EVALUATION ON THE REPRODUCTIVE PERFORMANCE AND SPONTANEOUS MALFORMATIONS AMONGST SD RATS IN THE INSTITUTE FOR MEDICAL RESEARCH COLONY

HUSSIN M.[1]*, SITI AMRAH S.[2], ZAKIAH I.[1], HASNAN J.[3] AND F.J.R. PAUMGARTTEN[4]

1 Toxicology and Pharmacology Unit, Herbal Medicine Research Centre, Institute for Medical Research, 50588 Kuala Lumpur.

2 Department of Pharmacology, School of Medical Sciences, USM, Health Campus, Kelantan.

3 Department of Pathology, School of Medical Sciences, USM, Health Campus, Kelantan.

4 Laboratory of Environmental Toxicology, National School of Public Health, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil.

* Corresponding author: hussin@imr.gov.my

ABSTRACT. This study was performed to investigate the reproductive performance and spontaneous malformations of female Sprague Dawley (SD) rats obtained from the Animal Resource Unit, Institute for Medical Research for their use in reproductive toxicity assessment. One hundred and thirty-five virgin female rats with a body weight ranging between 190 g and 210 g were randomly caged overnight with 60 fertile male rats in 1:1 basis. Vaginal smear was performed the next morning and the day of sperm positive was considered as gestational day (GD) 0. On GD21 caesarean hysterectomy was performed to examine the outcome of each pregnancy. The female fertility index (sperm positive) was 90.5% and the pregnancy index was 100%. The percentage of pre and post-implantation loss were 1.96 and 7.48 respectively with the number of early resorption outnumbered the late resorption (0.56 ± 1.0 and 0.36 ± 1.5 respectively). Out of 1,234 foetuses examined, only 5 foetuses (0.41%) presented with gross congenital malformation. The female SD rats obtained from the Institute demonstrated high fertility and pregnancy indexes with low incidence of resorption and malformation, therefore suitable to use for reproductive toxicity assessment.

Keywords: Reproductive performance, spontaneous malformations, Sprague Dawley rats

INTRODUCTION

A toxicological approach utilising *in vitro* and whole animal studies (*in vivo*) in chemical hazard assessments can provide specific toxicity data on reproduction by extrapolating the animal data based on examining dose-responses, routes of exposure and cellular/molecular mechanisms (Hoyer, 2001). In evaluating human reproductive toxicity risk, laboratory animals like rats and mice are chosen because the metabolism, distribution and transportation process

are similar to human (Schardein *et al*., 1985;Wilson, 1975).

Sprague Dawley (SD) rat is an outbred stock strain which basically maintains as closed colonies of rats of undefined genotype (Kacew and Festing 1996). The SD rat is one of the species that has proven to be most useful in pharmacological and toxicological research due to its many similarities to human metabolism pathways. In addition there are many anatomical and physiological characteristics similar to humans thus allowing comparisons in absorptions, distribution and excretion of ingested compounds. Furthermore, the SD rat also has a short life span and gestation period, high fertility rate, is less expensive and economic to maintain. Most importantly there is also a large database of its characteristics which is very useful in the interpretation of the relevant animal data to human.

In reproductive toxicology research, the rat is highly recommended to be utilised as an animal model in view of the fact that other animals have some weaknesses, for instance the higher frequency of spontaneous malformation in mice and rabbits. Hence, a large number of animals are needed which would increase the cost and duration of experiments. In comparison to SD rats, the duration of gestation and the number of foetus produced by guinea pigs, hamsters and ungulate animals like sheep and pigs limit their laboratory use. Despite the fact that monkeys are genetically closer to humans, the use of this animal in reproductive studies would be considerably more expensive (Kotwani *et al*., 1995).

As far as the author is concerned, there is no historical control data on reproductive performance and congenital malformation available, particularly in the Animal Resource Unit, Medical Resource Centre, Institute for Medical Research (IMR) and animal facilities from local universities or institutes in general. The present study was conducted to investigate the status of reproductive performance of female SD rats obtained from the Animal Resource Unit, IMR, for their use in reproductive toxicity assessment and also in order to provide a set of historical control data for developmental toxicity studies using SD rats which could be adopted and used as reference data by other Malaysian researchers in conducting similar studies.

MATERIALS AND METHODS

Chemicals

Formaldehyde (38% w/w) and ammonium sulphide were purchased from Merck, Germany. Ether and sodium chloride were obtained from Fisher Scientific and Sigma, USA.

