The Detrimental Effects of Corticosterone Administration on Post-Implantation Embryonic Development in Mice

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Abstract - Stress is a common problem encountered by females that could influence fertility. Several stages of normal reproductive process could be disturbed by stress. An increased in corticosterone (CORT) levels is an indication of stress in rodents. Chronic CORT administration in rats has been reported to induce oxidative stress condition and subsequently resulted in tissue damage in the brain. However, reports on the effects of stress or stress hormones in inducing oxidative stress in early pregnancy are still few. Thus, this study aims to investigate the effects of exogenous CORT in inducing oxidative stress in pregnant mice. Mice were given intraperitoneal injection of 5mg/kg body weight (BW), 10mg/kg BW and 20mg/kg BW CORT from day one to day seven of pregnancy. On day seven of pregnancy, mice were subjected to laparotomy to determine the number of implantation sites and blood were taken to analyze the plasma levels of progesterone and malondialdehyde (MDA) following treatment with three different doses of CORT. Litter size were then counted at birth and compared with the number of implantation sites to determine the resorption rate in the treatment and control group. Results showed that lower number of implantation sites ($p<0.05$), higher resorption rate ($p<0.001$) and lower plasma progesterone levels ($p<0.05$) in CORT-treated groups as compared to control. The plasma MDA levels on the other hand were not significantly different in CORT-treated groups as compared to control. This study concluded that CORT administration is detrimental to the post-implantation embryonic development. However, the plasma MDA levels, one of the biomarker for oxidative stress were not changed, thus suggesting that doses and duration of CORT administration in this study is not sufficient to induce oxidative stress condition.

Keywords: Stress, Corticosterone, Embryo

INTRODUCTION

Stress is an attribute of modern life style. It affects reproductive system that could influence fertility in men and women [1]. Fertility issues on couples have been widely discussed around the world. It has been estimated that in 2002, about 35 to 70 million couples worldwide are infertile and have turned to Assisted Reproductive Technology (ART) to overcome their infertility problem [2]. Several stages of normal reproductive process could be disturbed by stress [3]. It has been reported that early pregnancy loss in human is attributed by maternal stress as indicated by increased cortisol levels [4]. Increased glucocorticoid levels, i.e. cortisol in human and corticosterone (CORT) in rodents are indication of stress [5]. Stress results in enhanced release of glucocorticoid due to activation of sympathoadrenals and hypothalamic-pituitary-adrenal axis [6] Previous study defined "stress" as teratogenic [7] and CORT was the most probable adrenal steroid hormone involved in mouse under stress condition [8]. Exogenous CORT administration during second term of pregnancy in rodents resulted in an increased frequency of totally resorbed litters, where the effect seen is directly proportional to the dose of CORT administration [8]. However, the mechanisms that affect the reproductive system in that study remain unclear. This study aims to investigate the effects of CORT administration in inducing oxidative stress during the first term (first seven days) of pregnancy in mice. It is suggested that the mechanism in which CORT induce its effect is through stress-induced oxidative stress condition [9,10]. It was previously reported that an increase in free radical formation following stressful conditions influences the entire reproductive span in the life of a female which may lead to infertility, arrested embryonic development and spontaneous abortion [11].

MATERIALS AND METHODS

i. Animal Treatment

Female mice (*Mus musculus*) of seven to eight weeks old with an average body weight (BW) between 26-30 gm were housed at 27ºC with 12 hour light-dark cycle. Animals were given food pellets and water *ad libitum* and randomly divided into four groups. Vaginal smear were taken daily and regularly cyclic animals were divided into four different groups. Animals then were mated with experienced male after a proestrous smear. The presence of

vaginal plug was defined as day one of pregnancy [12] . Animals in Group 1 (control) were given intraperitoneal (ip) injection of corn oil [MP Biomedicals, USA] for 7 days from day one to day seven of pregnancy. On the other hand, animals in Group 2, 3 and 4 received daily ip injection of CORT [Sigma Aldrich, USA] at the dose of 5mg/kg BW, 10mg/kg BW and 20mg/kg BW, respectively for 7 days from day one to day seven of pregnancy. The experimental protocol was in strict accordance with regulations and prescribed animal ethical procedures outline by The Medical Research and Ethics Committee of Faculty of Medicine, UiTM.

ii. Implantation Sites and Resorption Rate

The numbers of embryo implanted following corticosterone treatment were determined by counting the number of normal and resorbed embryos [13] in the uterus from laparatomy on day seven of pregnancy. The animals were then sutured and continued their pregnancy towards term. Litter size then were counted at birth and compared with the number of implantations sites; to determine the resorption rate in treatment and control group [3]. Resorption or post-implantation loss was defined as the number of implantation sites (normal embryos and resorbed embryos) minus live fetuses × 100 / number of implantation sites [14].

iii. Sample Collection and Hormonal Measurement

Blood samples were collected during day seven of pregnancy via retro orbital sinus puncture after mice were anaesthetized using Ketamine/Xylazine solution [15]. Plasma was separated by centrifugation (3000rpm, 4ºC for 15minutes) and frozen at -70ºC until progesterone and malondialdehydes (MDA) analysis. Plasma progesterone were measured using the automated Elecsys immunoanalyser (RocheDiagnostics, Mannheim, Germany) .

iv. Determination of Oxidative Stress Biomarker, MDA.

