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*Research Article*

# Impact of rapid eye movement sleep deprivation during pregnancy on survival, oxidative status and corticosterone level in offspring of Wistar rats

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**Keywords:**
*corticosterone, fetal programming, physiological adaptions, rapid eye movement sleep deprivation.*

**ABSTRACT**

**Background**: Prenatal exposure to sleep deprivation involves complex communication between the maternal compartment, placenta, and the growing fetus. This study explored the role of rapid eye movement sleep deprivation (REMSD) during different pregnancy stages on survival, blood pressure, corticosterone, oxidative stress indices, and C-reactive protein in sleep-deprived dams and their offspring. **Methods**: To investigate this, 10 pregnant rats (respectively) were subjected to REMSD from gestational day 1 (GD 1) - 20, GD 7 - 20, and GD 14 - 20. After parturition, on postnatal days 1 and 60, blood pressure was determined in dams and offspring, respectively. Serum and heart tissues were collected to determine circulating corticosterone, C-reactive protein, malondialdehyde, and levels of antioxidant enzymes. **Results**: Values of corticosterone, mean arterial blood pressure, and malondialdehyde in the offspring showed comparable patterns of increase observed in their sleep-deprived mothers. Similarly, values of corticosterone, mean arterial blood pressure, malondialdehyde, and antioxidant enzymes in sleep-deprived dams and their offspring presented a positive correlation. Offspring of dams subjected to REMSD were predisposed to prepartal stress. Nonetheless, the offspring of dams exposed to REMSD from GD 1 - 20 revealed the most detrimental effects, following their low number of survivors and birth weight. **Conclusion**: This study identified the potential consequence of continued maternal sleep deprivation-induced stress, sustained exposure to corticosterone, and the corresponding effect it may have posed on the offspring's development.



## INTRODUCTION

Several studies support the importance of sleep, its invigorating and regulatory effects on cognition, oxidant-antioxidant balance, endocrine functions, emotional stability, and the immune system (Ward *et al*., 2017; Kecklund and Axelsson, 2016). Sleep Deprivation (SD) occurs when the accumulated "sleep debt" is not attended to. This increases homeostatic sleep pressure, alters the working processes of sleep, and creates an imbalance in the sleep-wake homeostat (Borbely, 1982). Hormonal mediators, released into circulation to help maintain a continuous active state in SD, can be dysregulated due to their sustained release, hence, compromise the function of the hypothalamic-pituitary-adrenal (HPA) axis and/or the peripheral organs that they stimulate. This is an unfavourable instance to maternal coordination and the developing fetus (Howerton and Bale, 2012).

Pregnancy is associated with several hormonal changes. Sleep disorder, lifestyle, or work schedule-induced SD during pregnancy can cause an additional variation to the release of these hormones. Sleep deprivation is severe life stress where physiological adaptions (the stressor) are less productive; hence, a disruption in bodily functions (Schiavone *et al*., 2013). Impaired regulation of the HPA axis can initiate an increased activation of corticotropin-releasing factor (CRF) firing neuron, an increase in peripheral glucocorticoids (GCs), and a suppression of the immune system (Arnett *et al*., 2016).

In humans and rats, mechanisms (which include an increase in inflammatory cytokines) associated with immunological induction are initiated during SD (Aho *et al*., 2013). Although the placenta protects the fetus from

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an unfavourable maternal environment, the high maternal level of cytokines reaching the placenta can be the basis for a disease state in the offspring (Howerton and Bale, 2012).

The HPA axis and placental circuit have been reported to increase the levels of GCs in maternal and fetal circulations due to maternal stress during gestation (King *et al*., 2001). Glucocorticoids, necessary for the development of various aspects of the fetal brain, freely pass through the placenta and cause modifications in fetal developmental programming when there are vast changes to the mechanisms that regulate the appropriate amount of the hormone released (Howerton and Bale, 2012). Thus, high levels of GCs can alter the placenta's action, and in extension, its communication with the fetus (Howerton and Bale, 2012). Likewise, high levels of pro-inflammatory cytokines in the fetal compartment may well promote oxidative stress and program hypertension in such offspring's later life.

Furthermore, SD in pregnancy cause gestational hypertension, pre-eclampsia, prolonged and preterm labor, increased inflammatory cytokines, and oxidative stress (Owusu *et al*.; Facco *et al*., 2013). In the same vein, maternal SD caused smaller infant birth weight, hypertension, altered renal function, inhibited neurogenesis, impaired neurocognitive performance, and lowered sexual behavior in rats offspring (Aswathy *et al*., 2018; Zhao *et al*., 2015; Lima *et al*., 2014; Alvarenga *et al*., 2013).

