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Reproduction, Fertility and Development

Supplementary Material

Anti-tuberculosis drugs used in a directly observed treatment short course (DOTS) schedule alter endocrine patterns and reduce the ovarian reserve and oocyte quality in the mouse

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Hormone analysis at 8 weeks recovery gap

To understand whether the changes induced by the ATDs on reproductive hormones are transient or long lasting, reproductive hormone level was assessed at 8 weeks after the completion of treatment with ATDs as explained in main manuscript. After the treatment the animals were given the recovery gap of 8 weeks. The animals were dissected after 8 weeks of recovery gap and assessed for gain in body weight and reproductive hormones in the serum.

***In-vitro* maturation of oocytes**

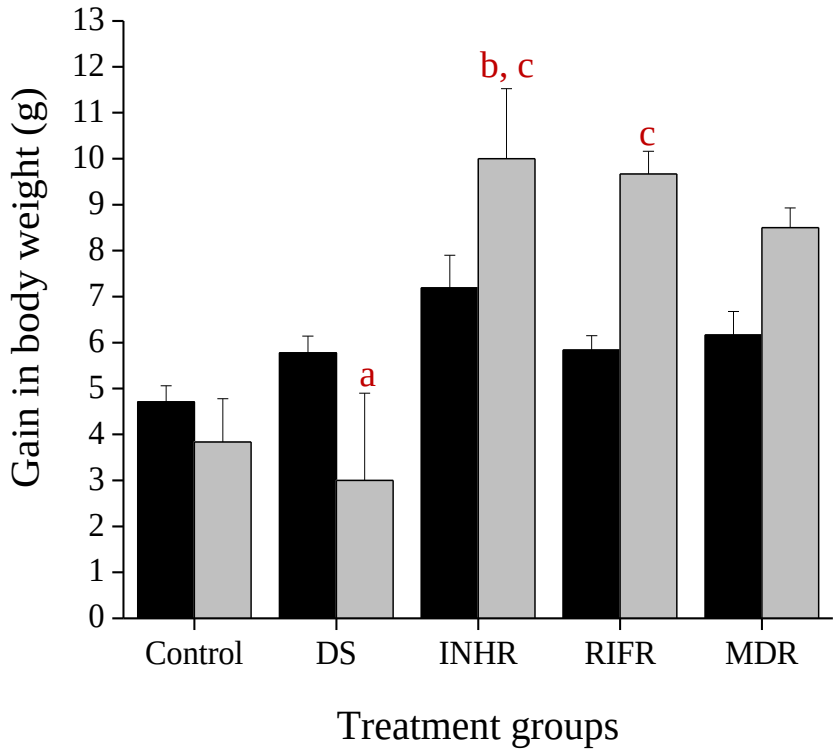

Figure S1: Effect of ATDs used in DOTS strategy on gain in body weight at 2 weeks and 8 weeks after completion of treatment. The data are represented Mean ± SEM (2 weeks after completion of treatment: n=Control-48; DS-36; INHR-37; RIFR-49 and MDR-37, 8 weeks after completion of treatment: n=Control-6; DS-6; INHR-6; RIFR-6 and MDR-6). [a]P<*0.05* and [b]P<*0.0001* vs 2 weeks after completion of treatment, [c]P<*0.05* vs control group of 8 weeks after completion of treatment.

2 weeks recovery gap 8 weeks recovery gap

The animals were sacrificed by cervical dislocation and the ovaries were collected in prewarmed DMEM. Ovaries were teased carefully to release the germinal vesicle (GV) stage oocytes from antral follicles. The GV oocytes were then washed in DMEM media droplets covered with prewarmed light paraffin oil after which they were put for culture in droplets (20 µL) of *in-vitro* maturation (IVM) medium supplemented with different concentrations of ATDs (10, 20, 40 and 80 µg/mL). These oocytes were incubated at 37 °C, 5 % CO2 for 24 h to check the nuclear maturation rate. Since ETH is not soluble in water, 1 % dimethyl sulfoxide (DMSO) was used as vehicle control and the working solutions of ETH were prepared in IVM medium.

