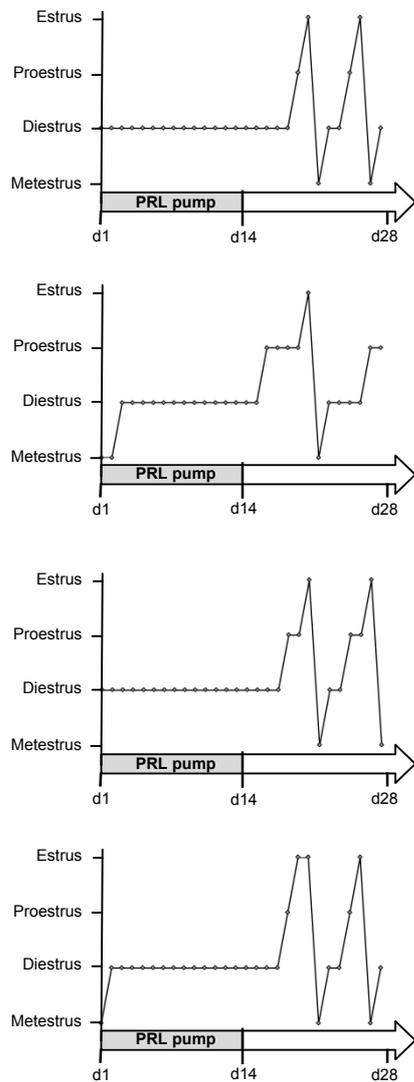
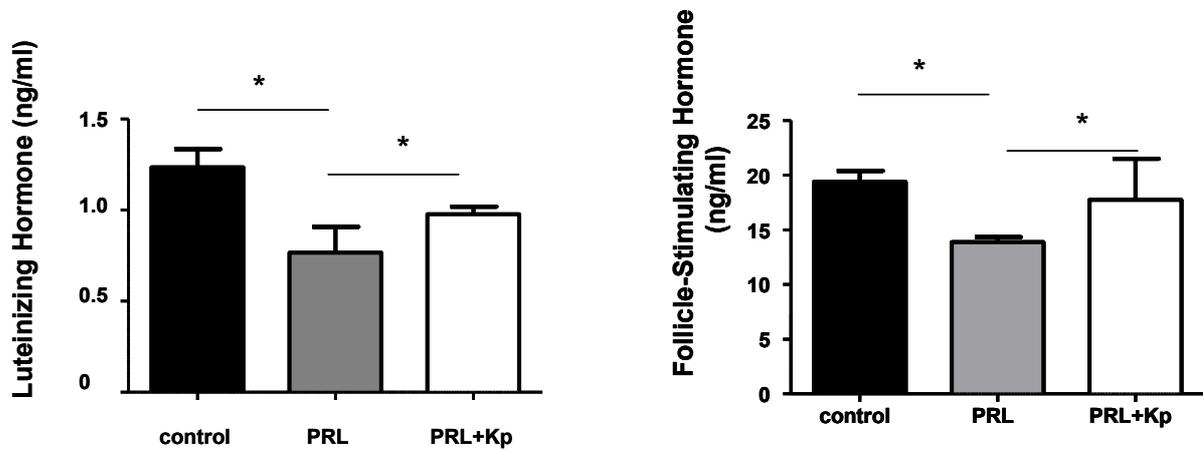


Supplemental Figure 1 :



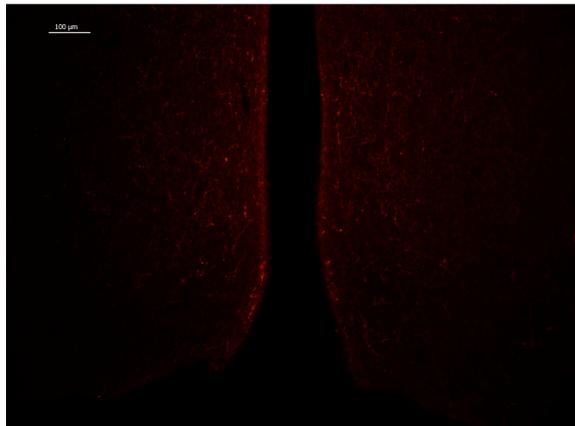
Suppl. Figure 1: Representative cycling profiles of four mice with pumps delivering PRL only for 14 days. Mice showed a resumption of cyclicity after 14 days of PRL treatment (grey boxes) demonstrating the reversibility of the gonadotropic deficiency.