Slow-wave sleep (SWS) and rapid eye movement (REM) are the two forms of sleep that can be deprived. However, rapid eye movement sleep deprivation (REMSD) has mostly been practiced in experimental rats because of its consequences on physiological functions. The duration of SD can also influence the severity of alteration and the associated risks in pregnancy. This study investigated the effect of REMSD during varied gestational periods on corticosterone (CORT), blood pressure (BP), oxidative stress indices, and C-reactive protein (CRP) in sleep-deprived dams and their offspring. It also examined the effect of REMSD during pregnancy on a plausible relationship between the measured parameters in the sleep-deprived dams and their offspring.

## MATERIALS AND METHODS

*Rapid Eye Movement Sleep Deprivation*

Sleep deprivation technique was based on muscle atonia that accompanies paradoxical sleep using multiple modified platforms (Argeri *et al*.; Raimundo *et al*., 2016; Lima *et al*., 2014; Thomal *et al*., 2010). A base panel with 20 narrow platforms (6.5 cm in diameter and 10 cm in height) was placed inside a tank (123 x 44 x 44cm) filled with water. The platform protruded 2 cm above the water surface, to enable the animals to move from one platform to another inside the tank. Food pellet containers lowered into the tank just above the platforms were hanging from the tank's cover. Two days before the commencement of rapid eye movement sleep deprivation (REMSD), the animals were adapted to the water tank and platform for 1 hour to avoid unnecessary falls in the water. Throughout the experiment, the experimental room was maintained at an atmospheric temperature of 25.2 - 27.3 $^{o}$C.

*Experimental procedure*

After 4 regular estrous cycles, virgin female rats were housed overnight with males on their proestrus evening. A vaginal smear was taken the following morning; the presence of spermatozoids indicated a positive smear, and the day was defined as day 0 of gestation (Lima *et al*., 2014). Ten (10) animals each were randomly assigned to the following groups: control (CTRL) - housed in their home cages for the entire period of the experiment, SD - submitted to sleep deprivation from gestational day 1 (GD 1) to 20 (SD1), GD 7 to 20 (SD2) and GD 14 to 20 (SD3).

The pregnant females were placed on REMSD platforms in the water tank for 12 hours from 7 am to 7 pm daily, after which they were transferred to their home cages. Also, CTRL animals (in the same room where REMSD was carried out) were transferred to temporary cages to allow for daily cleaning of their home cages. This routine ensured that handling stress was unified across all groups. The procedure was repeated daily until GD 20, when the pregnant rats were returned to their cages, following the REMSD, and moved to the animal facility awaiting parturition. Animals were allowed free access to food and water throughout the entire period of study.

*Offspring groups*

After birth, the mother pups ratio was maintained at a maximum of 1:4 for standardization, since only dams from SD3 had adequate prolificacy among the SD groups (see Table 1). Offspring were left with their mothers for 21 days (Argeri *et al*., 2016). After weaning, separated male and female offspring were categorized as subgroups according to their maternal groups: O-CTRL as offspring of CTRL, O-SD1 as offspring of SD1, O-SD2 as offspring of SD2, and O-SD3 as offspring of SD3 (see Figure 1). Offspring were allowed free access to food and water in their home cages without SD until they were 60 days old. As a result of no or few male offspring across the SD groups (none in O-SD1 and O-SD2), only female offspring were used in this study.