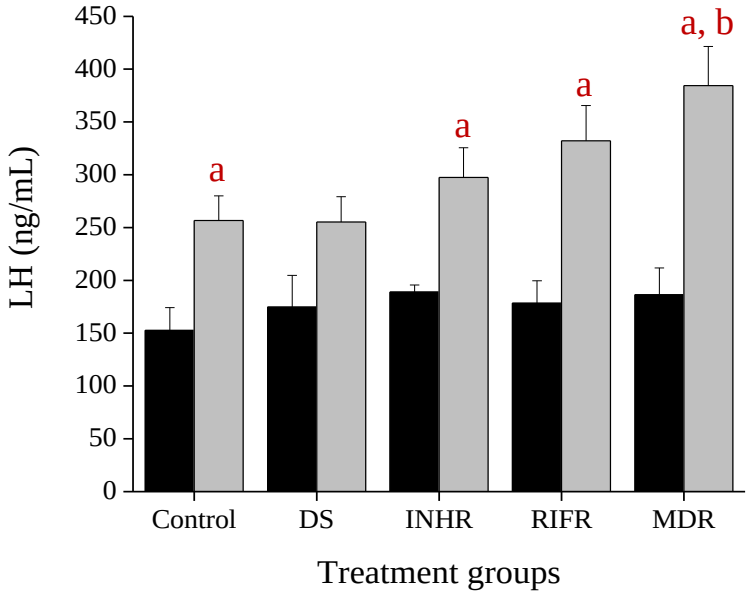

Figure S2: Effect of ATDs used in DOTS schedule on LH level at 2 weeks and 8 weeks after completion of treatment. The data are represented Mean ± SEM (2 weeks after completion of treatment: n=Control-5; DS-5; INHR-5; RIFR-5 and MDR-5, 8 weeks after completion of treatment: n=Control-6; DS-6; INHR-5; RIFR-6 and MDR-5) [a]P<*0.01* vs 2 weeks recovery gap in respective treatment group, [b]P<*0.05* vs control group of 8 weeks after completion of treatment.

