumption						
Current vs. none	-0.17 (-0.35, 0.01)	-0.18 (-0.38, 0.01)	-6.14 (-26.61, 14.34)	-7.95 (-29.21, 13.30)	0.05 (-0.24, 0.34)	0.02 (-0.27, 0.30)
CHQ12						
Middle and high vs. low	0.08 (-0.04, 0.20)	0.08 (-0.05, 0.22)	0.43 (-13.38, 14.23)	0.34 (-14.22, 14.91)	-0.07 (-0.26, 0.12)	-0.06 (-0.25, 0.14)

Table 7. Associations between work groups, confounders and urinary FSH, and E1C levels in luteal phase.

	FSH		E1C		PdG	
	Crude	Adjusted	Crude	Adjusted	Crude	Adjusted
	β (95%CI)	β (95%CI)	β (95%CI)	β (95%CI)	β (95%CI)	β (95%CI)
Area						
Array vs. panel	0.01 (-0.03, 0.05)	0.004 (-0.04, 0.04)	5.24 (0.07, 10.41)	6.70 (1.45, 11.94)	-0.01 (-0.56, 0.54)	0.06 (-0.52, 0.64)
Module vs. panel	0.01 (-0.02, 0.04)	0.01 (-0.01, 0.04)	4.35 (0.81, 7.89)	4.35 (0.72, 7.97)	0.25 (-0.16, 0.61)	0.23 (-0.16, 0.62)
Age (years)						
35+ vs. <35	-0.002 (-0.04, 0.03)	0.01 (-0.03, 0.04)	0.34 (-4.31, 5.00)	-0.98 (-5.60, 3.63)	0.07 (-0.39, 0.53)	-0.02 (-0.53, 0.48)
BMI (kg/m^2)						
<19.0 vs. 19.0-24.0	0.05 (0.01, 0.09)	0.05 (0.01, 0.10)	-4.08 (-10.18, 2.03)	-5.17 (-11.03, 0.69)	-0.03 (-0.63, 0.57)	-0.11 (-0.74, 0.52)
>24.0 vs. 19.0-24.0	-0.03 (-0.05, -0.01)	-0.03 (-0.13, 0.00)	0.43 (-2.86, 3.73)	-0.13 (-3.36, 3.11)	-0.12 (-0.45, 0.21)	-0.15 (-0.50, 0.20)
Smoking						
Current vs. none	-0.02 (-0.08, 0.03)	-0.01 (-0.07, 0.04)	3.88 (-3.29, 11.05)	4.22 (-2.73, 11.16)	-0.26 (-0.98, 0.46)	-0.23 (-1.00, 0.54)
Alcohol consumption						
Current vs. none	-0.06 (-0.13, 0.01)	-0.06 (-0.13, 0.01)	-6.93 (-16.10, 2.25)	-7.76 (-16.54, 1.02)	0.31 (-0.62, 1.25)	0.38 (-0.60, 1.35)
CHQ12						
Middle and high vs. low	0.01 (-0.04, 0.06)	0.02 (-0.03, 0.06)	-3.09 (-9.29, 3.11)	-4.01 (-10.02, 2.01)	-0.41 (-1.03, 0.20)	-0.39 (-1.06, 0.27)