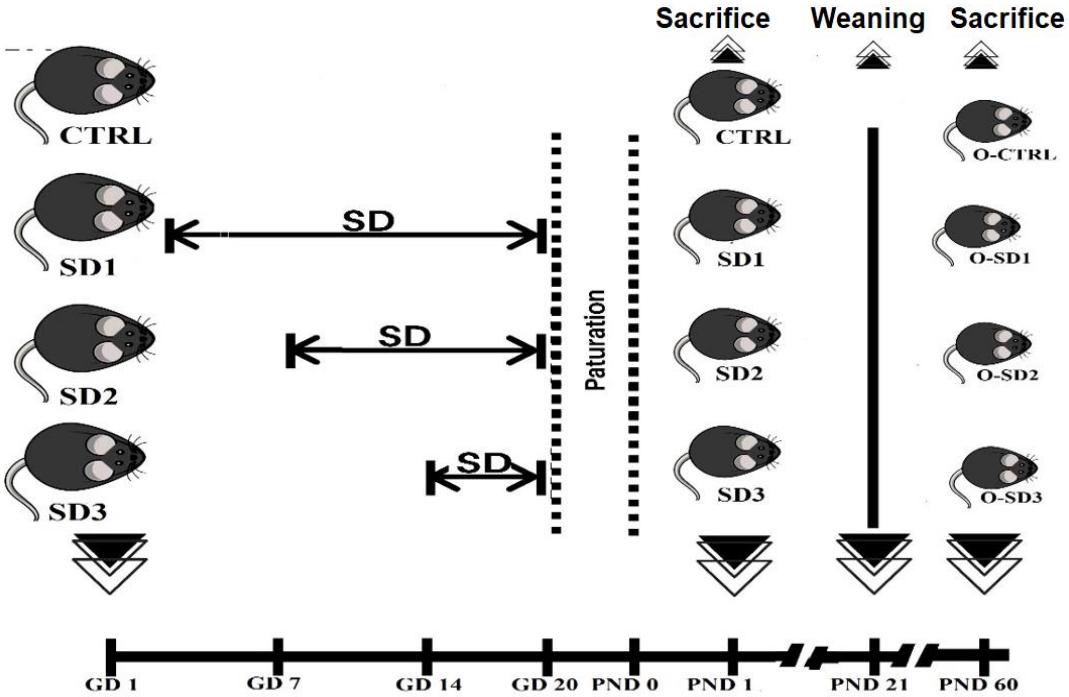



**Figure 1**: Chronological order of animal groupings, rapid eye movement sleep deprivation, and the duration of the experiment. CTRL - control; SD1 - sleep deprivation from GD1 to GD20; SD2 - sleep deprivation from GD7 to GD20; SD3 - sleep deprivation from GD14 to GD20; O-CTRL - offspring of CTRL; O-SD1 - offspring of SD1; O-SD2 - offspring of SD2; O-SD3 - offspring of SD3; SD - sleep deprivation; GD - gestational day; PND - postnatal day.

*Sample collection*

The day of parturition was referred to as postnatal day 0 (PND 0). At PND 1, blood pressure (BP) was measured in the sleep-deprived mothers (n = 4) using the non-invasive tail-cuff method, and they were sacrificed afterward by cervical dislocation. Blood was collected immediately in plain tubes, centrifuged at 3,000 rpm for 10 minutes. The separated serum and harvested heart tissues were stored at -20 $^0$C until when needed for assay. This was done in all the groups. Offspring of the sacrificed mothers were distributed evenly among other mothers of the same group for spontaneous lactation until litter were 21 days old and ready for weaning. At PND 60, BP was measured in offspring using the non-invasive tail-cuff method, and they were sacrificed by the same procedure stated above. Likewise, serum and heart tissues were collected for further analysis. Parameters essayed for include CORT, CRP, malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH).

*Determination of blood pressure*

The non-invasive tail-cuff system and a volume pressure recording sensor (Kent Scientific Software: CODA 4.1) was used for BP determination. Animals were placed in the rear of the holder, and the rear hatch to the holder was secured. The nose cone was in a position to limit the animals from turning around while inside the holder. The animals were allowed about 5 minutes to acclimatize to the holder and also thermoregulate. Cuff placement, the cuff attachment to the CODA controller, and BP determination were carried out as previously described by Daugherty *et al*. (2009). The systolic and diastolic BP were obtained from the CODA spreadsheet after the cycle was completed. Mean arterial blood pressure (MABP) was determined by using the formula: (SBP + 2×DBP)/3 (Daugherty *et al*., 2009).

*Measurement of corticosterone and C-reactive protein*

Corticosterone (CORT) and CRP were measured in serum using the ELISA method according to the manufacturer's instruction and protocol. Commercially available ELISA kits (mybiosource.com, USA, catalog numbers MBS761865 and MBS268328, respectively) were used to determine CORT and CRP levels. Absorbance was measured at 450 nm.

*Determination of malondialdehyde level and oxidative enzymes*

Heart tissues were weighed and homogenized in 5 volumes (by weight of tissue) of 20 mmol sodium phosphate buffer, 120 mmol KCL, and 1:100 PMSF. The

sample was centrifuged at 3000 rpm for 10 minutes. The supernatants were used to determine lipid peroxidation and the respective oxidative enzyme levels. Lipid peroxidation was determined by the formation of thiobarbituric acid reactive substances (TBARS), whereas MDA concentration was calculated using the method of Beuge and Aust (1978). The absorbance was measured at 535 nm.