2 weeks recovery gap 8 weeks recovery gap

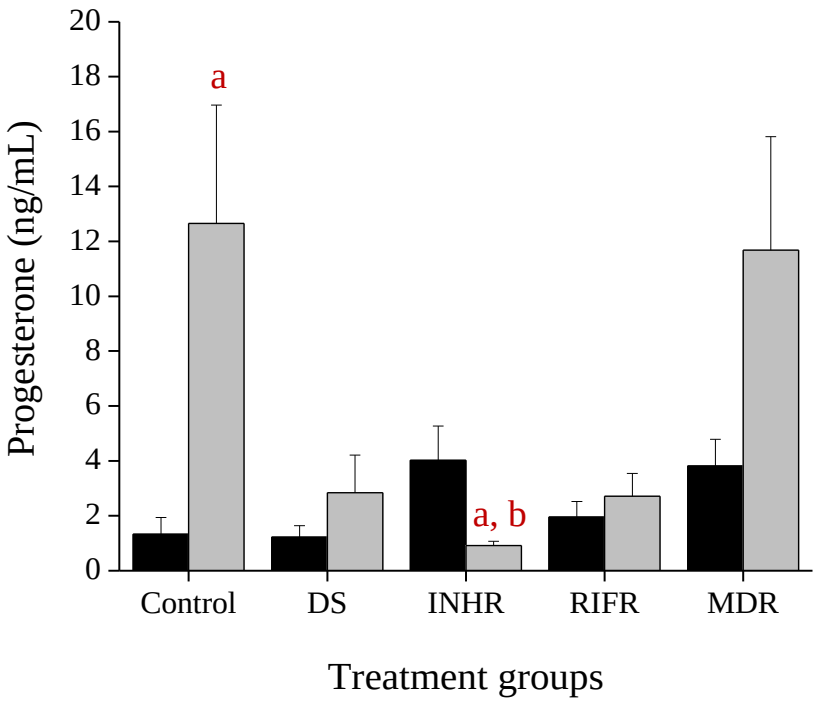

Figure S3: Effect of ATDs used in DOTS schedule on progesterone level at 2 weeks and 8 weeks after of treatment. The data are represented Mean ± SEM. One-way ANOVA was used for the statistical evaluation, 2 weeks after completion of treatment: n=Control-5; DS-4; INHR-5; RIFR-5 and MDR-5, 8 weeks after completion of treatment: n=Control-5; DS-5; INHR-6; RIFR-5 and MDR-6. [a]P<*0.05* vs 2 weeks recovery gap in respective treatment group, [b]P<*0.05* vs control group of 8 weeks after completion of treatment.

2 weeks recovery gap 8 weeks recovery gap

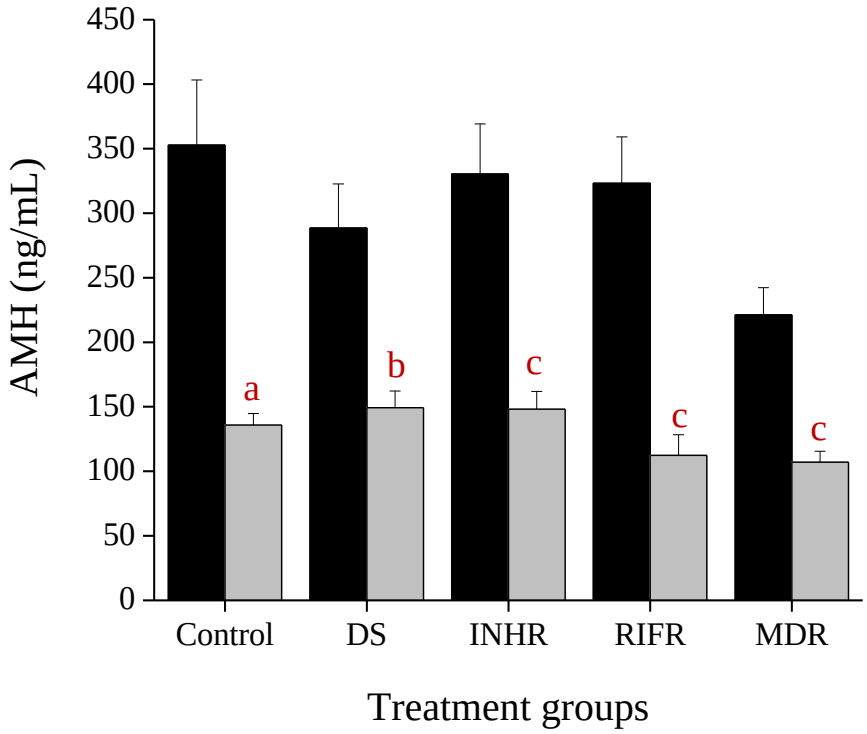

Figure S4: Effect of ATDs used in DOTS schedule on AMH level at 2 weeks and 8 weeks after completion of treatment. The data are represented Mean ± SEM (2 weeks after completion of treatment: n=Control-5; DS-6; INHR-6; RIFR-6 and MDR-6, 8 weeks after completion of treatment: n=Control-5; DS-5; INHR-6; RIFR-6 and MDR-6) [a]P<*0.05,* [b]P<*0.01,* [c]P<*0.001* vs 2 weeks recovery gap in respective treatment group.

