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Effects of paraquat on development of preimplantation embryos in vivo and in vitro

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Paraquat can cause oxidative stress through redox cycling, and preimplantation embryos are sensitive to oxidative stress in vitro. In this study, the effects of paraquat on preimplantation embryo development were examined. Exposure of preimplantation embryos (collected on the day after ovulation) to paraquat in vitro for 24 h at concentrations as low as caused a signi cant decrease in the percentage of 8-cell embryos and an increase in the percentage of compacted morulae, but the content of reduced glutathione (GSH) in embryos was not changed. Altered embryo development was most likely due to premature compaction because a 42% decrease in cell number per compacted morulae was observed in embryos exposed to paraquat at 1 mM. Exposure of preimplantation embryos to paraquat in vitro for 4 days at 200 M or higher eliminated development beyond the blastocyst stage. Exposure of bred female mice to paraquat at 30 mg/kg on day 2 after ovulation led to a small but signi cant decrease in the percentage of 8-cell embryos on day 3 without a detectable increase in the percentage

static during development. Nasr-Esfahani et al. [10] showed that glutathione levels drop through fertilization and development of mouse embryos by 45% to the 2-cell stage. Moreover, Gardiner and Reed [11] showed that GSH levels drop by 90%

Table 1
Effects of paraquat exposure on breeding outcomes

Measurement	Treatment	
	Saline	Paraquat
Number of females with copulation plugs	62	63
Dam weight on day 17 (g)	51.7 0.8	50.0 l.l
Dam liver weight (g)	2.8 0.1	2.7 0.1
Dam uterine weight (g)	16.7 0.7	15.8 0.8
Number of fetuses/dam	12.0 0.6	11.4 0.7
Number of resorptions/dam	2.0 0.4	2.1 0.4
Fetal weight (g)	0.91 0.01	0.91 0.01
Number of fetal malformations	0	0
Total fetal weight per dam (g)	11.1 .5	10.3 0.6
Percent of dams pregnant on day 17 (full-term)[a]	93.1 4.4%	70.6 10.2*

Bred dams were injected (i.p.) with saline or paraquat (30 mg/kg) on the day of ovulation (d0). Data are presented as mean SEM.

[a] The percent of dams maintaining pregnancy was calculated by taking the number of pregnant dams on day 17 divided by the total number of dams with copulation plugs on day 0 multiplied by 100.

Indicates that the mean is signi cantl980 5.-u9sMb80 5.-u9sMb80 5.-u9sMbi0o-u9sMbi0n%0

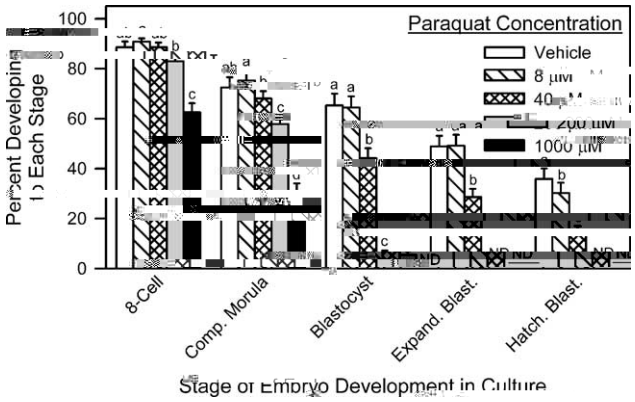

Fig. 3. Paraquat inhibited embryo development throughout the preimplantation period. Embryos were collected from bred, superovulated female mice 1 day after ovulation and cultured in medium supplemented with paraquat at a set concentration for 4 days. The percentage of embryos at each stage of development (8-cell, compacted morula, blastocyst, expanded blastocyst, or hatching blastocyst) was determined at 24-h intervals for each treatment group. Data are presented as the percentage of total embryos (S.E.) that had developed to the given developmental stage by day ve post ovulation (22 embryo pools per treatment group). Different letters indicate a signi cant difference between means within each developmental stage. ND, none detected.

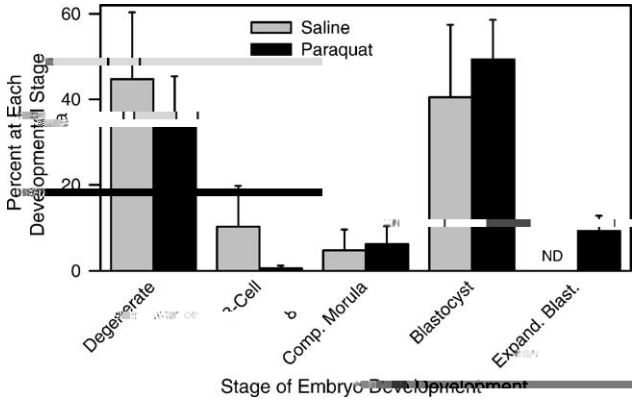

Fig. 5. Paraquat exposure on the day of ovulation (day 0) did not signi cantly alter embryo developmental day 3. Bred female mice were exposed to paraquat as in Fig. 2. Three days later, dams were killed and embryos were collected for assessment of developmental stage by microscopy: degenerate, 8-cell, compacted morula, blastocyst, or expanded blastocyst. Data are presented as mean S.E. (9 mice per treatment group). ND, none detected.