Levels of SOD and CAT in serum and heart tissues were determined by the methods of Misra and Fridovich (1972) and Sinha (1972), respectively. The exponential disappearance of $H_2O_2$ was at 570 nm. Reduced glutathione (GSH) level was determined using Ellman's method modified by Jollow *et al*. (1974). The absorbance was measured immediately at 412 nm. The GSH contents were calculated using GSH as standard and expressed as µmol $mg^{-1}$ protein.

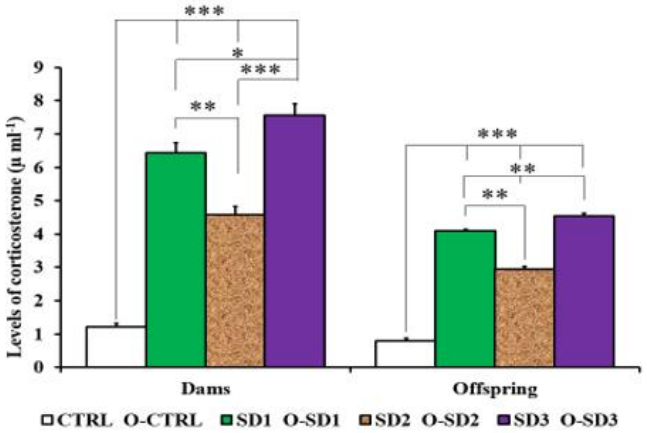
*Statistical Analyses*

Data obtained were statistically analysed by one-way analysis of variance (ANOVA), followed by Student's Newman-Keuls post-hoc test using GraphPad Prism 5 (GraphPad Software, San Diego, CA). All the values are expressed as mean ± standard error of mean (SEM). The correlation coefficient (r) of Spearman was used to estimate the association between MABP, CORT, MDA, and other measured variables in the selected populations studied. P values less than 0.05 ($p < 0.05$) were considered significant.

**Table 1:** Pregnancy outcome, litter size, survival rate, and birth weight of offspring after maternal sleep deprivation

| | **CTRL** | **SD1** | **SD2** | **SD3** |
|---|---|---|---|---|
| Pregnancy outcome | 90% | 30% | 40% | 70% |
| Number of litter | 11 | 4 | 5 | 9 |
| | **O-CTRL** | **O-SD1** | **O-SD2** | **O-SD3** |
| Survival rate of offspring | 90% | 18% | 44% | 71% |
| Average weight at birth | 4.96 g | 3.61 g | 4.02 g | 4.57 g |

Note: The table shows that all SD groups have reduced number of litter, while their subgroups have low survival rate and birth weight compared with their respective control groups. CTRL - control, O-CTRL - offspring of CTRL; SD1 - sleep deprivation from GD1 to GD20; SD2 - sleep deprivation from GD7 to GD20; SD3 - sleep deprivation from GD14 to GD20; O-SD1 - offspring of SD1; O-SD2 - offspring of SD2; O-SD3 - offspring of SD3; GD - gestational day.