Plasma were processed accordingly for evaluation of MDA levels using the thiobarbituric acid reactive substances (TBARS) method [16]. The absorbance was measured photometrically at 632 nm and the concentration were expressed as nanomoles MDA per gram protein (nmol/g).

v. Statistical Analysis

Data were analyzed using the SPSS package program (SPSS 16.0, Chicago, IL, USA). A Kolmogorov-Smirnov test was used to test the normality of data distribution. Implantation and progesterone data were determined by Anova test. The significance of difference in the resorption rates was tested by a x^2 test (Chi-Square Test). *P* values of < 0.05 were considered statistically significant. Box plots were used to show the median value of plasma MDA levels in all four groups.

RESULTS

i. Effects of CORT on the Number of Implantation Sites

All mice were pregnant and implantation sites were evaluated at laparatomy on day 7. All healthy embryos and resorbing embryos were indicated by gross observation of uterus at laparotomy. The resorbed embryos were identified by their small size and necrotic hemorrhagic appearance and it was compared with that of healthy embryos [17]. Healthy embryos showed normal decidual swelling alongside the right and left horn of uterus. The total number of healthy embryos and resorbed embryos were counted and indicated as the number of implantation sites.

Based on the observation following laporotomy on day 7, none of the mice in control group (Group 1) and in group treated with 5mg/kg BW CORT (Group 2) showed sign of resorbing embryos. On the other hand, in group treated with 10mg/kg BW CORT (Group 3) and 20mg/kg BW CORT (Group 4), a mixture of healthy and resorbing embryos were detected following laporotomy on day 7.

Figure 1 showed that the number of implantation sites were significantly lower in groups treated with CORT at the dose of 10mg/kg BW and 20mg/kg BW ($p<0.05$) as compared to control.

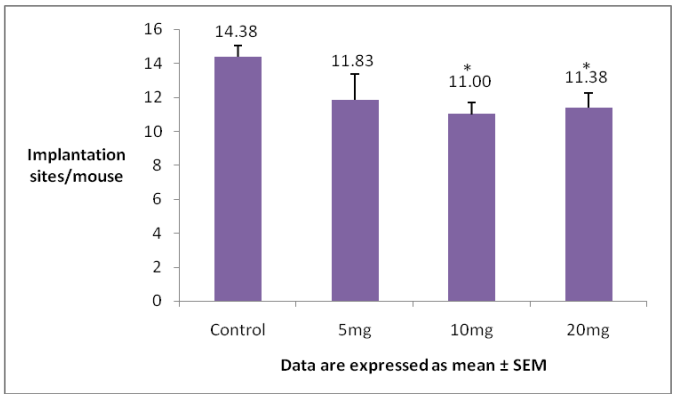

Figure 1: Number of implantation sites in mice following different doses of CORT treatment

Data were expressed as mean ± SEM and analyzed by One-way Anova, *$p<0.05$ significantly different from the control group.

This data showed that CORT administration for the duration of seven days and starting at the dose of 10mg/kg BW is able to reduce the number of implantation sites in the treated mice.

ii. Effects of CORT on the Resorption Rate.

Resorption or post-implantation loss was defined as the number of implantation sites (normal embryos and resorbed embryos) minus live fetuses × 100 / number of implantation sites [14]. Thus, resorption were interpreted either by the presence of resorbed embryos at laparotomy on day 7 or by the lost of fetuses after counting the litter size and compare it with the number of implantation sites. Table 1 shows the effect of CORT on the resorption rate in mice.

Table 1: Resorption percentage in mice following different doses of CORT treatment

Experimental Groups	No. of live fetuses	No. Resorbing Embryos	% Resorption
Control	109	6	5.2
5mg/kg BW CORT	64	7	9.9
10mg/kg BW CORT	39	27	40.9***
20mg/kg BW CORT	55	36	39.6***

x^2 test; (Chi Square Test), ***p<0.001, significantly different from the control group

The resorption rate in pregnant mice was increased following seven days of CORT administration starting at the dose of 10mg/kg BW.

iii. Effects of CORT on plasma progesterone level

Figure 2 showed the effect of CORT on plasma progesterone level at day seven of pregnancy in mice. This figure showed that progesterone levels were significantly lower (p<0.05) only in groups treated with CORT at the dose of 10mg/kg BW as compared to control.

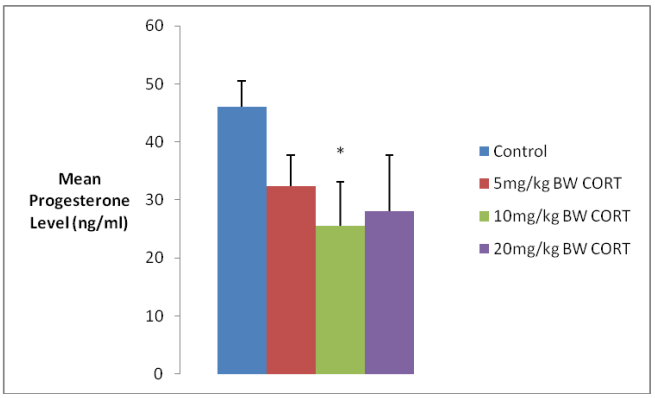

Figure 2: Progesterone level in mice on day seven of pregnancy following different doses of CORT treatment.

