DEVELOPMENT OF A LABEL RETAINING CELL METHOD TO IDENTIFY STEM CELLS IN MOUSE ENDOMETRIUM

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Epithelial and stromal stem/progenitor cells have been demonstrated in human endometrium by their clonogenic activity (1). To understand the regulation and growth kinetics of endometrial stem cells (SCs), an animal model is being established using the label retaining cell (LRC) approach. Tissue SCs are quiescent and will retain a DNA synthesis label (BrdU), while the label is diluted out in more mature dividing cells during a chase period. Those cells retaining the label over long period of time are LRCs and have been identified as SCs (2). An optimum time to initially label the majority of the cells is critical. We hypothesised that during mouse endometrial development, there is a growth window for maximum labelling of epithelial and stromal cells. The aim of this study was to determine the optimum age and length of time for maximum endometrial BrdU labelling. Two stages of endometrial growth were exploited: *(1)* postnatal growth before gland development (3 days old – P3); and *(2)* oestrogen induced growth in ovariectomised prepubertal mice (4–5 weeks old). C57/CBA P3 mice received subcutaneous BrdU injections twice daily for 3 consecutive days. Four to five-week-old ovariectomised mice received BrdU filled mini-osmotic pumps implanted subcutaneously for 7 and 14 days, with daily oestrogen injections. Mice were killed 4 h after the last treatment, uteri collected, and BrdU labelled cells were detected by immunohistochemistry. Results are shown in the table below:

Age (days)	P3	(OVX, prepubertal) 28	(OVX, prepubertal) 35
Total dose (μg/g)	306	277	277
Length of labelling (days)	3	7	14
Epithelial BrdU cells (%)	85.70 ± 2.27 (n = 3)	59.65 ± 0.70 (n = 4)	66.35 ± 2.64 (n = 2)
Stromal BrdU cells (%)	78.43 ± 2.98 (n = 3)	70.52 ± 1.05 (n = 4)	59.44 ± 4.80 (n = 2)

The data shows that maximum labelling for epithelial and stromal cells was achieved for P3 mice, possibly due to greater endometrial growth of the Müllerian duct compared to oestrogen induced endometrial growth following ovariectomy. Chase experiments are currently in progress on P3 labelled mice to identify the location of LRCs in mouse endometrial glands and stroma.

(1) Chan RW, Schwab KE, Gargett CE (2004) *Biol. Reprod.* **70**, in press. (2) Morris RJ, Potten CS (1994) *Cell Prolif.* **27**, 279–289.

10.1071/SRB04Ab256