

## **Developing a rabbit model for progestagen only related breakthrough bleedings**

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Breakthrough bleeding (BTB) is a common problem for women using progestagen-only contraceptives and is the main reason for discontinuing the use of this form of contraception. In order to develop new progestagens that do not induce BTB a predictive model is essential. Although menstrual bleedings occur only in primates we decided to examine the possibility of using rabbits, an animal model that is well known for testing the progestagenic activity of compounds on the uterus in the McPhail test. In an in vivo model, spayed rabbits were s.c. treated with placebo, the pure progestagens 3-keto-desogestrel and levonorgestrel, and the anti-progestagens mifepristone and Org 31710 for ten consecutive days. On day 11 the uteri, cervix and vagina were dissected and fixed. HE stained sections showed severe haemorrhages in the endometrium (but not cervix and vagina) of rabbits treated with 3-keto-desogestrel or levonorgestrel. No bleedings were observed in the other groups. Ex vivo tissue culture of these endometria revealed that the expression of proMMP-3 in the conditioned medium is increased in bleeding endometrium compared to the non-bleeding endometrium of the placebo group. This correlates with P-only effects observed in human endometria. To further study the regulation of proMMP-3 by progestagens in the rabbit endometrium an in vitro explant model similar to the human menses induction model established by Marbaix et al., (1996; PNAS 93:9120-9125) was developed. In this explant model, endometrium of estradiol primed immature rabbits is cultured in the presence or absence of steroids. Without steroids proMMP-3 expression is high. Pure progestagens like levonorgestrel can block this expression significantly, whereas anti-progestagens like RU486 can not. Together our data suggest that both the in vivo and in vitro rabbit model are useful for the development of new progestagens that do not induce breakthrough bleedings.