Animals

One hundred and thirty-five healthy virgin female and 70 confirmed fertile male SD rats with a body weight ranging from 180 g to 200 g and 200 g to 250 g, respectively were obtained from the Animal Resource

Unit, Medical Resource Centre, Institute for Medical Research. The animals were housed in polypropylene cages, lined with wood shaving and at controlled temperature (20 ± 2 °C), with 40% to 60% humidity under 12 hours of light and dark cycle. The animals were acclimatised for about a week prior to start of the study. Food and water were available *ad libitum*. Food for the animals was purchased from Specialty Feeds, Australia. Ethical approval for this study was obtained from the Animal Care and Ethics Committee, Ministry of Health Malaysia (ACUC No: ACUC/KKM/02 (2/2007). The research was carried out according to OECD Guidelines 414 and some of the methods were adapted from Wilson (1965).

Experimental design

This study was conducted in 5 batches. After a week of acclimatisation, vaginal smear was performed daily on all female rats. Rats in a proestrus phase were mated with fertile males on a one-to-one basis when each female rat was introduced to a male's cage. The animals were paired usually in the morning around 9.00 am, and they were then left overnight. A vaginal smear was performed again the next morning to confirm mating with the presence of spermatozoa.

Briefly, vaginal smears were performed in the morning around 9.00 am. A few drops of 0.9% sodium chloride solution were drawn into a pipette. The pipette tip was then inserted into the vagina of female rats. The solution was gently flushed into the vagina and drawn back into the pipette. A new clean pipette was used for each female in order to prevent carryover of sperm from mated female to non-mated female.

The fluid which consisted of cellular cells, was then smeared evenly onto a marked microscope slide. The wet and unstained smears were viewed and examined immediately under a light microscope at 40× magnification (MEIJI Techno). The female rat was considered mated when sperm were detected in the vaginal smear. The female rat was then designated as gestational day (GD) 0 and observed daily until day 21 (GD21) and sacrificed on GD21.

Mortality and maternal clinical observations

The dams were inspected daily for duration of 60 minutes and followed by 30 minutes observation for any signs of maternal toxicity such as piloerection, vaginal bleeding, diarrhea, alteration in locomotion, dull fur, emaciation, sedation, soft stool, urination or maternal deaths (Christian, 2001).

Maternal food and water consumption

The food and water intake were measured and recorded every week starting on GD0, 7, 14 and 21.

Maternal body weight, body weight gain and corrected maternal body weight gain

The body weight of dams was weighed and recorded every 3 days. Maternal body weight gain for GD0 to GD6, GD6 to GD9, GD9 to GD12, GD12 to GD15, GD15 to GD18 and GD18 to GD21 and corrected weight gain were calculated as follows:

Maternal body weight gain (i.e. GD0 to GD6)
= body weight at GD6 – body weight at GD0

Corrected weight gain
= (GD21 body weight – GD0 body weight)
– gravid uterus

Caesarean hysterectomy and assessment of maternal visceral organs, reproductive performance and foetal parameters.

On GD21, each dam was euthanised with a dose of diethyl ether following which a caesarean hysterectomy was immediately performed to ensure standardised duration of pregnancy and to prevent the mother from eating (cannibalism) malformed or stillbirth foetuses before they were examined (Roche, 2008).

Each dam was placed on a post-mortem board and the peritoneal cavity was opened via longitudinal and transverse incisions (Figure 1). The gravid uterus and ovaries were incised, taken out, adhering tissue removed and weighed. The gravid uterus was dissected longitudinally to expose the foetuses. The number of live and dead foetuses (absence of movement when touched) was recorded. The foetuses were then removed by cutting the umbilical cord close to the foetuses. The foetuses were dried from amniotic fluid using tissue paper and sexed. Determination of the sex of foetuses was based on the location of the genital papilla which was further away from the base of tail in the male than in the female. The sex of foetuses was then confirmed by performing visceral examination with the presence of testicles for the male. Each collected foetus was assigned a number according to its position in the uterine horn starting with number 1 at the end of the ovary of the right uterine horn and marked. The marking system was adapted from Chahoud and Kwasigroch (1977). Placentae and foetuses were weighed and recorded.

The ovaries which attached to the uterus were dissected, cleaned and weighed. The corpora lutea were fixed and kept in a bottle consisting of 5% formalin. The number of corpora lutea in both ovaries were then counted under a stereo microscope (LEICA MZ 6) in order to determine whether small size litters were due to a lower frequency of ovulation or a failure of fertilised ova to implant in the endometrium of the uterus.