Data were expressed as mean ± SEM and analyzed by One-way Anova, *p<0.05 significantly different from the control group.

iv. CORT administration and MDA concentration in plasma

The result of this study was summarized in Figure 3.

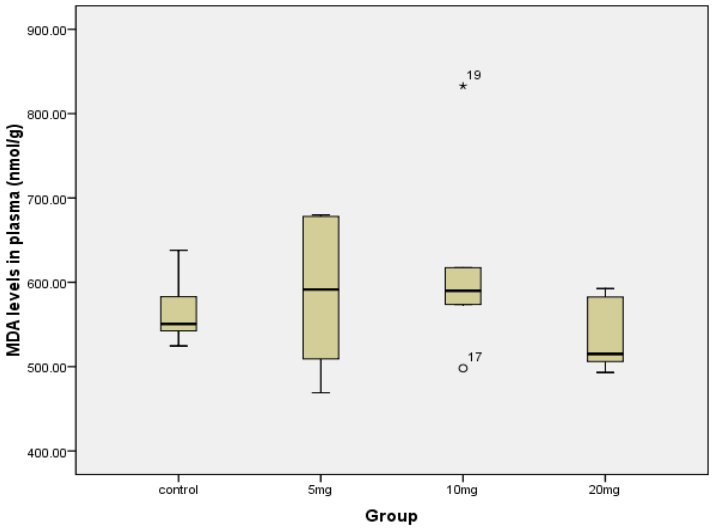

Figure 3: Plasma MDA levels in mice following different doses of CORT treatment.

Data were expressed as median ± SEM.

This figure showed that plasma MDA in all treated groups were not significantly different as compared to control group. This study showed that seven days of CORT treatment is not sufficient to affect the MDA levels in mice.

DISCUSSION

Previous study reported that, CORT administration to postnatal rats affect the reproductive development where it lead to irregular cycle and decreased lordosis quotients and subsequently decreased the sexual response in the treated animals [18]. This study proved that CORT administration adversely affects female reproductive system in rodents.

It has been documented that CORT treatment on day 11 to day 14 of pregnancy in mice induced resorption and resulted in an increased frequency of totally resorbed litters by increasing the dose of CORT from 1.25 mg to 5.00 mg [8]. In that study, different doses of CORT were given and the resorptions were counted by killing the animals on day 17 of pregnancy. The study aimed to determine the incidence of cleft palate in litters, thus CORT were given during second trimester (day 11-14 of pregnancy). Conversely, another study showed that the rate of resorption following injection of CORT given only at day 11 of gestation in hamsters were not significantly different as

compared to control group [19]. This may be due to single injection of CORT which did not affect the resorption rate in pregnant hamsters.

We therefore tempted to examine and compare whether different doses of CORT administration for a period of seven days during early gestation could affect the implantation and resorption rate in pregnant mice. Early seven day of gestation was chosen to avoid totally resorbed litters [8] and to mimick physiological stress during early pregnancy [20]. In our study, laparotomy was done once only (i.e. on day 7) to ascertain that post implantation loss in this study happened between the first trimester towards term. To avoid stress, mice were not subjected to two laparomies during pregnancy although one study proved that two laparomies done on day 8 (first trimester) and on day 14 (second trimester) did not affect the litter size [3].

In our study, it is suggested that the detrimental effect of CORT on the post-implantation embryonic development is related to the reduction in the progesterone levels. Progesterone is a principle hormone required to maintain the pregnancy. Previous study showed that restraint stress for the first six days of pregnancy decreased serum progesterone concentrations [20] and data presented here corroborate that finding i.e elevation of CORT levels following stress exposure during early stage of pregnancy decreased plasma progesterone levels in mice.

However, mechanisms of CORT in affecting the female reproductive system remain unclear. Two studies mentioned that CORT is teratogenic [8, 21] thus, showed its effect on rodents. Whereas Turner & Taylor [18] mentioned that CORT showed its effect on the female reproductive system through the disturbance in pituitary-adrenal function. In addition, CORT administration in experimental animals have been shown to induced oxidative stress condition [9,10] and resulted in tissue damage in the brain [9].

We therefore, tempted to examine the possibility that the mechanism of CORT in affecting the female reproductive system is via the oxidative stress condition. Our study showed that plasma MDA in all treated groups were not significantly different as compared to control group, most probably due to acute duration of CORT treatment (seven days). Previous study showed that MDA levels were affected only after chronic duration of CORT treatment [9, 10, 22].

In conclusion, CORT administration for early seven days of pregnancy is detrimental to the development of post implantation embryos in mice, where it decreased the number of implantations and increased the rate of resorption. However, different doses and duration of CORT treatment in this study were not able to affect the MDA levels.

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