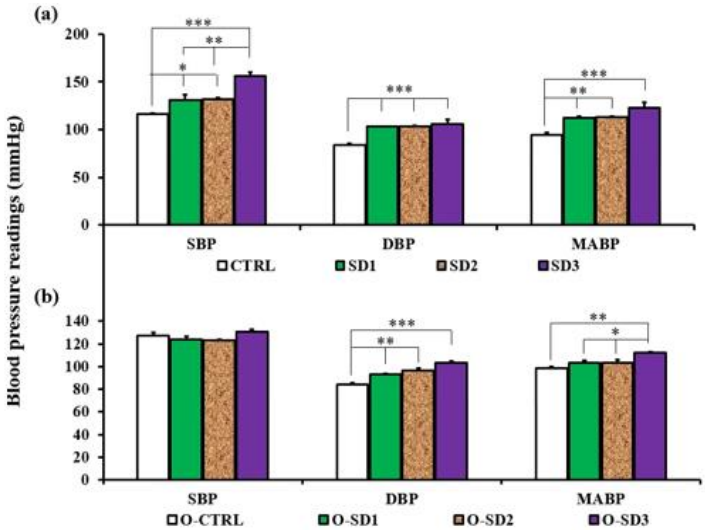




**Fig. 2 (Left):** Corticosterone levels in sleep-deprived dams and their offspring. Corticosterone was significantly increased in SD1, SD2, SD3 and O-SD1, OSD2, O-SD3 compared to CTRL and O-CTRL respectively. The level of significance is shown as *p < 0.05, **p < 0.01, ***p < 0.001. CTRL - control; O-CTRL - offspring of CTRL; SD1 - sleep deprivation from GD1 to GD20; O-SD1 - offspring of SD1; SD2 - sleep deprivation from GD7 to GD20; ; O-SD2 - offspring of SD2; SD3 - sleep deprivation from GD14 to GD20; O-SD3 - offspring of SD3; GD - gestational day.

**Fig. 3 (Right):** Systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial blood pressure (MABP) in **(a)** sleep-deprived dams and **(b)** their offspring. The level of significance is shown as *p < 0.05, **p < 0.01, ***p < 0.001. CTRL - control; SD1 - sleep deprivation from GD1 to GD20; SD2 - sleep deprivation from GD7 to GD20; SD3 - sleep deprivation from GD14 to GD20; O-CTRL - offspring of CTRL; O-SD1 - offspring of SD1; O-SD2 - offspring of SD2; O-SD3 - offspring of SD3; GD - gestational day.

## RESULTS

*Pregnancy outcome, litter size, survival rate, and weights at birth*

Seven dams in SD1, 6 in SD2, and 3 in SD3 failed to give birth after 20, 14, and 7 days of REMSD, respectively. Weight loss was most evident in SD1 dams, with an average loss of 41 g after 20 days of REMSD. Parturition occurred as early as on GD 17 among SD1 and SD2 dams. Although some SD3 dams reached GD 21, a few gave birth on GD 20.

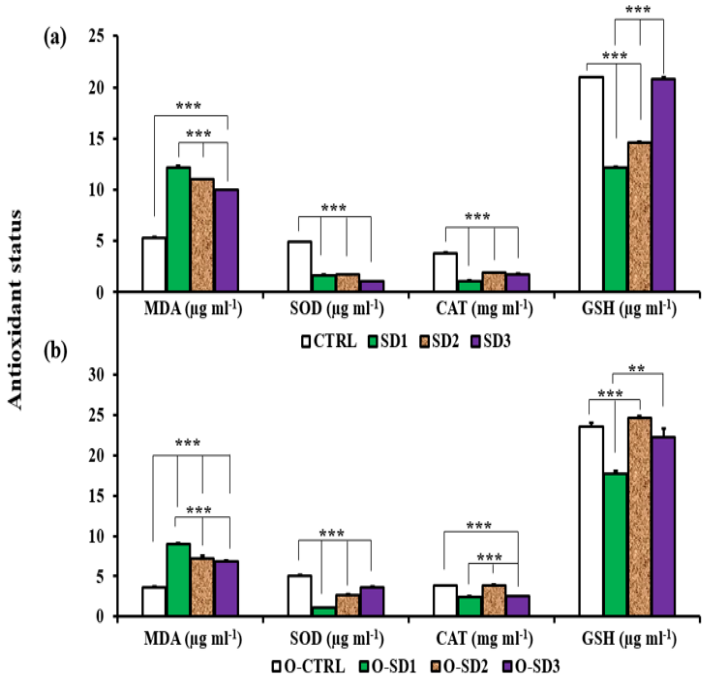
Litter size and weight were as reduced as 2 pups and 3.3 g in SD1 offspring. These specific pups were found dead on postnatal day 2. The survival rate was highest in O-SD3 (71%), with O-SD1 having only an 18% survival rate (Table 1).

*Effect of SD on corticosterone and blood pressure in pregnant mothers*

Result shows that CORT levels in SD dams were remarkably higher than their control dams ($p < 0.001$). Similarly, BP readings were increased across SD groups at $p < 0.01$ (SD1 and SD2) and $p < 0.001$ (SD3) compared with the control dams (refer to Figs 2 and 3a, respectively).

*Effect of SD on corticosterone and blood pressure in the offspring*

In the offspring of SD dams, CORT and BP were also higher than the offspring of control dams. The pattern of increase and significance level are similar to those seen in their sleep-deprived mothers except for MABP, were only O-SD3 was statistically significant compared with O-CTRL ($p < 0.01$). Also, the MABP level (just like the highest level was seen in SD3) was higher in O-SD3 compared with O-SD1 and O-SD2 ($p < 0.05$). Results of CORT and BP in the offspring are represented in Figs 2 and 3b, respectively.



