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Short communication

Effect of Heat Stress on Pregnancy Outcomes in Sprague Dawley Rats

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Abstract

Heat stressed animal may cause problem to the farmers as it will drop the production performance and reproductive efficiency such as animal's fertility and calving interval. In Malaysia, farmers tend to establish an open housing system or let their livestock to roam for grazing. Slightly increase in degree of hyperthermia from the environment factor could induce heat stress in animals. Hence, the aim of this study is to observe the effect of heat stress on pregnancy outcome in pregnant Sprague Dawley rats. Upon determination of oestrous, the female rats were mated. Once pregnancy was confirmed, the rats were divided into two groups. The treatment groups were exposed to the heat treatment at 41°C. While in control group, the rats were maintained at 24 °C. The treatment was given until delivery. Once delivered, the rats were euthanized. The reproductive organs were dissected and weighted. Pregnancy outcomes were observed and were seen to be affected. There was reduction in the reproductive organs weight which were the uterus and ovary, lower number of pup, alteration of offspring sex and also higher percentage of resorption. Although there was no significant finding between the two groups, but there was a pattern in the parameter observed that may indicate the effect of heat stress in pregnant rats. Therefore, heat stress may give negative effect to the pregnancy outcomes and reproductive system in rats.

Keywords: *Heat stress; Sprague Dawley rats; pregnancy; livestock; reproduction*.

1. Introduction

Being hot and humid throughout the year, Malaysia's climate is categorised as equatorial as it is located near the equator. Sometimes, the weather can be extreme hot and extreme cold. Animals that are reared in an open housing system are more affected by this climate changes. Farmers should put an effort in farm animal management in order to prevent exposing animals to heat stress. *El Nino* phenomenon can also cause heat stress or heat shock not only to human but also to livestock animal as the sudden increase in temperature will cause both physiological and psychological changes.

Heat stress is the sum of forces external that causing body temperature becomes higher from its resting state [1]. When not properly managed, animal that is affected by heat stress may be resulted in multitude of pathological and physiological responses [2]. The core body temperature of animals will increase during heat stress and when observed in humans, they will report unpleasant sensations [3]. When both temperature and humidity increase, the higher the risk of heat stress occur [4]. Quinn et al. [5] reported that a research done by using rat model demonstrates that early stages of heat stress are characterised by tachycardia and increased blood pressure with the latter falling in severe heat stress [5].

Heat stress is not confined only to hot regions. Slightly increase in degree of hyperthermia from the environment factor could induce heat stress in animals. Heat stress is economically significant with livestock production and reproductive losses. Due to increased demand for animal product in developing country, global climate change, population growth, limitation to livestock production and global food security caused by heat stress are likely to grow importance [6]. In cow fertility, heat stress includes an increased number of days open, reduce conception rate and larger number of cows suffering different type of anoestrous [7]. In dairy science, heat has been one of the primary stressors evaluated. As the high metabolic demand and associated endothermy makes high-producing dairy cows more sensitive towards heat-induced depression of production.

Animal welfare is an important issue in livestock production that farmers should really understand. To prevent exposing animal to heat stress, animals should be raised in the zone of thermal well-being where the animals are more comfortable and productive [8]. Providing shade shelter to animal especially livestock animals gives beneficial effect in terms of thermoregulatory responses and productive responses have been demonstrated on numerous occasions [9, 10, 11, 12]. The beneficial effects of providing shade shelter to livestock animals in improving their reproductive performance are also well established [13, 14]. Therefore, the objective of this study is to determine the effect of heat stress on pregnancy outcomes such as litter size and congenital abnormalities and also to determine the effect of heat stress on reproductive organs which are uterus and ovary and percentage of resorption in pregnant rats.

2. Materials and Methods

2.1. Animal Selection and Grouping

Twelve (12) sexually matured female Sprague Dawley rats (180-200 g) with the age of eight-week old were used in this study. All animals were housed individually in the cage that had been sup-

plied with clean and absorbent bedding along with commercial pellet food and water *ad libitum* and maintained on 12-hour light/dark cycle at 20-24 ^{O}C. The rats were randomly divided into two groups which were the control group and the treatment group, with each group consisted of six rats.

2.2. Mating and Pregnancy Diagnosis

Oestrus cycle of the virgin rats was checked daily for ten consecutive days to determine the regularity of the cycle. The procedure was done by filling the blunt-end dropper with approximately 1 ml of 0.9 % normal saline before placing it into the vagina and flushed several times. The rats were restrained by proper handling method in order to minimize stress and to prevent injury to the handler. Then, the collected vagina fluid was dropped on a ring glass slide, put under a light microscope, and examined with magnification of 10 to 40. Once the regularity of oestrus cycle was determined, females were mated with proven fertile rats to induce pregnancy. The rats were left to mate overnight undisturbed starting from the evening of pro-oestrous. In this study, two to one (female: male) mating ratio, i.e. two females mated with one male was used. On the next morning, each female was taken out and the vagina smear was collected to detect the presence of sperm. If the sperm were presence, female will be considered as day 0 of pregnancy [15].