To determine if preimplantation embryos are sensitive to paraquat-induced toxicity in vivo, embryos were isolated on day 1 from bred, superovulated female mice that were treated with saline or paraquat (30 mg/kg) on the day of ovulation (Treatment Protocol #1, Fig. 1). Non-degenerate embryos in both saline- and paraquat-treated mice were found to be predominantly at the 2-cell stage when isolated on day 1 (Fig. 4A), and there was no signi cant difference between the percentages of any developmental stages between the two treatment groups. However, the GSH content of non-degenerate embryos on day 1 was signi cantly reduced (by 18%) in paraquat-treated mice relative to embryos from control mice (Fig. 4B). These data suggest that paraquat exposure in vivo may induce oxidative stress in 2-cell embryos, leading to a signi cant reduction in GSH content but no signi cant change in development.

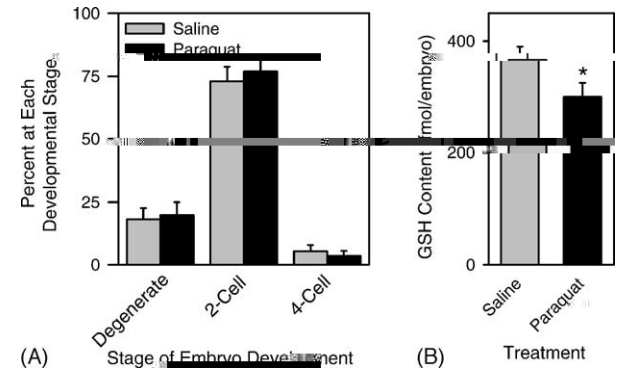

Fig. 4. Paraquat exposure on the day of ovulation (day 0) decreased embryo GSH content but did not alter embryo development at day 1. Bred female mice were injected with saline or paraquat (30 mg/kg body weight) at approximately 12:00 h after ovulation. Twenty-four hours later (A), dams were killed and embryos were collected for assessment of developmental stage by microscopy (26 mice per treatment group). Non-degenerate embryos were pooled (24 embryo pools per treatment group) for analysis of GSH content (B). Data are presented as mean S.E. * Indicates that the mean was signi cantly different from control.

To determine if paraquat exposure could alter the development of embryos beyond the 2-cell stage, embryos were isolated on day 3 from bred, superovulated female mice that were treated with saline or paraquat (30 mg/kg) on 1 of 2 different days: day 0 or day 2. After treatment on day 0 (the day of ovulation—Treatment Protocol #1, Fig. 1), non-degenerate embryos were found to be predominantly at the blastocyst stage, and there were no signi cant differences in the percentages of any developmental stage between saline- and paraquat-treated mice, nor was there a difference in the percentage of degenerate embryos (Fig. 5). Nevertheless, paraquat treatment seemed to cause a slight (but not significant) decrease in the percentage of 8-cell embryos (Fig. 5). After treatment on day 2 (Treatment Protocol #2, Fig. 1), non-degenerate embryos were again found to be predominantly at the blastocyst stage, and a decrease in the percentage of 8-cell embryos was seen in paraquat-treated mice that was statistically signi cant (Fig. 6A). A slight (but not signi cant) increase in the percentage of compacted morulae was also observed (Fig. 6A). These data suggested that development up to or through the 8-cell stage may be impacted by in vivo exposure to paraquat. GSH levels in day 3 embryos were also examined at 24 h after paraquat treatment on day 2. Unlike for day 1 embryos, paraquat did not signi cantly alter embryo GSH content on day 3 (Fig. 6B).

In a previous study by Dial and Dial [24], paraquat was shown to signi cantly decrease the number of litters pro-

to paraquat as liver does [25] and Hausburg, unpublished results), and oxidative stress may have occurred. It is not clear what the fate of GSH was in paraquat-exposed embryos, but it is likely to have formed mixed disul des and GSSG. Attempts to measure GSSG levels in day 1 embryos were unsuccessful. Gardiner and Reed [11] showed that exposure of 2-cell mouse embryos to BSH (13 M) in vitro for 15 min led to a greater than 80% decrease in cellular GSH content which could be entirely accounted for by increased levels of GSSG and protein mixed-disul des with the latter being the greatest component of the oxidized glutathione. When GSH levels in day 3 embryos were examined after in vivo paraquat exposure on day 2, they were found to be not significantly different from controls (Fig. 6B). This was expected because the predominant developmental stage on day 3 was the blastocyst, and mouse blastocysts are known to be able to both synthesize GSH de novo and reduce GSSG through the activity of glutathione reductase [12,14].

The experimental results presented here indicate that paraquat exposure can profoundly alter the development of preimplantation embryos in vitro leading to a failure of de-

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