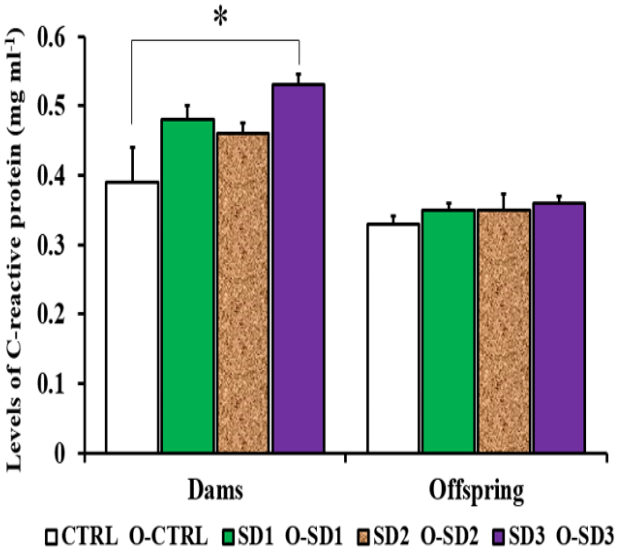




**Fig 4 (Left):** Levels of malondialdehyde (MDA) and oxidative enzymes in **(a)** sleep-deprived dams and **(b)** their offspring. MDA exhibited a significant increase in (a) sleep-deprived dams and (b) their offspring. The level of significance is shown as *p < 0.05, **p < 0.01, ***p < 0.001. SOD - superoxide dismutase; CAT - catalase; GSH - glutathione; CTRL - control; SD1 - sleep deprivation from GD1 to GD20; SD2 - sleep deprivation from GD7 to GD20; SD3 - sleep deprivation from GD14 to GD20; O-CTRL - offspring of CTRL; O-SD1 - offspring of SD1; O-SD2 - offspring of SD2; O-SD3 - offspring of SD3; GD - gestational day.

**Fig 5 (Right):** Levels of C-reactive protein (CRP) in sleep-deprived dams and their offspring. CRP was only statistically increased in SD3 compared with CTRL. The offspring groups showed no statistical difference. The level of significance is shown as *p < 0.05. CTRL - control; O-CTRL - Offspring of CTRL; SD1 - sleep deprivation from GD1 to GD20; O-SD1 - offspring of SD1; SD2 - sleep deprivation from GD7 to GD20; O-SD2 - offspring of SD2; SD3 - sleep deprivation from GD14 to GD20; O-SD3 - offspring of SD3; GD - gestational day.

*Effect of SD on malondialdehyde, antioxidant enzymes and C-reactive protein in pregnant mothers*

Malondialdehyde (MDA) was higher in SD dams compared with control dams ($p < 0.001$). Levels of antioxidant enzymes were considerably reduced in SD dams compared with control dams ($p < 0.001$). In contrast, the level of CRP was only statistically higher in SD3 dams when compared with the control dams ($p < 0.05$). Results of MDA and antioxidant enzymes are represented in Fig 4a, whereas CRP values in Fig 5.

*Effect of SD on malondialdehyde, antioxidant enzymes and C-reactive protein in offspring*

Malondialdehyde (MDA) and antioxidant enzymes levels in offspring rats are represented in Fig 4b and CRP values in Fig 5. MDA levels in the offspring patterned after levels seen in their mothers and were higher ($p < 0.001$), while levels of antioxidant enzymes were lower when compared with the levels seen in offspring of control dams ($p < 0.001$). CRP levels were not different between the offspring of SD dams and those of control dams.

