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## Effects of Busulfan Sustained-release Emulsion on Depletion and Repopulation of Primordial Germ Cells in Early Chicken Embryos

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The current study aimed to determine the optimal time of busulfan (1,4-butanediol dimethanesulfonate) administration for the preparation of suitable recipient embryos to generate germline chimeras in chickens. We compared two different administration times, with regards to the degree of endogenous primordial germ cell (PGC) depletion and donor PGC incorporation in recipient embryos. A dose of 75 µg busulfan in 50 µL sustained-release emulsion was injected into the yolk of chicken embryos incubated for 0h or 24h. Both busulfan treatments resulted in significant reduction in the number of PGCs compared with that of controls ( $P < 0.01$ ). The number of PGCs in the blood at stage 14 was significantly lower after busulfan treatment at 0h of incubation than after treatment at 24h of incubation ( $P < 0.01$ ). No significant difference was observed in the numbers of PGCs in the gonads at stage 29 between the two treatment groups. The number of donor PGCs after busulfan treatment at 0h was significantly higher than that after treatment at 24h and in controls ( $P < 0.01$ ). The ratios of donor to recipient PGCs in the gonads of both busulfan treated groups were higher than in controls ( $P < 0.01$ ). In conclusion, injecting busulfan sustained-release emulsion into the yolk of recipient embryos at 0h of incubation was a suitable method of preparing them for the generation of germline chimeras.

**Keywords:** [busulfan sustained-release emulsion](#), [early chicken embryos](#), [germline chimeras](#), [primordial germ cells](#)

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