2.3. Heat Stress Treatment

Heat stress was experimentally induced by placing six pregnant rats in an egg incubator (M & M Ternak). Egg incubator is initially used to incubate the egg as it produced controlled heat. The modified heat treatment was done by exposing the rats at 41 ^{O}C for 30 minutes [16]. After heat stress being administered, the rats were placed back in their home cage. The procedure was repeated daily until parturition. The day of birth (day 0) was determined by examining the cage daily.

2.4. Examination of the Pregnancy Outcomes

Following delivery, pregnancy outcomes were assessed. The parameters being assessed were number of pups and gross congenital abnormalities, and male and female ratio. All pups were counted and checked for external abnormalities. All pups were euthanised after being assessed.

2.5. Examination of the Rat's Reproductive Organs

Once delivered, each dam was euthanised. The rats were suffocated in an air tight jar filled with chloroform to subdue them into unconsciousness. Then, they were euthanised by cervical dislocation. The dam was dissected and the reproductive organs (uterus and ovary) were taken out for gross assessment. The weight of both organs was recorded. Implantation sites of the uterus were counted. The differences between number of implantation site and litter size was calculated to determine the percentage of resorption.

2.6. Data Analysis

All the data collected were analysed by using the Independent t-test by using SPSS software.

3. Results

The findings on the reproductive organs weight which are uterus and ovary were presented in Table 1. Both uterus and ovary weight in treated group were decreased compared with control group. However, there were no significant differences observed between the two groups.

Table 1: Mean reproductive organs weight in heat stress and control groups.

Organ weight (g/100 g b.w.)	Control	Heat stress
Uterus	1.186 ±0.204	0.971 ± 0.146
Ovaries	0.059 ± 0.006	0.058 ± 0.005

Data are presented as mean ± SEM (n= 6 per group). Significant differences determined by independent t-test with p< 0.05

The findings on pregnancy outcomes for both control and treatment groups are shown in Table 2. The percentage of resorption was seen higher in treated rats compared to control but the difference was not statistically significant. Stress group had lower litter size compared with control group. However, there was no significant difference between the two groups. No significant differences were observed in the percentage of female and male ratio between the two groups. In addition, no congenital abnormalities were observed in control and treated groups.

Table 2: Pregnancy outcomes in heat stress and control groups.

Parameters	Control	Heat stress
Percentage of resorption (%)	15.98 ± 5.25	24.64 ± 13.31
Number of pup	10.6 ± 1.03	8.29 ± 1.44
Percentage of female (%)	41.22 ± 2.92	47.85 ± 9.85
Percentage of male (%)	55.70 ± 5.05	29.20 ± 9.92
Congenital abnormalities	0	0

Data are presented as mean ± SEM (n= 6 per group). Significant differences determined by independent t-test with p< 0.05

4. Discussion

In this study, the weight of ovary in heat stressed rats is slightly reduced. Roth et al. (2000) stated that exposing rats to different stressor of 6 hours per day resulted in reduction of primordial, primary and pre-antral follicles in which can reduce the weight of ovary as well [17]. Another study, conducted by Myers et al. (2004) showed that more information of the ovary function can be provided from the follicle number [18]. Approximately, 20% to 50% healthy follicles depletion was shown in stressed rats compared to controls [18]. The previous study suggested that hormonal changes could lead to significant reduction in the ovarian weight [19]. According to Donoghue et al. (1989), heat stress was found to reduce the LH levels and hypothalamic gonadotropin-releasing-hormone-I (GnRH1) [19]. The delayed in timing of recruitment of ovulatory follicles and decreased LH receptor level and oestradiol synthesis activity in follicle were caused by heat stress [20]. Study done by Macfarlane et al. (2000) stated that infusion of stress like concentration of cortisol suppressed follicular development and LH surge in sheep [21]. In another study by Shimizu et al. (2005) revealed that FSH receptor level and aromatase activity in granulose cells and oestradiol levels in follicular fluid of early antral, antral and pre-ovulatory follicles were strongly inhibited and increased in apoptosis of granulose cells due to the heat stress [22]. These findings from previous study might support the findings in present study.

Although the weight of uterus is not statistically significant, but there is reduction in the weight of uterus in heat stressed rats compared to control. Previous study done by Hsueh et al. (1976) reported that decrease in the number of receptor oestrogen complexes may alter and greatly reduce the growth of uterine [23]. Oestradiol and progesterone are the primary hormones that are responsible for preparing the uterine endometrium for implantation. Weitlauf (1988) stated that oestrogen is important for the proliferation of the uterine epithelium and also sensitises it to progesterone action by inducing progesterone receptor (PR) gene [24]. However, there is still lack of study that could fully explain the effect of heat stress on oestrogen hormone that shows decrease in the weight of uterine.