2 weeks recovery gap 8 weeks recovery gap

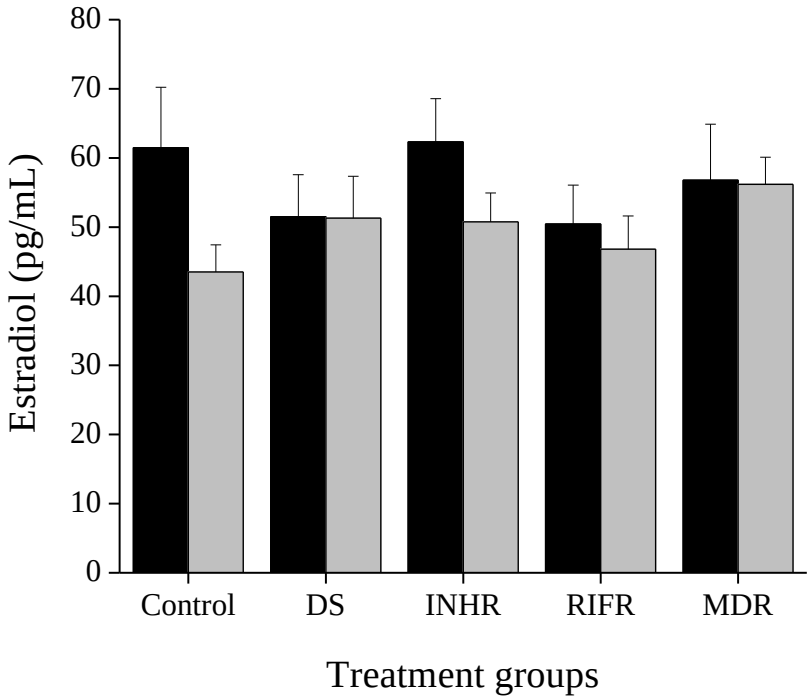

Figure S5: Effect of ATDs used in DOTS schedule on estradiol level at 2 weeks and 8 weeks after completion of treatment. The data are represented Mean ± SEM (2 weeks after completion of treatment: n=Control-6; DS-6; INHR-6; RIFR-6 and MDR-6, 8 weeks after completion of treatment: n=Control-6; DS-5; INHR-6; RIFR-5 and MDR-6).

2 weeks recovery gap 8 weeks recovery gap

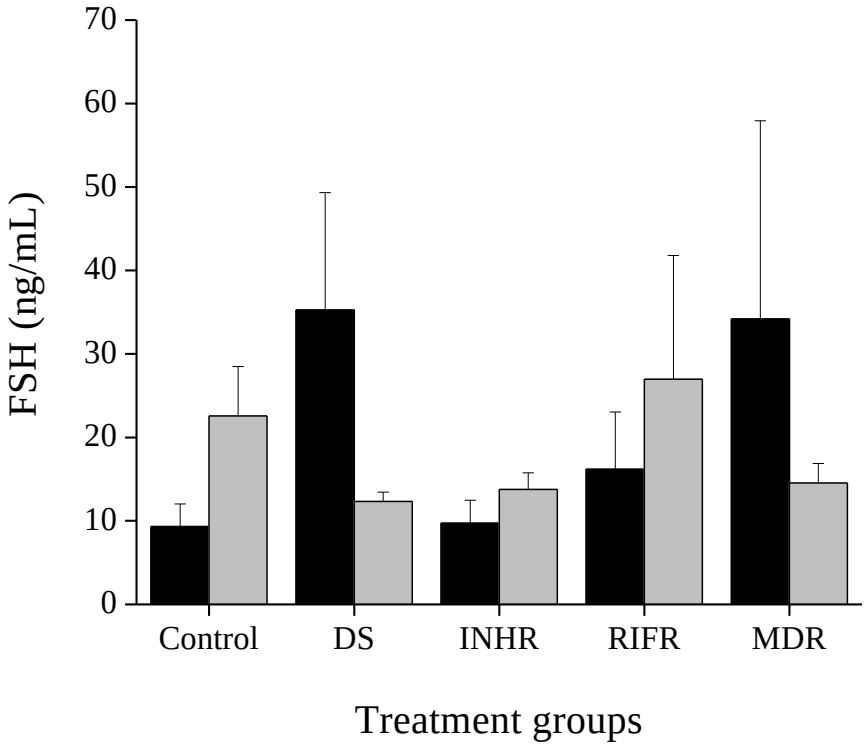

Figure S6: Effect of ATDs used in DOTS schedule on FSH level at 2 weeks and 8 weeks after completion of treatment. The data are represented Mean ± SEM (2 weeks after completion of treatment: n=Control-5; DS-5; INHR-5; RIFR-5 and MDR-5, 8 weeks after completion of treatment: n=Control-5; DS-5; INHR-5; RIFR-5 and MDR-4).

