

Effect of titanium exposure on embryonic development during pre-implantation period in rats

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Doses of titanium trichloride ($1/10^{\text{th}}$ and $1/5^{\text{th}}$ of LD_{50}) were administered once and daily to pregnant rats to assess their effect on embryonic development. $1/5^{\text{th}}$ dose of $TiCl_3$ administered once orally on 1st, 2nd and 3rd day *post-coitum*. Similarly $1/10^{\text{th}}$ of LD_{50} was administered daily. Results revealed that $1/10^{\text{th}}$ LD_{50} dose of $TiCl_3$ was more effective during pre-implantation period as number of 4 and 8-celled embryos decreased as compared to $1/5^{\text{th}}$. Delayed hatching of the blastocysts on day 5 was registered in $TiCl_3$ treated dam.

Identification of environmental agents that have adverse effect on reproductive health is particularly challenging. Environmental toxicants or xenobiotics interfere with reproduction. In laboratory animals, toxicants have been reported to impair the early gestational development of the embryos as revealed by *in vitro* studies. Titanium is a grey metal and its powder is highly flammable. It is used in explosive and chemical industries. Due to its corrosive resistance properties and lustering nature, it is used in electronic devices, paints, and cosmetics. Tipton *et al*¹ have observed toxic effect of some titanium compounds. Titanium dust in high concentration has been reported in workers manufacturing titanium hydride². Titanium induced mutagenicity has also been shown^{3,4}. Pre-implantation mammalian embryonic impairment by environmental chemicals has been reported^{5,6}. Some of the metals have shown their effect on this developmental stage^{7,8}. Till date no work has been reported on titanium metal whether it effects the embryos during pre-implantation period. Oral administration of titanium trichloride to female rats is slightly toxic⁹, which may effect the reproductive system in rats. Therefore, the present study is an attempt to elucidate the effect of titanium trichloride on the embryos when exposed during earlier days of gestation (day 1-5 *postcoitum*).

Adult cyclic female rats of Wistar strain (140 ± 10 g) were obtained from the animal colony of the department and were kept under uniform husbandry conditions of light (14L : 10D), and temperature ($26^\circ \pm 1^\circ\text{C}$). These animals were given "Lipton Indian Ltd". Gold Mohur rat pelleted diet and water *ad libitum*.

Female rats were caged with adult males of Wistar strain of proven fertility in the ratio of 2:1 and next morning the vaginal smear was examined through microscope as well as morphologically. The presence of vaginal plug and the spermatozoa in the smear confirmed the mating and designated as day 1 of pregnancy. Mated female rats were kept in separated cages and randomly divided into different groups.

Titanium trichloride (Thomas Private Limited, Bombay) was diluted with water and $1/5^{\text{th}}$ and $1/10^{\text{th}}$ of LD_{50} (4.30g/kg) doses were prepared freshly at the time of administration⁹. Animals were divided into following four sets.

Set -I—Animals of set 1 were further divided into 4 groups. Group 1 served as control which received vehicle only (po) Animals of group 2 were administered with single dose of $1/5^{\text{th}}$ of LD_{50} of titanium trichloride (po) on day 1 of pregnancy. Animals of group 3 received single dose of $1/5^{\text{th}}$ of LD_{50} of titanium trichloride on 2nd day of pregnancy. Similarly the animals of group 4 received/dose of

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titanium trichloride ($1/5^{\text{th}}$ of LD_{50}) once (po) on 3^{rd} day of pregnancy.

Set-2—Animals of set 2 were divided into 4 groups. Group 1 animals served as control which received vehicle only (po). Animals of group 2 received $1/10^{\text{th}}$ of LD_{50} of titanium trichloride through po on day 1 of pregnancy. The animals of group 3 were administered (po) $1/10^{\text{th}}$ of LD_{50} of titanium trichloride daily for 2 days (day 1 and 2 *post-coitum*). The animal of group 4 received the same amount of titanium trichloride daily for 3 days (day 1, 2, and 3 of *postcoitum*).

On day 4 the animals of set 1 and 2 were sacrificed by cervical dislocation. Pre-implantation embryos were collected by rupturing the ampulla of the fallopian tube and were flushed with phosphate buffer. Even the uterine horns were also flushed. These embryos were collected and kept in 1% solution of aqueous sodium citrate for about 5 min and then were replaced in 5 μ l citrate solution on a clear microslide. Mixture of methanol and acetic acid in 3:1 ratio was added dropwise on one side of the slide and was allowed to flow over the embryos. The excess of the citrate solution was removed with the help of pipette and soaked with filter paper strip and finally the embryos were spread over the slide. Slides were dried, stained with 1% aqueous solution of toluidine blue for 15 min. The embryos were washed with water and cells in the embryos were counted under microscope.

Set-3 and 4—Treatment was same as in set 1 and 2. The animals of set 3 and 4 were sacrificed on 5^{th} day of pregnancy and their uterine horns were flushed with phosphate buffer and the embryos were collected. Although the fallopian tube may not show the presence of embryos, it was also flushed. Embryos were collected and treated with 1% aqueous solution of citrate for 5 min. These were processed in the similar manner as describe under set 1 and 2. Finally the embryos were observed for the presence zona pellucida. The embryos which did not show zona pellucida (shedded zone pellucida) were considered as hatched-out blastocysts. Results were analysed statistically using *t* test.

