**Effect of Antenatal Iron Supplementation on Rat Morphology**

**Umbreen Noor**, Khadija Qamar**, Uzma Shahid*, Irum Iqbal ***, Ifra Saeed***, Shadab Ahmed Butt**

*Anatomy Department, Wah Medical College, Wah Cantt;**Anatomy Department, Army Medical College, Rawalpindi; ***Anatomy Department, Islamabad Medical and Dental College, Rawalpindi*

**Abstract**

**Background**: To study the effect of routine antenatal daily iron supplementation on gross morphology of placenta.

**Methods**: In this randomized control trial thirty two adult female and ten adult male Sprague-Dawley rats (weighing 200-250 gm) were taken. They were distributed randomly into two equal study groups. Ferrous sulphate was administered orally at 0 and 10mg/kg/day starting from gestation day (GD) 0, and half of the animals were sacrificed on GD 17 and half were sacrificed on GD 20. Placentae were removed and morphometric study was done for both qualitative and quantitative parameters.

**Results**: The results were assessed on GD 17 and 20. There was no significant change in placental weight, diameter and thickness on GD 17 between the two groups. There was significant increase in placental weight, diameter and thickness on GD 20 in non-supplemented (control) rats as compared to iron replete rats.

**Conclusion**: Antenatal daily iron supplementation is effective to prevent anaemia and iron deficiency.

**Keywords**: Iron supplementation; Placenta.

**Introduction**

Placental structure and function determine the growth trajectory of the fetus. Abnormal placental growth is associated with adverse pregnancy outcomes. Disproportionately heavy placentas may indicate an adaptive response to an adverse intrauterine environment and it may occur in the presence of conditions such as maternal anemia, hormonal incoordination, exposure to some chemicals such as ethanol and cigarette smoking. Conversely, a disproportionately small placenta may indicate poor nutrient supply to the placenta resulting in placental growth restriction and subsequently fetal growth restriction. 1,2

When a woman becomes pregnant, her iron needs increase to support her pregnancy and the growth of her baby. Iron supplements are almost universally prescribed for pregnant women at doses ranging from 30mg/day to as high as 240mg/day where prevalence of anemia is high. Iron (Fe) deficiency in pregnancy has serious consequences for both the mother and the baby. In the immediate postnatal period, these include increased risk of low birth-weight and increased morbidity. In the neonatal period, there is an increased risk of impaired motor development and coordination. In children, language development and scholastic achievement can be affected; there are significant psychological and behavioral effects and decreased physical activity. 3,4 As adults, the effects persist and can result in elevated blood pressure and cardiovascular problems. Iron supplementation of pregnant individuals with adequate iron status may aggravate oxidative stress, a factor which could contribute to preterm delivery. Iron supplements may raise the risks of gestational diabetes, hypertension, and metabolic syndrome in some women.5,6

The premise of employing any animal model system is that if the process being studied is fundamental it will likely demonstrate conservation across species. Because of the short estrus cycle, brief gestation period, and low cost of maintenance, the rats have been used extensively in studying reproduction.

**Material and Methods**

Ten adult male and thirty two adult female Sprague Dawley rats weighing 200-250 grams were taken from the animal house of National Institute of Health (NIH), Islamabad. Pregnant rats were kept individually in cages with wood shavings as bedding and kept at standard room temperature that was maintained on 12 hour light/dark cycle. Animals were given routine laboratory diet (NIH) in the form of pellets supplemented with vitamins and water ad libitum. Female rats were given chance to mate in the ratio of 4:1 with males overnight. The presence of the vaginal plug on the following morning indicated pregnancy and was designated as gestation day 0 (GD...
The pregnant rats were randomly allocated into two groups of sixteen rats each. The first group remained on the control diet throughout the experiment, whilst the second group was treated with ferrous sulphate in a dose of 10 mg/Kg/day. Group A (Control Group) – (16 Rats) was further sub-divided into two sub-groups of eight rats each. Group A-I (Control Group) – (8 Rats), which were given 5 ml distilled water by oral gavage tube at 9 am daily for 17 days starting from GD 0 to GD 16 and then sacrificed on the next day after the last dose and Group A-II (Control Group) – (8 Rats), which were given 5 ml distilled water by oral gavage tube at 9 am daily for 20 days starting from GD 0 to GD 19 and then sacrificed on the next day after the last dose.

Group B (Exposed to iron supplements) – (16 Rats) was further sub-divided into two sub-groups of eight rats each. Group B-I (Exposed to iron supplements) – (8 Rats), which were given 5 ml distilled water by oral gavage tube containing ferrous sulphate 10 mg/kg of body weight/day (same as the normal human dose) at 9 am daily for 17 days starting from GD 0 to GD 16 and then sacrificed on the next day after the last dose. Group B-II (Exposed to iron supplements) – (8 Rats), which were given 5 ml distilled water by oral gavage tube containing ferrous sulphate 10 mg/kg of body weight/day (same as the normal human dose) at 9 am daily for 20 days starting from GD 0 to GD 19 and then sacrificed on the next day after the last dose.