Supplemental Figure 2 :

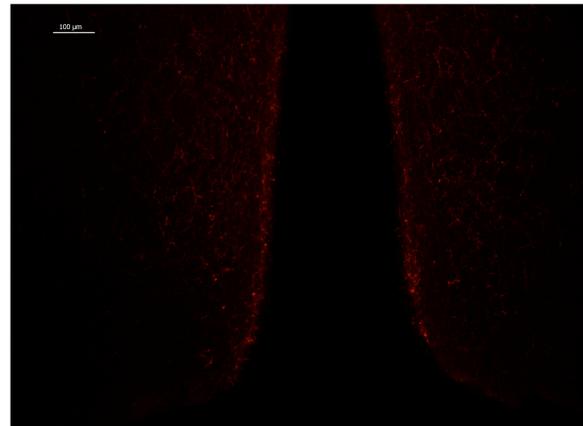


Suppl. Figure 2: Hormone levels in control, PRL and PRL+Kp mice. Five to twelve mice were used in each assay group. P values are provided for statistically significant differences; all P values were calculated with nonparametric Kruskal Wallis and post test Dunn's multiple comparison tests.

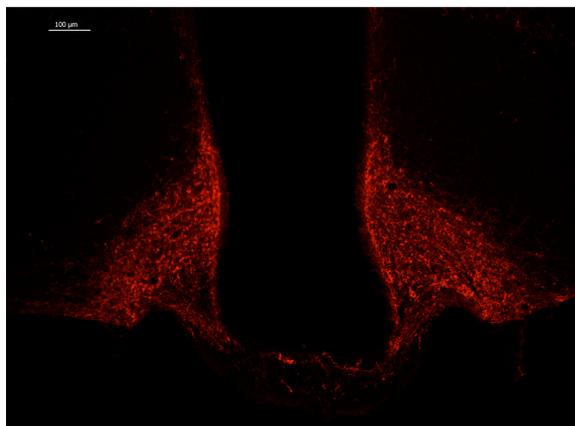
Supplemental Figure 3 :



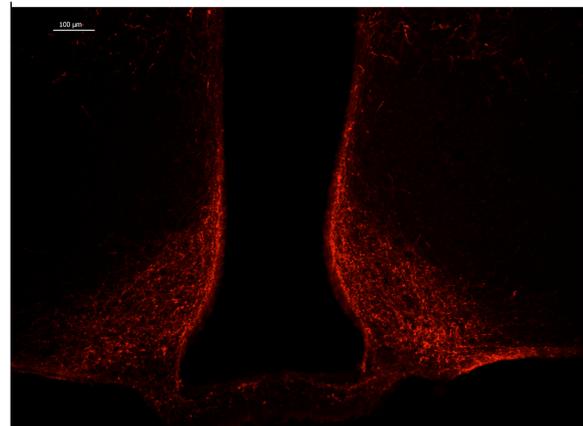
Control AVPV



PRL treated AVPV



Control ARC



PRL treated ARC

Suppl. Figure 3: NKB immunostaining in control and PRL mice. NKB neurons staining in ARC and AVPV sections from control and PRL-treated mice (PRL) (Scale bar, 100μm).

The NKB immunoreactivity looks similar in both the AVPV and the ARC.

Methods:

Sections (40 μm thickness) were permeabilized in PBS-Tween-20 (0.1%), followed by 10 min of heat-induced epitope retrieval in a microwave using 10 mM citrate buffer (pH6). Sections were blocked for 45 min at RT using (3% normal donkey serum in PBS with 0.4% Triton X-100). Sections were then incubated overnight at 4°C in blocking solution with a highly specific mouse neurokinin B antibody that was raised in rabbits (1:850, NB300-201, Novus Biologicals, Cambridge, UK). Immunoreactive sites were visualized using a donkey anti-rabbit IgG Alexa Fluor 555 (1:400, A31572, Invitrogen, USA) and a Cy3 filter set. The same microscope exposure settings were used to image each section.

Supplemental Table 1

Table 1
Primer Sequences of Genes Analyzed in quantitative RT-PCR

Name	Accession number	Amplicon (bp)	Sense primer	Antisense primer
<i>18S</i>	NR_003278.3	66	CCCTGCCCTTTGTACACACC	CGATCCGAGGGCCTCACTA
<i>Kiss1</i>	NM_178260.3	116	GCTGCTGCTTCTCCTCTGTGT	GATTCCTTTTCCCAGGCATTAA
<i>fsh-β</i>	NM_008045.2	103	GGCTACTGCTACACTAGGGA	GCAATCTTACGGTCTCGTAT
<i>gnrh</i>	NM_008145.2	193	AGCACTGGTCCTATGGGTTG	CCTGGCTTCTCTTCAATCA
<i>lh-β</i>	NM_008497.2	131	CCTAGCATGGTCCGAGTACT	AAGGAGACTATGGGGTCTACA
<i>nkb</i>	NM_001199971.1	122	CTGTGTGGGATGTAATGGAG	TGGATAGCTGACAGGATCAG

The abbreviations of the genes, their GENBANK accession number and 5'- to 3'- nucleotide sequences of the sense and antisense primers are presented.