Total implantation sites, plus early and late resorptions were counted and recorded. Early resorption was classified when only placental tissue was visible together with a dark brown blood clot. A resorption site with both placental and embryonic tissue visible was classified as late resorption. Uteri from rats that

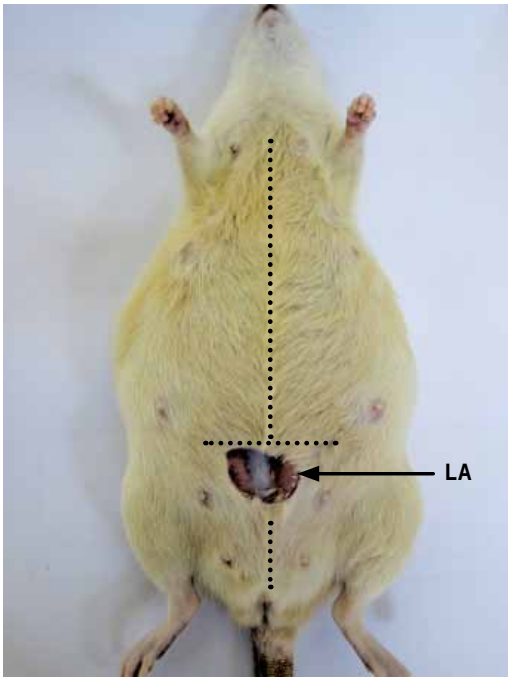
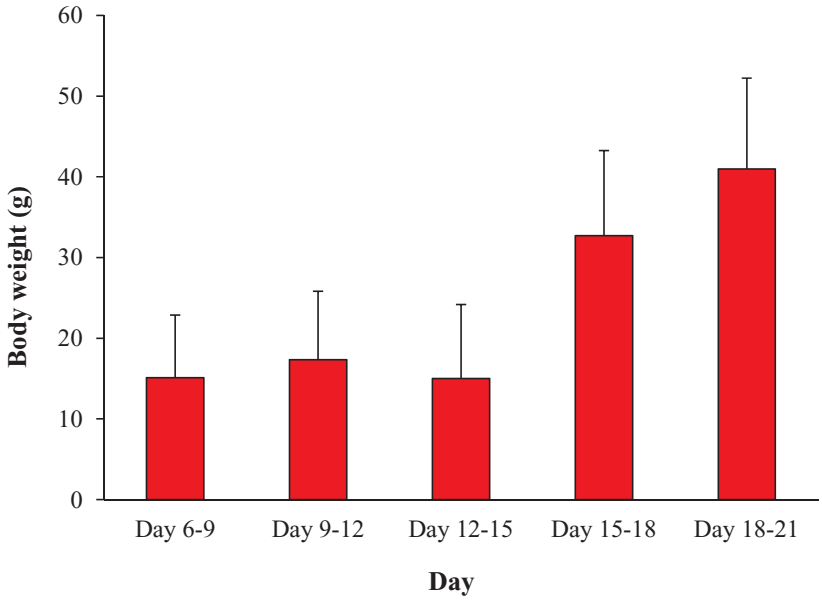


Figure 1. Photograph of pregnant rat. Longitudinal incision was made through the white line or Linea Alba (LA) on GD 21. Transverse incision was made to expose the gravid uterus.

Table 1. The relative maternal organ (%) weight of SD rats orally administered with distilled water (Control) from GD6 to GD20 of gestation. Each value represents the mean ± SD of 105 animals.

Parameters	Weight (%)
Gravid uterus	24.06 ± 3.80
Liver	3.64 ± 0.56
Lung	0.43 ± 0.06
Heart	0.24 ± 0.03
Right Kidney	0.27 ± 0.04
Left Kidney	0.26 ± 0.04
Right Ovary	0.02 ± 0.01
Left Ovary	0.02 ± 0.01

Figure 2. Bar chart showing the body weight gain of dams measured on GD0 to GD21 of gestation. The body weight was measured every 3 days. Each value represents the mean ± SD of 105 animals. Animals were sacrificed and caesarean hysterectomies were performed on GD21.



showed positive vaginal smears but were not visibly pregnant at caesarean section were placed in 10% ammonium sulfide solution for 15 minutes under a chemical fume hood to determine the number of implantations. The percentage of pre- and post-implantation loss was calculated as follows:

Pre-implantation loss

$$= \frac{\text{No. of copora lutea – no. of implantation}}{\text{No. of corpora}} \times 100$$

Post-implantation loss

$$= \frac{\text{No. of implantation – no. of live foetuses}}{\text{No. of implantations}} \times 100$$

Meanwhile, the maternal organs such as liver, kidney, lungs, and heart were dissected, weighed and recorded. The relative organs weight (ROW) of each animal was then calculated as follows:

ROW

$$= \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rat on sacrifice day (g)}} \times 100$$

Live foetuses were then euthanatised with diethyl ether. External examination of foetuses for morphological abnormalities was performed by assessing the eyes, ears, tail, head, mouth, and limbs and recorded.

