Effects of selenium toxicity on oestrous cyclicity, ovarian follicles, ovulation and foetal survival in rats

R K Parshad

Department of Zoology, Punjab Agricultural University, Ludhiana 141 004, India

Received 12 October 1998; revised 1 February 1999

Effects of intraperitoneal injections of sodium selenite (2.0 and 4.0 mg/kg body weight) to normally cycling female albino Wistar rats daily for 30 days, and of single injection either during proestrous or oestrous and at each stage of the 4-day oestrous cycle were determined on oestrous cyclicity, ovarian follicles, ovulation, implantation and pregnancy outcome on day 14 of gestation. Administration of selenite for 30 days had no effect on the duration of first two oestrous cycles but afterwards the rats remained at the dioestrous stage. Their ovaries developed cystic follicles. Selenite treatments during the oestrous cycle preceding mating affects the implantation and pregnancy outcome in a dose-related manner. Its single dose containing 2.0 mg/kg body weight administered either at proestrous or oestrous, though had no effect on different reproductive parameters investigated in this study but its daily dose during the 4 day oestrous cycle reduced the number of corpora lutea and implantations as compared to saline injected control female rats. Similar effects of a single dose of selenite (4.0 mg/kg body weight) when injected at proestrous were recorded. Higher dose of selenite at oestrous or throughout the cycle decreased the number of implantations, but in addition, also increased the resorption rate/litter on day 14 of gestation. The present studies clearly show that high selenium levels in the body during the oestrous cycle preceding mating affects the number of ovulations, implantations and live embryos depending upon its dose and stage of administration.

Selenium is an essential micronutrient for humans and livestock required at low concentration of 0.02 ppm or less in diet and has maximum tolerable limit of 2 mg/kg. It is generally derived through food chain from soils where it is unevenly distributed. Low and high levels of selenium in soils are associated with several animal and human health problems. Because of its being an integral part of the active site of a widely dispersed enzyme glutathione peroxidase, which helps to keep low levels of cellular peroxidase that damages cell membranes, selenium deficiency often results in skeletal and cardiac myopathy and several reproductive disorders. Numerous studies have also shown that excess dietary selenium which may be ingested as L-selenomethionine, sodium selenite or sodium selenate, accumulate in different tissues and causes toxic effects and impair reproductive functions in domestic animals and humans. Some studies suggest its mutagenic, clastogenic, carcinogenic and/or anticarcinogenic effects. Selenium intoxication also impairs reproductive functions and causes congenital malformations. In male rats it affects the differentiation and development of spermatozoa in the testis and morphology and functions of caudal epididymal spermatozoa. Selenium is reported to deposit underneath the zona pellucida of secondary and mature Graafian follicles of selenized female rats and reduce the fertilization rate of ova of ewes and its fetoplacental accumulation during gestation cause maternal deaths. However, the effects of selenium intoxication during reproductive cycle on ovarian follicles, ovulation and foetal survival are not known which have been investigated related to stages of follicle maturation and ovulation during the oestrous cycle of rats.

Wistar female rats reared under standard laboratory conditions were used. They had ad lib access to pelleted feed throughout the experimental period. Oestrous cyclicity was checked by microscopic examination of vaginal smears. Rats showing regular 4 day oestrous cycle were selected for treatments. They were grouped on the basis of stage of reproductive cycle.

To determine the effect of long-term treatment of selenite on oestrous cycle and ovaries, its intraperitoneal injections were given daily to two groups of rats (2.0 and 4.0 mg/kg body weight) for 30 days. After 24 hrs of last injection animals were sacrificed and their ovaries were examined. Mortality rate on different days during the experimental period was also recorded. Four intraperitoneal doses of sodium selenite (2.0 mg/kg body weight) at metoestrous, dioestrous, proestrous and oestrous stages were given to a group of 25 rats. After these
treatments, they were mated with males of proven fertility. Pregnancy was determined next morning by the presence of spermatozoa in the vaginal smears and was designated as Day 1 of gestation. On day 14 of gestation, 9 females were sacrificed, their ovaries were trimmed off and the number of corpora lutea was counted. The number of live foetuses was determined in utero and resorption frequency was assessed by gross inspection of uteri. Similarly effect of higher dose of selenite (4.0 mg/kg body weight) during the oestrous cycle has been determined on another group of rats. Two groups of rats of 12 each were administered with a single dose of selenite (2.0 mg/kg body weight) intraperitoneally either at proestrous or at oestrous. After these treatments they were mated and subsequent observations were made as mentioned above in pregnant females. Same experimental plan was followed for another two groups of animals where the dose of selenite was adjusted to 4.0 mg/kg body weight) and the control groups were administered with equal amount of saline.

Selenite exposure to cycling rats daily for 30 days has no effect on the duration of first two oestrous cycles but for the rest of the period of study till day 31, females remained at the dioestrous stage. Mortality of animals was 13.6% and 40% respectively for low and high doses and it occurred mainly after day 21 of the treatment. Previous studies have revealed selenite as extremely toxic element, but in comparison to males, it is better tolerated in females and its level of toxicity is variable depending upon the route of its administration. Examination of the ovaries from the treated rats on 31st day of study revealed the presence of cystic follicles in 21% (with low dose) and 60% (with high dose) of females survived up till this period. The ovaries of other animals, where cysts were absent, showed no signs of corpora lutea. It appeared that continuous treatment with selenite for 30 days rendered the ovaries nonfunctional and ovulation had not occurred. Daily administration of selenite though has no effect on first two oestrous cycles, but the possibility of causing intra-oocytic changes as suggested by Thorlacius-Ussing et al. cannot be negated. Therefore, reproductive performance of the females was studied after four doses of selenite during different stages of oestrous cycle preceding mating and also after a single dose of selenite either at proestrous or at oestrous stage during which final maturation of oocytes and ovulation takes place.

