REPRODUCTIVE PERFORMANCE IN *CLOCK* MUTANT MICE

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The relationship between circadian rhythmicity and rodent reproductive cyclicity is well established, but the impact of disrupted clock gene function on reproduction has not been investigated. This study evaluated the reproductive performance of melatonin deficient and proficient mice carrying a mutation in the core circadian gene, *Clock*. In natural matings, melatonin deficient *Clock* mutant mice took 2 to 3 days longer to mate and to subsequently deliver pups than their control line. The melatonin proficient mutants (*Clock-MEL)* had a smaller, but still significant delay ($P < 0.05$). The *Clock* mutation resulted in smaller median litter sizes compared to the control lines (7 *v.* 8 pups, $P < 0.05$) while melatonin proficiency reversed this difference. Survival to weaning was 84% and 80% for the melatonin deficient and proficient *Clock* mutant lines respectively, compared to 94 to 96% for their control lines. When immature mice were subjected to a standard PMSG/HCG superovulation protocol, *Clock-MEL* mice had lowered fertility and significantly fewer ovulations than their control line although embryo development appeared to be only slightly affected (Table 1).

Table 1. Embryo development following superovulation in *Clock-MEL* mice

	Fertile matings	Embryos recovered at 96 h post HCG	Degenerated or unfertilised	2 cell to morulla	Blastocyst	Hatched blastocyst
WT-MEL	80% (12/15)	29 ± 4	10%	60%	29%	1%
Clock-MEL	53% (8/15)	19 ± 5	17%	48%	32%	3%

When kept in constant darkness, 7 of 15 *Clock-MEL* mice, became arrhythmic, but still became pregnant. The 7 mice that free ran for at least 14 days in constant darkness with a period of 27.1 h also became pregnant.

This study has shown that a mutation in the *Clock* gene that results in a protein incapable of initiating the transcription of target genes has significant, but subtle effects on reproductive performance. The capacity to produce melatonin or additional genes introduced along with the genes for the melatonin synthesising enzymes reduced the impact of the mutation further. It would appear that redundancy within the circadian timing system allows the reproductive cyclicity to persist in *Clock* mutant mice, albeit at a suboptimal level.

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