Fertility and pregnancy indexes were calculated as follows:

Fertility index (%)

$$= \frac{\text{number of pregnant females}}{\text{Number of sperm-positive females}} \times 100$$

Pregnancy index (%)

$$= \frac{\text{Number of females with live born}}{\text{Number of females pregnant}} \times 100$$

The summary of maternal reproductive performance and foetal parameters recorded through the experimental period are listed below.

Maternal reproductive performance parameters:

1. Fates of females survival
2. Fertility index (%)
3. Pregnancy index (%)
4. Maternal clinical observations
5. Maternal food and water consumption
6. Maternal body weight (GD0, 6, 9, 12, 15, 18, 21)
7. Maternal weight gain (GD0-6, GD6-9, GD9-12, GD12-15, GD15-18, GD18-21)
8. Corrected maternal body weight gain
9. Maternal visceral organ examination
10. Number of corpora lutea per litter
11. Number of implantation sites per litter
12. No. of resorption sites per litter
    a. Early: no embryonic tissue visible at termination
    b. Late: placental and embryonic tissue visible at termination
3. Percent of pre-implantation loss (%)
4. Percent of post-implantation loss (%)

Foetal parameters

5. No. of live and dead foetuses
6. No. of live and dead foetuses per litter
7. Sex distribution (ratio)
8. No. of male and female foetuses

9. Foetal weight :male and female
10. External malformations
11. Placental weight

Statistical analysis

The data were assessed and expressed as mean ± standard deviation (SD) using SPSS version 16.

RESULTS

Mortality and maternal clinical observation

All dams were healthy throughout the gestation period without any changes in their general health and behaviour. Caesarean hysterectomy was performed on GD21. No gross lesions were observed on the visceral maternal organs (gravid uterus, liver, lungs, heart, kidneys and ovaries) in all female rats. Nine of the dams delivered on GD21 prior to arranged caesarean section and therefore were excluded from the experiments.

Maternal food and water consumption

The food and water consumption was measured every week throughout the gestation period. No changes on weekly maternal food and water intake were observed in all dams.

Maternal body weight, body weight gain and corrected maternal body weight gain

The maternal body weight increased proportionally to the day of pregnancy. The body weight gain of animals started to increase on GD12 until GD21 as shown in Figure 2.

Maternal visceral organs examination

Maternal organs were collected, weighed and examined for any morphology changes after performing caesarean hysterectomy on GD21. There were no morphological changes to the gravid uterus, liver, lung, heart, kidneys and ovaries as well as the relative and absolute weights as shown in Tables 1 and 2.

Maternal reproductive performance

Maternal reproductive parameters are shown in Table 3. The SD female rats showed high fertility and pregnancy indexes. The percentage of post-implantation loss is higher than pre-implantation loss at 7.48% and 1.96% respectively.

Foetal examination

All foetuses were healthy and only one dead foetus was found after performing the caesarean hysterectomy on GD21. The average foetal body weight was 5.24 ± 0.8 g with male foetuses showing a higher average body weight than female

Table 2. The absolute maternal organ weight of SD rats orally administered with distilled water (Control) from GD6 to GD20. Each value represents the mean ± SD of 105 animals.

Parameters	Weight (g)
Gravid uterus	84.76 ± 17.8
Liver	12.70 ± 2.0
Lung	1.48 ± 0.2
Heart	0.85 ± 0.1
Right Kidney	0.94 ± 0.1
Left Kidney	0.91 ± 0.1
Right Ovary	0.07 ± 0.02
Left Ovary	0.07 ± 0.02

Table 4. Foetal parameters in SD rats orally administered with distilled water (Control) from GD6 to GD20 of gestation. Each value represents the mean ± SD of 1234 foetuses.

Parameters	Results
Foetal body weight (g)	5.24 ± 0.8
Male foetuses weight (g)	5.290 ± 1.0
Female foetuses weight (g)	5.114 ± 0.6
No. of male : female foetuses	580 : 654
Male: female ratio	0.88

Table 5. Summary of congenital malformations observed in foetuses of control SD rats

Parameters	Results
Total number of foetus examined	1234
Percentage of foetuses showing malformations (%)	0.41
Syndactyly (%)	
Hindpaw Forepaw	0.08 0.00
Ploydactyly (%)	
Hindpaw Forepaw	0.24 0.16

Table 3. Maternal reproductive performance parameters in SD rats orally administered with distilled water (Control) from GD6 to GD20 of gestation. Each value represents the mean ± SD of 105 animals.

