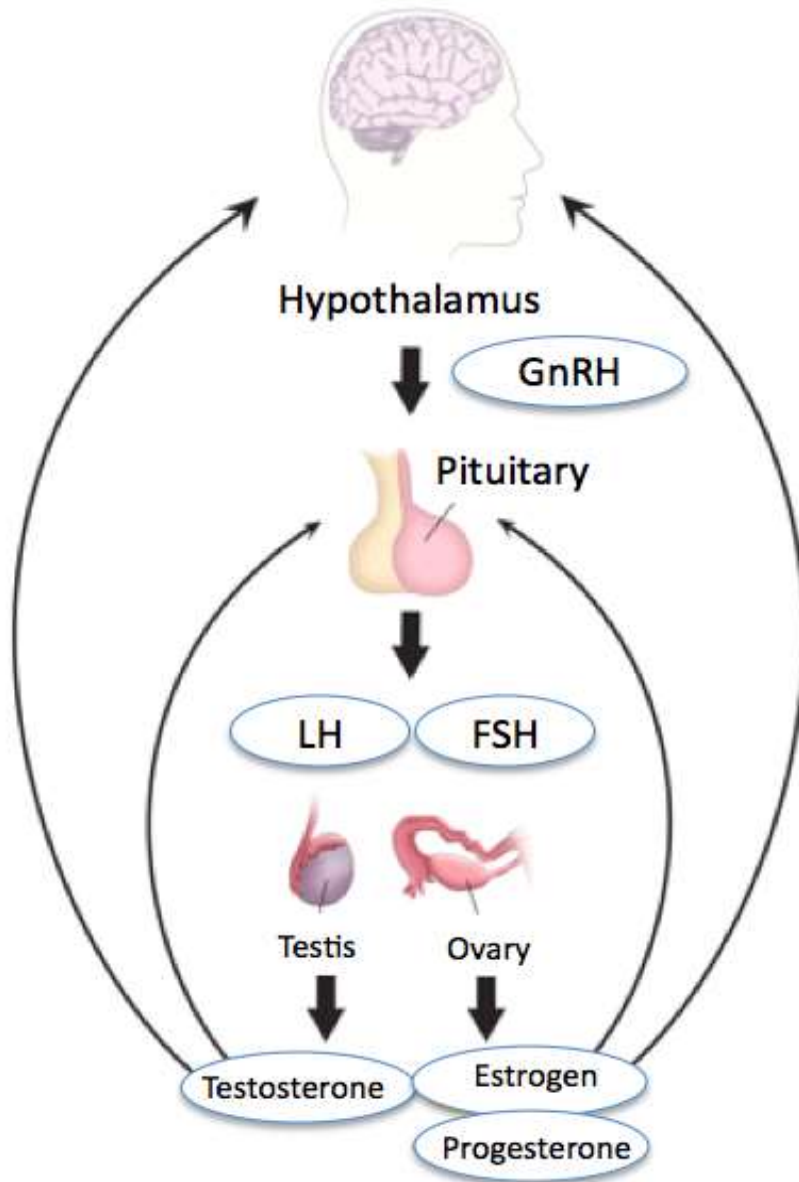


Gonadotrope Specific Ablation of JNK 1/2 Leads to Impaired Fertility

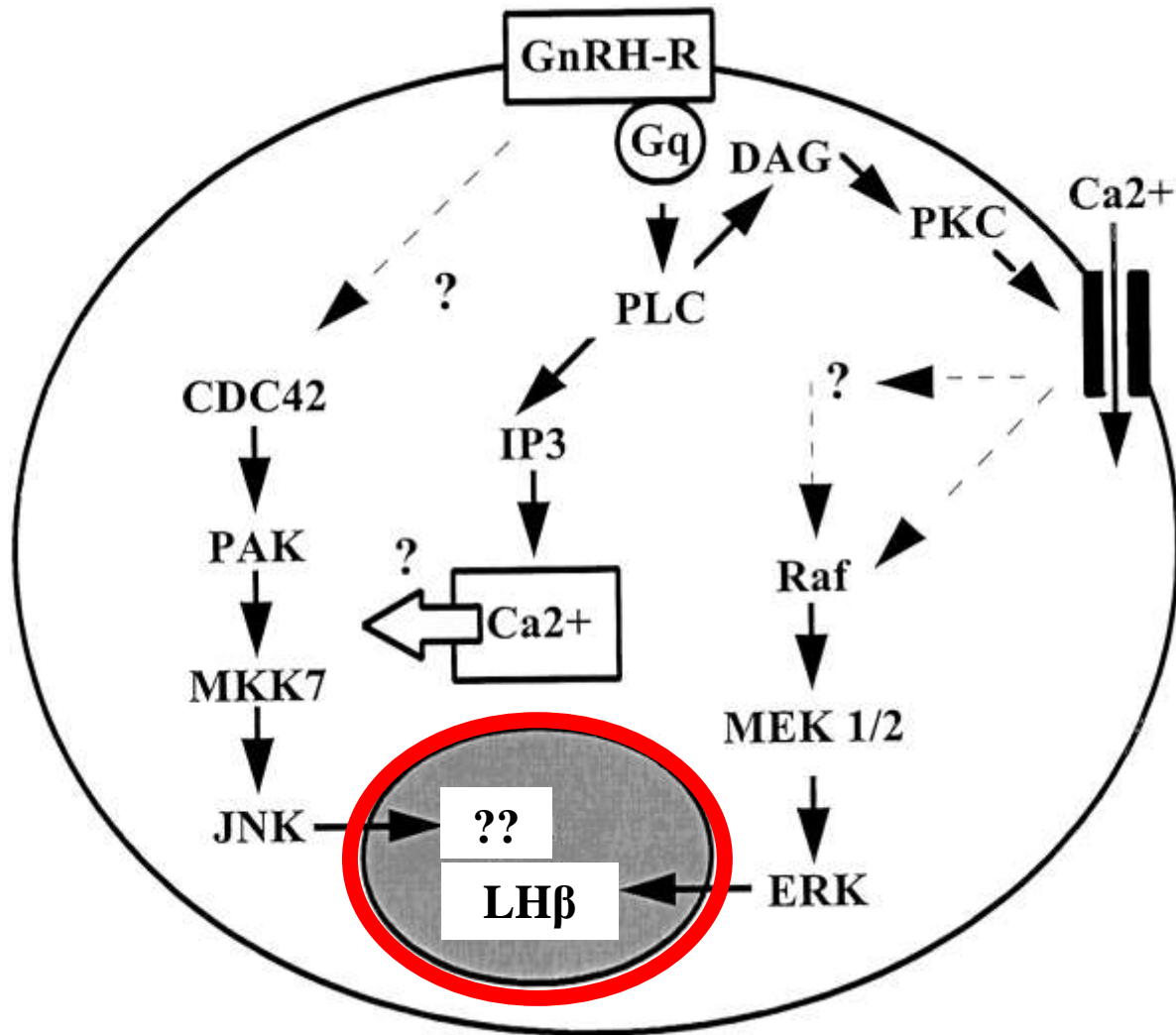
Presentation by: Kelsea Zukauckas
INBRE Undergraduate Research Day
April 30, 2016