The results are presented in Tables 1 and 2.

Pre-implantation exposure of titanium trichloride ($1/5^{\text{th}}$ and $1/10^{\text{th}}$ of LD_{50}) from day 1 of gestation till sacrifice exhibited reduced number of cells in rats embryos on day 3 of pregnancy as compared to control rats. This incidence was more in $1/10^{\text{th}}$ of LD_{50} treated titanium trichloride rats.

Pre-implantation period in the mammalian reproduction process is a unique phase when developing embryos are freely floating in uterine fluid having no contact with mother. At this stage embryos gets all nourishment through the uterine fluid via maternal luminal epithelium. It has also been reported that number of metals when administered before implantation impair the embryonic development through uterine fluid^{5,6}. The

Table 1—Effect of titanium trichloride on pre-implantation period (1 to 5^{th} day) of embryonic development in rats. (Observation on celled stages)

Treatment	No. of embryos examined	No. of rats used	Celled stages observed after 72 hr		
			2 celled	4 celled	8 celled
1/5 th LD ₅₀ , once (po)					
Control (vehicle only)	85	6	20(23.5)	22(25.8)	43(50.5)
On 1 st day <i>post- coitum</i>	90	6	23(25.5)	21(23.3)	46(51.1)
On 2 nd day <i>post- coitum</i>	75	5	19(25.3)	18(24.0)	38(50.6)
On 3 rd day <i>post- coitum</i>	80	5	36(45.0)*	24(30.0)	20(25.0)*
1/10 th LD ₅₀ , daily administered, po					
Control (vehicle only)	90	6	21(23.3)	24(26.6)	45(50.0)
On 1 st day <i>post-coitum</i>	85	6	21(24.7)	22(25.8)	42(49.4)
On 1 st and 2 nd day <i>post-coitum</i>	70	5	25(35.7)	21(30.0)	24(34.2)*
On 1 st ,2 nd and 3 rd day <i>post-coitum</i>	75	5	34(45.3)*	18(24.0)*	23(30.6)*

*Values are statistically significant with respect to their control ($P<0.05$). Figures in parentheses are percent celled stage.

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Table 2—Effect of titanium trichloride on pre-implantation period (1 to 5th day) of embryonic development in rats. (Observations for hatching of blastocysts)

Treatment	No. of embryos examined	No. of rats used	No. of hatched blastocyst	Hatched (%)
1/5 th LD ₅₀ , once, po				
Control (vehicle only)	77	5	75	97.4
On 1 st day post-coitum	73	5	70	95.8
On 2 nd day post-coitum	62	4	56*	90.3
On 3 rd day post-coitum	65	4	43*	66.1*
1/10 th LD ₅₀ , daily administered, po				
Controlled (vehicle only)	93	6	90	96.7
On 1 st day post-coitum	70	5	67*	95.7
On 1 st and 2 nd day post-coitum	73	5	59*	80.8
On 1 st , 2 nd and 3 rd day post-coitum	78	5	51	65.3*

*Values are statistically significant with respect to their control ($P < 0.05$).

administration of the toxicants may be blastocidal/blastotoxic and this activity is exclusively mediated through the transportation of toxicants from uterine fluid to the transportation of toxicants from uterine fluid to the blastocysts as there is no mechanism of filtration of toxicants like placenta.

It has also been observed in present studies that the administration of titanium trichloride to female rats before implantation at 1/5th and 1/10th of LD₅₀ doses reduced the number of cells in embryos. The number of 2 and 4-cells in embryos was increased significantly (Table 1). On the basis of these findings it may be explained that administration of titanium trichloride may interfere with the division of cells in the embryos and arrest the cell-division at 2 or 4-celled stage. Consequently the number of 8-celled embryos was decreased. This may also be due to the delay in cell division following conception, or delay in first division or delay in part of population which would then be eliminated before implantation. On the basis of these finding it can be said that these embryos may lost their vitality before implantation. Jacquet *et al.*,¹⁰ have reported similar findings when lead was administered at zero day of pregnancy. Metals have effect on embryos¹¹. Nilsson *et al.*,¹² have also reported the transportation of lead from uterine epithelium to uterine fluid which was then taken up by the blasocysts. This supports the present findings, that the delayed hatching of blastocysts on day 5 was

registered in titanium trichloride treated dams. Effects was observed in both the doses of 1/5th and 1/10th of LD₅₀ of titanium trichloride (Table 2). Implantation is one of the complex process in which the blastocysts first hatch from zona pellucida and then implant on the crypts of luminal epithelium. Without hatching the further development of embryos is liable to be inhibited which may result in defective invasion in uterine endometrium. Trophoblast invasion is reported to be more susceptible to some metals than early post-implantation development. Wide *et al.*,¹³ reported that after examination of the surface of day 5 mouse blastocysts showed that shedding of zona pellucida in some cases failed to occur. It is thus concluded that the administration of titanium trichloride before implantation to female rats caused embryo toxicity and results in the impairment of embryonic development by arresting the cell division.

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