Parameters	Results
Total No. of females No. of sperm-positive females (n)	135 126
No. of pregnant females (n)	114
No. of naturally delivered females on day 21 pc prior to arranged caesarean hysterectomy (n)	9
Fertility index (%) Pregnancy index (%)	90.5 100
Gravid uterus weight (g)	84.76 ± 17.4
Maternal weight (g)	
Day 0	185.80 ± 25.1
Day 6	221.93 ± 23.7
Day 9	236.77 ± 22.5
Day 12	254.60 ± 23.4
Day 15	272.00 ± 27.9
Day 18	298.16 ± 45.9
Day 21	342.01 ± 37.3
Pregnancy weight gain (g)	
Day 0-6	36.12 ± 9.2
Day 6-9	14.84 ±7.0
Day 9-12	17.61± 7.1
Day 12-15	16.21±6.2
Day 15-18	32.36±10.2
Day 18-21	39.27±13.3
Day 0-21	157.95 ± 28.2
Day 0-21 (minus uterus weight)	71.68 ± 24.2
Implantation sites	
Total (N)	1332
Per litter	12.68 ± 2.4
Resorptions	
Early	0.56 ± 1.0
Late	0.36 ± 1.5
Pre-implantation loss (%)	1.96 ± 6.3
Post-implantation loss (%)	7.48 ± 13.2
No. of corpora lutea/litter	12.93 ± 2.3
No. of dead foetuses	1
No. of live foetuses	1234
No. of implantation sites per litter No. of foetuses/litter	12.69 ± 2.4 11.75 ± 2.6

foetuses. One thousand two hundred and thirty four foetuses were examined for any external abnormalities. Only 5 of the foetuses were found to have gross congenital malformation such as syndactyly (abnormal fusion of the fingers or toes) and polydactyly (extra fingers or toes) on forepaw and hindpaw. Foetal parameters are shown in Table 4 and percentange of malformation in Table 5.

DISCUSSION

The use of animals for hazard identification in human risk assessment is widely considered to be the best approach currently available. Any adverse effects seen in the animal studies have been assumed to indicate a potential risk to humans. Several authors have reviewed the literature on agents known to cause developmental effects in humans, although the data is limited, their finding strongly supports this assumption (Schardein, 1993). Although there is no definite suggestion regarding the choice of the animal species to be employed in foetal development research, it is desirable to use animals having similarities with humans in respect to placentation, reproduction pattern, metabolism and enzyme systems (Sisodia, 1972). In order to simulate the human condition, it is necessary to choose species that are comparable to human physiology. Toxicity tests are normally performed on either mice or rats due to their availability, low cost and the established toxicology data in the literature for these species (Rollo, 1977). SD rat is one of the species that has proven to be useful in pharmacological and toxicological research due to its many similarities to human metabolism pathways (Kotwani *et al*., 1995).

Controlled experiments in laboratory animals play a crucial role in the testing and regulation of drugs, chemicals and other substances with potential teratogenic risks to humans. Therefore, data on fertility and pregnancy is needed to provide the comprehensive and direct insight into reproductive capability. Developmental toxicity experiments are typically conducted in genetically homogeneous animal strains. Thus, the large historical data base of control information that has built up over time can often prove useful in interpreting the results from current experiments (Ryan, 1993).

The present study showed that the reproductive performance of our SD rats is similar to other colonies that have been reported and shown to have good reproductive performance. Spontaneous delivery occurred in nine animals on GD21 prior to caesarian hysterectomy and therefore was excluded from the study. No implantation site was noted in the non-pregnant sperm positive rats. Five foetuses (0.41%) presented with external malformation (syndactyly and polydactyly) could be considered as incidental cases as similar observation was demonstrated by Manson and Kang (1994). According to Helen and Ernest (2004), external malformations could be observed in the

control group of untreated animals. These malformations could also be due to the general stress from restraining of animals, noise, light and heat during the gestation period which may affect developmental outcome. Several studies have indicated a positive correlation between general stress and adverse developmental effects including low birth weight and congenital malformations (Stott, 1973; Gorsuch and Key, 1974). The questions remains, on the other hand, as to whether abnormal development of the foetuses during pregnancy is because of vascular, hormonal or other changes associated with stress, a factor which certainly needs to be investigated (Scialli, 1988).

CONCLUSION

In conclusion, data provided by this study showed that the female SD rats obtained from IMR demonstrated high fertility and pregnancy indexes with low incidence of resorption and malformation. Hence, the SD colony available in IMR is suitable to be used for evaluation of test materials in reproductive toxicology assessment.

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