**Table 2:** Correlation Coefficients (r) in measured parameters between SD3 and O-SD3

| Offspring / Mothers | CORT | MABP | CRP | MDA | CAT | SOD | GSH |
|---|---|---|---|---|---|---|---|
| CORT. | *0.74 | | | | | | |
| MABP | 0.54 | **0.98 | | | | | |
| CRP | -0.23 | **0.96 | 0.33 | | | | |
| MDA | ***0.99 | 0.46 | ***1.0 | *0.98 | | | |
| CAT | 0.46 | 0.64 | 0.17 | ***0.99 | 0.47 | | |
| SOD | *0.83 | *0.83 | *0.87 | 0.50 | ***0.99 | 0.45 | |
| GSH | *0.79 | 0.26 | 0.60 | *0.80 | 0.01 | *0.83 | *0.89 |

Note: The table shows the comparison of parameters measured in SD3 dams and their offspring (O-SD3). The level of significance is shown as *p < 0.05, **p < 0.01, ***p < 0.001. CORT - corticosterone; MABP - mean arterial blood pressure; CRP - C-reactive protein; MDA - malondialdehyde; CAT - catalase; SOD - superoxide dismutase; GSH - glutathione; SD3 - sleep deprivation from GD14 to GD20; O-SD3 - offspring of SD3; GD - gestational day.

## DISCUSSION

This study demonstrated that varied duration of REMSD in pregnancy imposed a relative increase in circulatory CORT level in Wistar rats. This agrees with Barzegar *et al*. (2015), Amugongo and Hlusko (2014), and Calegare *et al*. (2010), who independently reported increased CORT in rats after noise stress, immobilization stress, and gentle handling protocol. Like in this study, pregnancy-related GC levels can be intensified by stress. If the stress persists for too, high GC level can lead to a dysfunctional HPA axis. However, we speculate that the relatively reduced CORT level seen in SD2 (compared with SD1 and SD3) may be attributed to the particular time in pregnancy that SD was introduced, hence an adaptation adjustment for HPA axis activity in SD2 dams.

Early and long-lasting gestational stress has been linked with a dysfunctional HPA axis activity (Del Cerro *et al*., 2015). Stimulatory effect of placental CRF on HPA axis activity and the natural decline in placental 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD-2) activity from GD 15 till term (Vaughan *et al*., 2012), respectively are perhaps responsible for the higher CORT levels of SD1 and SD3, with respect to SD2.

In addition to its decline from GD 15, the placenta expression level of 11β-HSD-2, an enzyme capable of converting CORT to an inactive metabolite, is reduced by maternal stress (Argeri 2016). As such, 11β-HSD-2 becomes saturated with excess CORT. This can expose the fetus to an increased CORT supply (especially during GD 14 - 20). From this study, the levels of CORT seen in the offspring were, interestingly, modeled after CORT levels in their SD mothers. Likewise, Mueller and Bale (2008) asserted a resultant increase in CORT level of offspring after early prenatal exposure to stress. Overexposure to GC has been proposed as a trigger for fetal adaptation in response to unfavorable intrauterine environment (Cottrell *et al*., 2014). The same trigger can lead to diseases later in the offspring; due to permanent changes in some organs' structure and functions. Exposure of the fetus to increased CORT beyond physiological range can alter the placenta's functional tone (Howerton and Bale, 2012), and such can form the basis for HPA axis dysfunction in offspring (Raimundo *et al*., 2016).

Our findings reveal that REMSD from GD 1 and GD 7 was associated with increased risk of death, preterm birth, and reduced birth weight in Wistar rats' offspring. Maternal stress and small for gestational age have been linked with prenatal mortality, especially in early to mid-pregnancy (Khashan *et al*., 2014). Altered HPA axis activity may as well explain the reduced birth weight and small for gestational age seen in offspring of SD1 and SD2. These outcomes might have been sex-dependent since the surviving offspring of SD1 and SD2 were only females. This also buttresses the fact that male placenta could be more susceptible to maternal stress (Khashan *et al*., 2014). Furthermore, GC's long-term catabolic

actions might have also contributed to the reductions in the birth weight of the offspring.

Increased CORT levels and the presumed altered HPA axis activity, somewhat may be linked to the increased MABP in the REMSD groups. Short term SD increased sympathetic tone and BP (Rajendiran *et al*., 2015). Likewise, altered cardiovascular autonomic regulation (Raimundo *et al*., 2016), increased BP and renal dysfunction (Argeri *et al*., 2016; Lima *et al*., 2014), and reduced hypothalamic angiotensin-converting enzyme 2 (ACE-2) activity have been reported in offspring of sleep-deprived dams (Lima *et al*., 2014). The present study shows that MABP increased proportionately in the offspring as in their REMSD dams. Taken together, it is possible, therefore, that because of REMSD insult, increased CORT levels initiate BP related changes in the maternal physiological environment during fetal development by setting in motion central modifications that perhaps persisted to a certain extent in adulthood of the offspring and, therefore, capable of generating cardiovascular risks. Moreover, the positive correlation of CORT and MABP between SD3 and O-SD3 confirms that the levels of these measured parameters in the offspring were undoubtedly directly related to the levels seen in their mothers.

