43. CALBINDIN-D9K AND -D28K: ARE CRITICAL FOR EMBRYO IMPLANTATION

Kien Luu, Guiying Nie and Lois Salamonsen
Prince Henry's Institute of Medical Research, Clayton, Victoria 3168

Calbindin-d9k (d9k) and calbindin-d28k (d28k) are highly upregulated in uterine epithelium at the time of implantation in mice[1,2]. This study aimed to investigate their functional roles in this process. Both wildtype (W/T) C57Bl6 and C57Bl6^{tmpin} mice (d28k-/-) were used, the latter strain can breed but with reduced fertility[3]. Uterine CaBP-d9k translation was disrupted using morpholino-modified anti-sense oligonucleotides (MO). Antisense d9k MO (30 nM/20µL in special delivery reagent EPEI) was administered by injection into one uterine horn day on either d2.5 or d3.5 prior to implantation (d4.5), (plug = d0). The other horn was injected with an irrelevant MO (n = 3/4 mice per treatment group). Numbers of implantation sites (N) were counted on d5.5. In preliminary studies intraluminal injection of FITC labelled MO showed their high integration into uterine luminal epithelium (the site of both d9k and d28k at implantation). Injection of specific MO into d28k-/- mice on d2.5 completely blocked implantation (Table 1), while no effect was seen following injection on d3.5. Similarly, no effect of MO injection was seen in W/T mice. The results show that endometrial expression of both d9k and d28k is necessary for implantation. The functions of these two as implantation can occur successfully when only one is present (i.e. in d28k-/- mice and in W/T treated with anti-d9k MO). The calbindins can now be added to the very small number of proteins shown to be critical for the process of implantation. These studies have clear implications for fertility regulation.

Table 1. Comparison of no. implantation sites in W/T and d28k-/- mice, treated with MO

Phenotype	*n*	Injection	N/Control Horn	N/Treated Horn
W/T	3	d2.5	3.5	2.5
d28k-/-	4	d3.5	3.5	3.5
W/T	4	d2.5	4.7	0
d28k-/-	3	d3.5	3.3	4.7

(1) Nie *et al. Biol. Reprod*. 2000; (2) Luu *et al.* Proc. ASRB 2001; (3) Luu *et al. Reprod. Fertil. Dev.* Suppl. **14**, 2002.

10.1071/SRB03Ab43