2 weeks recovery gap 8 weeks recovery gap

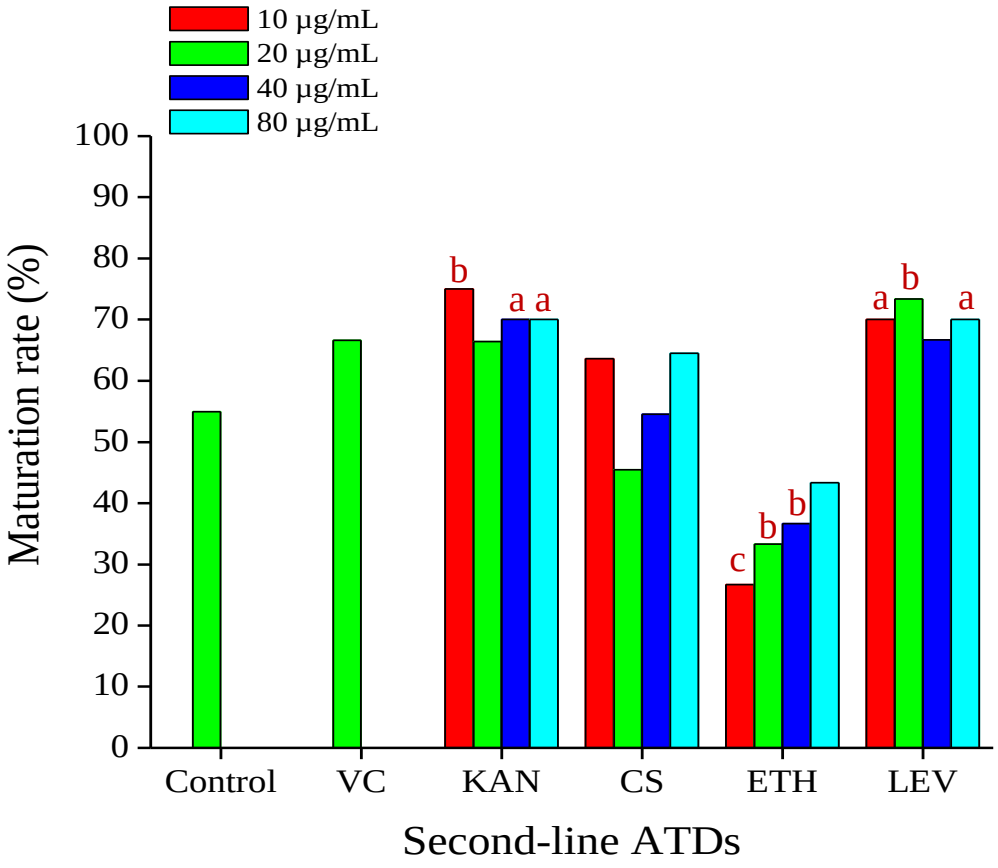

Figure S7: Effect on maturation rate after exposing the GV oocytes to Second-line ATDs during IVM. The data are represented in percentage (n=control-1340, VC-450, KAN-10 µg/mL-60, KAN-20 µg/mL-51, KAN-40 µg/mL-30, KAN-80 µg/mL-30, CS-10 µg/mL-22, CS-22 µg/mL-22, CS-40 µg/mL-22, CS-80 µg/mL-31, ETH-10 µg/mL-30, ETH-20 µg/mL-30, ETH-40 µg/mL-30, ETH-80 µg/mL-30, LEV-10 µg/mL-30, LEV-20 µg/mL-30, LEV-40 µg/mL-30, LEV-80 µg/mL-30). $^{a}P<0.05$, $^{b}P<0.01$, $^{c}P<0.0001$ vs control.