Iron (crystalline ferrous sulphate FeSO4.7H20) was finely grounded by mortar and pestle. Dose was adjusted on the basis of human dose which is 10 mg/Kg body weight/day and weighed by means of electric balance and mixed in 5 ml of drinking water. Before the animals were euthanized their final weights were taken. The animals were sacrificed by an overdose of ether anaesthesia. Cotton soaked in ether was placed into the jar. The animal to be sacrificed was lifted by tail and dropped into the jar. The gravid uterine horns were exposed by lower midline abdominal incision. The placenta and fetuses were examined in situ. The uterus, along with its contents, was rapidly removed and blunt probes were used to tease away the surrounding adipose tissue. It was then cross-sectioned to view the individual implantation sites. One fetus and placenta were taken from each uterine midhorn to avoid biasing selection of embryos by weight. The umbilical cord was cut and the fetuses separated. Placenta was carefully examined for any abnormality, and location of umbilical cord was observed. Placenta from each animal was removed and the uterine wall was trimmed around margins of the placenta and the latter was dried by dabbing on a blotting paper and weighed on an electronic balance. Placentae were placed on clean white paper and diameter of placenta was measured by using vernier caliper. The placentae were cut into two equal halves by bisecting at right angles and thickness of placenta at the central thickest part was then measured by using vernier caliper.

**Results**

All rats showed no abnormal clinical signs during the experimental period. Morphology of the placenta did not reveal any abnormality. Placentae were soft in consistency and discoid in shape. The placenta had two surfaces. The fetal surface was flat, smooth and covered by amnion. The maternal surface was convex and attached to the uterine musculature. It was thickest at its centre and its thickness decreased at its periphery. Each placenta had thin umbilical cord, which arose from its centre containing the vitelline vessels clearly visible within the substance. Unlike the human placenta, it was not divided into cotyledons.

**Table 1:** Effect of antenatal iron on gross morphology of Placenta: Comparison between control (A-I) and experimental group (B-I) (8 rats in each)

<table>
<thead>
<tr>
<th>Placental Parameters</th>
<th>Control</th>
<th>Experimental</th>
<th>Statistical Significance p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (gm)</td>
<td>0.3436 ± 0.19</td>
<td>0.335 ± 0.022</td>
<td>&gt;0.05•</td>
</tr>
<tr>
<td>Diameter (mm)</td>
<td>12.5 ± 0.19</td>
<td>12.37 ± 0.26</td>
<td>&gt;0.05•</td>
</tr>
<tr>
<td>Thickness (mm)</td>
<td>4 ± 0.19</td>
<td>3.875 ± 0.30</td>
<td>&gt;0.05•</td>
</tr>
</tbody>
</table>

• Statistical difference between the two groups is insignificant with p > 0.05.

**Table 2:** Effect of antenatal iron on gross morphology of Placenta: Comparison between control (A-II) and experimental group (B-II) (8 rats in each group)

<table>
<thead>
<tr>
<th>Placental Parameters</th>
<th>Control</th>
<th>Experimental</th>
<th>Statistical Significance p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (gm)</td>
<td>0.607 ± 0.024</td>
<td>0.533 ± 0.018</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Diameter (mm)</td>
<td>16.25 ± 0.16</td>
<td>15.313 ± 0.3</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Thickness (mm)</td>
<td>5.5 ± 0.19</td>
<td>4.625 ± 0.26</td>
<td>&lt;0.05*</td>
</tr>
</tbody>
</table>

* Statistical difference between the two groups is significant with p < 0.05.

Results showed insignificant change in placental weight, diameter and thickness (p>0.05) of control and experimental groups on GD 17. (Table 1). There was an
increased in placental weight, diameter and thickness (p<0.05) of control as compared to experimental groups on GD 20. (Table 2).

Discussion

Iron remains a commonly prescribed supplement in pregnancy. Blood Haemoglobin and serum ferritin values decrease in the iron deficient rats as compared to iron supplemented group. Various epidemiological studies indicate that placenta continues to increase in size if faced with an unfavourable maternal environment, such as pregnancy at high altitude, hormonal in coordination, exposure to some chemicals such as ethanol, cigarette smoking and maternal anemia. There are concerns regarding the severity of the maternal dietary regime used, citing it as an explanation for the inconsistent result of a decrease in placental: fetal ratio. A milder dietary restriction in pregnancy is likely to show no significant effect on placental morphology.

At GD 20, the placentae of group A-II showed significant increased (p < 0.05) in weight, diameter and thickness, as compared to group B-II. In our previous study we have demonstrated that there is significant fetal weight gain in GD 20 group as compared to GD 17 and as this group had a longer exposure to adverse intrauterine environment, a degree of adaptive compensation has occurred leading to significant changes in the histomorphology of placentae. Our results coincides with that presented by Huang et al., which demonstrated that large placental weight was associated with a low maternal haemoglobin.

References