Supplemental Material

***in vivo* efficacy testing protocol**

Female BALB/c mice (6 to 8 weeks old; Charles River) in anestrus or the diestrus stage of the reproductive cycle were acclimated to the animal facility for 10 days after which stained vaginal smears were prepared and examined using a light microscope to identify the stage of estrous. Mice in anestrus or the diestrus stage of the estrous cycle were randomized into cages (3-5 mice/cage) and inoculated intraperitoneally (IP) with 0.5 mg of Premarin 2 days prior to bacterial inoculation (d-2), the morning of bacterial inoculation (d0), and two days post-bacterial inoculation (d2) as shown in Fig. S1. Trimethoprim (TMP) was administered orally (0.4 g/L drinking water) on the first day of Premarin treatment; mice were also given 3.6 mg/kg of streptomycin (STM) by IP injection on days -2 through day 1. STM was provided in the drinking water along with TMP (5 g STM and 0.4 g TMP per liter of water) starting on day 2 until the end of the experiment. On day 0, mice were anesthetized with a mixture of ketamine/xylazine and 30 µL of PPCM formulated in 2.7% HEC, 2.7% HEC alone, Gynol-II®, or Yaso-GEL™ were applied vaginally using a positive displacement pipette. Thirty seconds later, mice were challenged with 10 µl of a PBS suspension containing 10^5 CFU of *Ng* strain MS11, which is a dose that infects 80-100% of mice. Vaginal mucus was collected on days 1, 2 and 5 post-inoculation using a sterile rayon swab (Puritan Medical Products Company, LLC; Ref. #25-800 R 50) and quantitatively cultured for *Ng* on GC-VCNTS agar. A small portion of sample was also cultured on HIA plates to monitor the presence of potentially inhibitory facultatively anaerobic commensal microbiota, which might cause clearance that is unrelated to the treatment. No inhibitory commensal flora were isolated from any of the mice this study. Differences in the percentage of culture-positive mice at each time point and the average number of CFU recovered per milliter of vaginal swab suspension over time were compared between experimental groups using the Log-Rank test with Bonferroni correction and a repeated measures ANOVA, respectively. p values < 0.05 were considered significant. For each experiment, 5-7 animals were tested in each experimental group for a total of 68 animals used in this study. Sample size was based on historical data that show 5-7 animals is sufficient for detecting a significant difference in the percentage of mice colonized over time for nonoxynol-9 versus no treatment. The study was not blinded and no data points were excluded from the study.

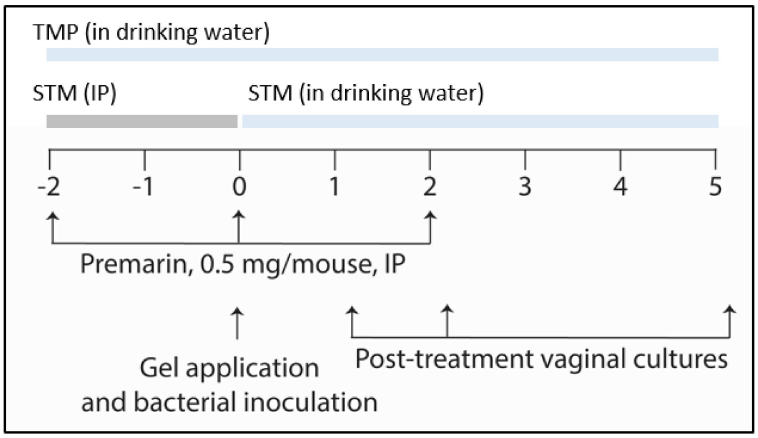

Figure S1: Schematic of *in vivo* efficacy testing of PPCM or Yaso-GEL™ against *N. gonorrhoeae.*