Selenite treatment throughout the oestrous cycle preceding mating caused dose-related mortality of females on the following day after the introduction of males. Conception rate (percent females conceived) in both the treatment groups injected with 2.0 mg and 4.0 mg selenite decreased from 92.0 % (control) to 72.8%

Table 1—Effects of sodium selenite treatment during oestrous cycle of rats on pregnancy outcome on day 14 of gestation (n=9) [Values are mean±SE.]

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (saline treated)</th>
<th>Selenium treated female rats</th>
<th>2.0 mg/kg b.w.</th>
<th>4.0 mg/kg b.w.</th>
</tr>
</thead>
<tbody>
<tr>
<td>% mortality after mating</td>
<td>0.0</td>
<td>0.0</td>
<td>12.0</td>
<td>28.0</td>
</tr>
<tr>
<td>% rats conceived</td>
<td>92.0</td>
<td>72.8</td>
<td>50.0</td>
<td></td>
</tr>
<tr>
<td>No. of corpora lutea/female</td>
<td>11.89±0.64 a</td>
<td>9.67±0.38 b</td>
<td>8.56±0.55 b</td>
<td></td>
</tr>
<tr>
<td>No. of live embryos</td>
<td>11.33±0.65 a</td>
<td>7.90±0.46 b</td>
<td>3.60±0.41 c</td>
<td></td>
</tr>
<tr>
<td>No. of implantation sites/litter</td>
<td>11.67±0.60 a</td>
<td>8.10±0.42 b</td>
<td>8.21±0.34 c</td>
<td></td>
</tr>
<tr>
<td>No. of resorption sites/litter</td>
<td>0.33±0.60 a</td>
<td>0.22±0.13 a</td>
<td>2.66±0.35 b</td>
<td></td>
</tr>
</tbody>
</table>

Mean values followed by the same letter in a row are not significantly different at 5% level of significance as determined by analysis of variance followed by multiple comparison test.

Table 2—Effects of sodium selenite treatment of proestrous and oestrous rats on pregnancy outcome on day 14 of gestation [Values are mean±SE]

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (saline treated)</th>
<th>Selenium treated female rats (n=9 rats in each group)</th>
<th>2.0 mg/kg b.w.</th>
<th>4.0 mg/kg b.w.</th>
</tr>
</thead>
<tbody>
<tr>
<td>% rats conceived</td>
<td>91.6</td>
<td>91.6</td>
<td>75.0</td>
<td>83.3</td>
</tr>
<tr>
<td>No. of corpora lutea</td>
<td>12.8 a</td>
<td>11.67±0.44 a</td>
<td>11.77±0.43 a</td>
<td>10.40±0.61 a</td>
</tr>
<tr>
<td>No. of live embryos</td>
<td>11.50±0.83 a</td>
<td>10.80±0.55 a</td>
<td>10.50±0.57 a</td>
<td>8.80±0.49 b</td>
</tr>
<tr>
<td>No. of implantation sites/litter</td>
<td>11.70±0.73 a</td>
<td>11.44±0.47 a</td>
<td>10.80±0.61 a</td>
<td>9.10±0.61 b</td>
</tr>
<tr>
<td>No. of resorption sites/litter</td>
<td>0.22±0.14 a</td>
<td>0.33±0.23 a</td>
<td>0.33±0.22 a</td>
<td>1.89±0.33 b</td>
</tr>
</tbody>
</table>

Mean values followed by the same letter in a row are not significantly different at 5% level of significance as determined by analysis of variance followed by multiple comparison test.
The number of implantations and of live embryos/litter also decreased significantly (Table 1). The effect appears to be more severe with daily higher dose during the oestrous cycle as it also increased the frequency of resorption. Administration of lower dose of selenite (2.0 mg/kg body weight) at proestrus or oestrous has no significant effect on number of corpora lutea, implantation sites, live embryos and resorption rate/litter, but it decreased the conception rate when injected at the oestrous stage (Table 2). Contrarily, higher dose of selenite (4.0 mg/kg body weight) administered at proestrus or oestrous significantly reduced the conception rate, number of live embryos and implantation sites on day 14 of gestation. Resorption rate/litter was higher only in females injected during oestrous stage (Table 2). Exposure to selenium at proestrus stage appears to have its effect on the reproductive events prior to implantation as indicated by the reduced number of implantation sites and of live embryos as compared to those of control. Once the pregnancy is established, prior treatment at the proestrus stage do not cause embryonic mortality or induce resorption. Reduced fertility may result either due to the detrimental effects on ovum or other associated events in the pre-implantation period but the number of ovulations remained unaffected as no change was recorded in number of corpora lutea in the control and treated groups. A higher dose of selenite at oestrous also increased frequency of resorption/litter which reflects that selenium exposure for few hours prior to mating has its adverse effects on the pre-implantation events and mother-fetal interactions after implantation. Selenium compounds are known to exhibit inhibitory effects on cellular growth and proliferation and possibly, selenium through its similar effect may be causing changes in the development and implantation of the embryo and consequently the pregnancy outcome in rats.

References
6 Watanabe T & Endo A, Mutat Res, 262 (1990) 93.
9 Noda M, Takano T & Sakurai H, Mutat Res, 66 (1979) 175.