

***In Vivo* Effects of Levonorgestrel Containing *In Situ* Gelling Drug Delivery Systems on Rat Pregnancy**

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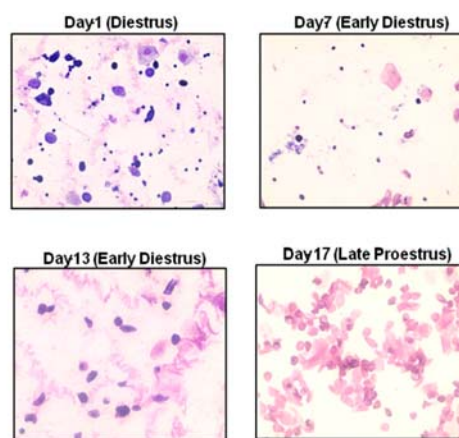
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Statement of purpose: Injectable contraceptives have been increasingly broadly used for preventing unintended pregnancies as they can be administered by patients themselves, and do not require surgical implantation and removal. Currently, over 40 million women worldwide use injectable contraceptives. However, the currently commercially available injectable contraceptives are only effective for 1-3 months and require patients to return to their provider 4-12 times per year. Therefore, there is an unmet need to develop injectables capable of providing more than 3-month contraception. In our group, we have developed proprietary injectable *in situ* gelling polymeric drug delivery systems that can continuously release Levonorgestrel (LNG) in the female rat blood for at least seven months. The purpose of this work is to assess the effects of the developed drug delivery systems on rat pregnancy during 7 months of treatment through vaginal cytology, body weight and mating studies.

Methods: *In situ* gelling polymeric delivery systems, F-55, F-64 and F-96 containing LNG or LNG pro-drug levonorgestrel-butanoate (LNG-B) were prepared using a combination of poly (lactide-co-glycolide) and polylactic acid and a mixture of solvents containing N-methyl-2-pyrrolidone and benzyl benzoate or triethyl citrate. Sprague dawley rats were subcutaneously injected with the above drug delivery systems. Vaginal cytology examinations were conducted at different time intervals post injection using DIFF staining kit (IMEB Inc) containing Azure A & Azure B along with Eosin stains. Smears were observed under fluorescence microscope (EVOS fl. AMG). Representative images of the vaginal cells are shown in Figure. Body weights of both treated and control were also measured at regular time intervals. After the 7 months of treatment period, the female rats were subjected to mating study. Breeding pair was selected and kept in a single cage for a period of 14 days. Occurrence of pregnancy was identified by observing the presence of vaginal mucous plug, body weight increase or palpation of the abdomen.

Results: Vaginal cytology study results showed that all of the vaginal cells are either at early diestrus, diestrus or proestrus stages but not estrus stage after the injection of *in situ* gelling systems in the female rats.

Body weights in the rats treated with LNG containing drug delivery systems F-55 LNG and F-96 LNG were higher than that of the control rats. However, in contrast, body weights in the rats treated with LNG-B containing drug delivery system F-55 LNG-B were lower than that of the control rats during the study period of time.



Vaginal cytology after injection of LNG (96) formulation.

Mating results show that only one out of 8 female rats in either rat group treated with F-96 LNG or F-55 LNG-B was pregnant. These results suggested that LNG release from the delivery systems into the rat blood might be the factor for the 7 month prevention of pregnancy.

Conclusions: The developed *in situ* gelling polymeric drug delivery systems showed contraceptive effect in female rats for 7 months with 87.5% efficiency in preventing pregnancy. They have great potential to be developed as clinically useful injectable contraceptive in the future.