The high MDA and low antioxidant enzyme values in the SD dams suggest that the dams were oxidatively stressed. Rajendiran et al. (2015), using sleep quality assessment, reported increased MDA values in pregnant women, while D'Almeida et al. (1998) observed no substantial MDA level changes after a total SD of 96 hours in male rats. Indeed, SD elicited oxidative processes in the body's organs, and initiation of oxidative stress during pregnancy (especially in the mitochondrial of the placenta) leads to congenital malformation and in some cases, fetal death (Cottrell et al., 2014; Wells et al., 2009).

The offspring of SD dams, in the same manner, exhibited increased levels of MDA, with level higher in O-SD1 (Fig 4b). In its ability to support pro-oxidant state and increase the generation of oxidants (Stark *et al*., 2011), CORT in this study might have caused a reduction of antioxidant enzymes in SD dams, and on the long run, affected embryonic development plus oxidative state of the offspring (Howerton and Bale, 2012). Since a respective increase and decrease in MDA and antioxidant enzymes can intensify stress burden, the increased oxidative stress may have resulted in the reduced number of litter in this study. This is especially evident in SD1 (the SD group with the highest MDA value), owing to its high pregnancy loss and death rate observed among its fetuses. This finding points out some salient factors; the duration of SD insult and the animals' response to the stress factors are crucial regarding the results of this study.

Despite the independent direct link between GC, oxidative stress, and inflammatory response, it is worthy to note that our work did not observe any significant difference in CRP levels, except in SD3. While some authors (Moller-Levet *et al*., 2013; Meier-Ewert *et al*., 2004) may have reported a solid inflammatory system response to short term SD, it will be rational to assert that in this study, increased GC in SD1 and SD2 (due to chronic stress during pregnancy) resulted in the insignificant values of CRP, by inhibiting the production of lymphocytes and its response to pro-inflammatory cytokines (Bandoli *et al*., 2018). On the other hand, the individual increase in the values of CORT, BP, and body weight may explain the significant increase in the value of CRP seen in SD3 (Meier-Ewert *et al*., 2004). The level of CRP may have initiated a low-level grade systemic inflammation in SD3. Still, it could not instigate the same in O-SD3 since the CRP level in SD3 had no substantial correlation with CRP in O-SD3.

Predictive adaptive responses have been reported to be adequate in female than in the male placenta (Togher *et al*., 2018), even though such adjustments can pose permanent (functional and structural) changes through increased GC fetal programming. As mentioned earlier, the degree of variation in the measured parameters may lie with the different durations of SD employed in this study. We discovered that some of the offspring's outcomes varied according to the duration of REMSD in the dams, and only SD3 presented male offspring, which suggests a high mortality rate of male fetuses in SD1 and SD2 (Togher *et al*., 2018). Undeniably, the level of CORT was highest in SD3 offspring. However, the duration of REMSD insult that SD1 and SD2 offspring were exposed to during gestation might have conditioned them to survive later life as it did them in the stressful maternal environment, due to the predictable adverse programming of CORT that persisted for a long time, especially in SD1 offspring.

There are certain limitations of this study. First, although the handling process was uniform across all the experimental groups, this study failed to distinguish the SD variable from other stress factors and the multiple platform method's environmental parameters. Second, there are chances that the consequences of the SD insult from dams to offspring may have continued after birth since the same set of mothers were allowed to nurture the offspring until weaning. Transferring the offspring at birth to foster mothers would have restricted our findings to SD insults during gestation alone. Lastly, sex hormones play vital roles in implantation. Based on the

parameters that we measured in our experiments, it will be difficult to tie pregnancy loss solely to CORT's actions, since progesterone and 17b-estradiol (not determined in this study) are crucial factors to pregnancy loss.

In conclusion, this study recounts the long-lived modifications in some parameters measured in the offspring of rats exposed to REMSD during the different stages of pregnancy. These modifications seem to be significantly associated with REMSD than to other stress factors connected with the methods employed in this study. The long-lived changes in the offspring of sleep-deprived rats could primarily have been due to alterations in fetal programming induced by the sustained and high circulating CORT level.

## ACKNOWLEDGMENTS

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## CONFLICT OF INTEREST

The authors declared that they have no conflict of interest.

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