Decrease in embryo development has been reported to be affected due to the high ambient temperature where the pre-attachment stage embryos were also affected [25]. For the embryo to attached, intricate signalling interactions between conceptus and mother is

required [26, 27, 28], thus, any stress factor, including heat stress, can disrupt this process [29, 30]. Previous study showed that exposing pre-implantation embryos to heat stress caused their development to decrease in mice [31] and cows [32], and also decreased in total cell number of blastocysts [33]. Maternal body that had been exposed to heat stress during the short period (4-8 days) between fertilisation and implantation is likely to cause the pre-implantation development to be severely affected directly by heat stress itself or indirectly by the deleterious change in reproductive tracts. The embryo development at an early stage in mice [34, 35] and cows [25] is inhibited by in vivo maternal heat stress thus causing the embryo being reabsorbed back during pre-implantation. Edwards [36] stated that high incidence of foetal resorption occurred when acute hyperthermia was induced in pregnant rats [36]. It is found that foetal or embryonic resorption were not a common feature and has occurred most frequently after heat treatment during a relatively short interval (days 11 to 15).

Although the results from the present study was not statistically significant, there were proves from previous study that showed high incidence of foetal resorption may occurred when pregnant rats were exposed to high ambient temperature. From the result obtained, it showed that heat stress reduced the number of pups when compared to control rats. According to Patin et al., [37], lower number of pups in stressed female rats could be due to abortion and some litters died a few hours or a few days later after delivery [37]. Previous study by Omtvedt et al. [38] has demonstrated that conception rate and litter size were reduced when heat stress were exposed to pregnant gilts from day 0 to 16 of pregnancy [38]. Also, from the same study, the number of stillborn piglets increased as heat stress induced during 102 to 110 days of pregnancy in pig. Another experimental study done by Wettermann and Bazer (1985) suggested that there was an alteration in reproductive endocrine system when introduce to heat stress during early pregnancy, especially the control of luteal function [39]. Other than that, Wettermann and Bazer [39] also found that reduction of litter size occurred due to the reduction of the amount of embryonic tissue present at day 16 of pregnancy [39].

Another study on the effect of heat stress on litter size reduction found that one potential explanation on the reduction of litter size in mothers that being exposed to 30 °C could be due to heat load or heat stress that might cause overheating leading to abnormal maternal behaviour or less viability of the offspring [40]. However, litter size reductions might have arisen from several factors including that mothers might have actively reduced their litter by killing and consuming their own pups. The same study propose that the reduction may originated from physiologically imposed energetic constraints that prevent mothers from producing milk at required rates and that it relates the reduction in number of pups to metabolism and growth of offspring [41]. Other findings by Brummelte and Gaela [42] has demonstrated that litter size in rats decreased when the dam was administered with corticosterone during pregnancy [42]. Corticosterone is a stress hormone that known to exert strong effects on peripheral blood vessels and placenta [43]. Stress could activate the sympathetic nervous system. This activation may cause lower blood flow to the uterus and foetus thus restricting foetal growth. Therefore, this finding supported the present study that heat stress may induce increasing in corticosterone level and may results in reduction of litter size.

The findings from this study showed that there was a variation in the percentage of female and male pup that had been exposed to high temperature compared with the rats that were maintained in normal temperature. Previous study reported that stress hormone has been linked to sex ratio skews through their physiological actions. Such maternal effects may result in parents that are physiologically constrained in their ability to alter sex ratios in response to current conditions. According to Navara, [44], and Mousseau and Fox (1998), stress responses provide a link between the proposed mechanisms of sex ratio adjustment [44, 45]. From the same study, they proposed that physiological effect of maternal gestational stress on developing offspring include changes in hypothalamic-pituitary-adrenal (HPA) axis function, body condition and adult reproductive behaviour and function in the offspring [46, 47, 48]. Offspring sex ratios and survival of the offspring could be influenced due to changes to the HPA axis (and thereby sensitivity to stress). Previous study showed the evidence that cause the variation in the sex ratio of the offspring is based on three propositions that there are equal numbers of X- and Y-bearing sperms, the probability that sperm will fertilize an ovum is unrelated to its sex chromosome and any variation in sex ratio at birth is therefore caused by sex-related foetal mortality. This evidence might support the result from the present study. Study conducted by Lane and Hyde [49] showed that rat exposed to maternal stress resulted in the production of significantly fewer males [49]. Perez-Crespo et al. [50] stated that in mouse, human, and bovine embryos, males tend to be more susceptible to oxidative stress than in female [50]. In fact, Kraemer (2000) reviewed that male foetuses are at greater risk of many developmental disorders, including prenatal death [51]. This finding relates to why the percentage of female pups from rats exposed to heat stress is high than male pups in the present study.

5. Conclusion

In conclusion, heat stress may give negative effect to pregnancy outcomes and female reproductive system. Although the finding of the effect of heat stress is not statistically significant but there is reduction in the reproductive organ weight which are uterus and ovary, increased the percentage of resorption thus reduce the number of pup and alter the offspring sex ratio. However, there is no congenital abnormalities in the pups were observed. Further investigation needs to be done on a larger sample size or duration of heat exposure. The level of reproductive hormones should be determined to give a better understanding in the effect of heat stress on pregnancy outcomes.

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