HPG Axis Overview



- ❖ Hypothalamus secretes gonadotropin releasing hormone (GnRH)
- ❖ Adenohypophysis secretes luteinizing hormone (LH) and follicle stimulating hormone (FSH)
- ❖ Gonads secrete testosterone (T) and estrogen (E)
- ❖ Negative feedback occurs to keep axis in physiological balance

Importance of MAPK in Gonadotropin Signaling



❖ GnRH facilitates extracellular-signal-regulated kinases (ERK) activation via PKC mediated extracellular calcium influx

❖ Previous works shows that ERK is important for LH β synthesis through the immediate early gene - EGR-1

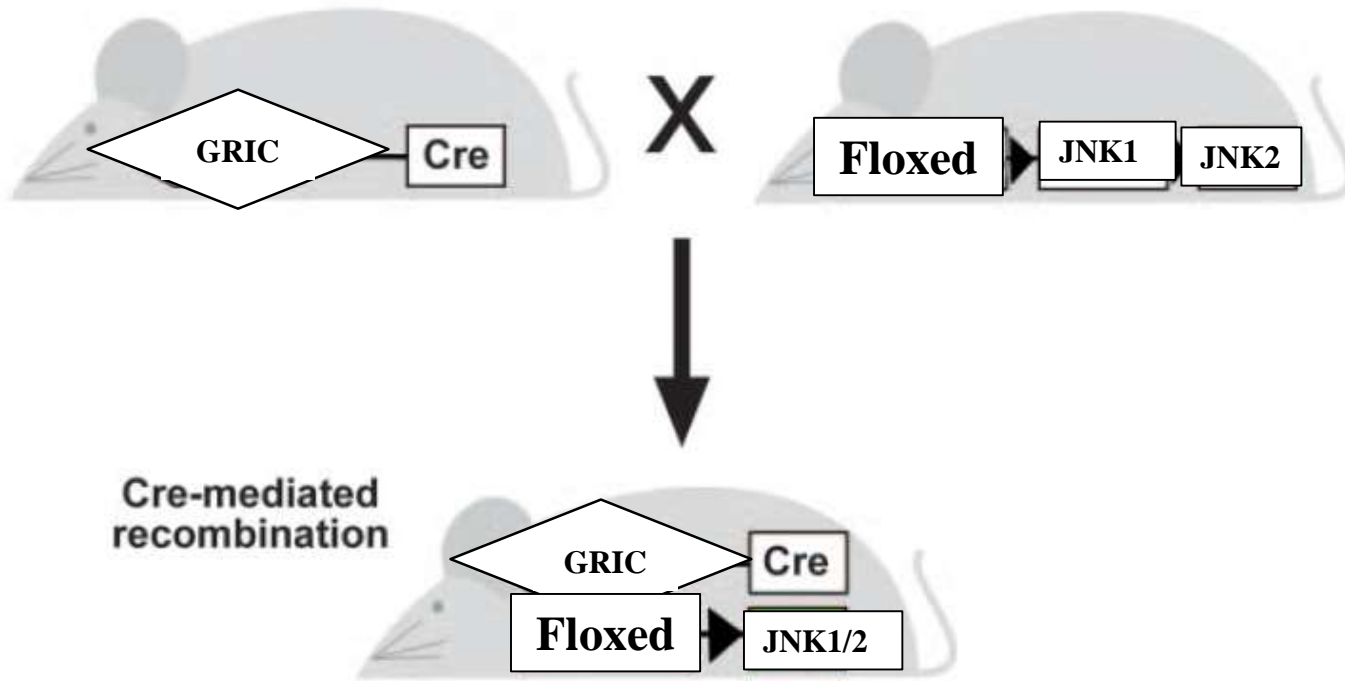
❖ While the ERK signaling cascade has been extensively studied the downstream physiological functions of c-Jun N-Terminal Kinase (JNK) has yet to be determined

JNK Role in Gonadotropin Synthesis

❖ Initial *in vitro* studies show that JNK has an effect on gonadotropin synthesis and secretions of LH and FSH, but the quantitative and magnitude of effects is unknown *in vivo*

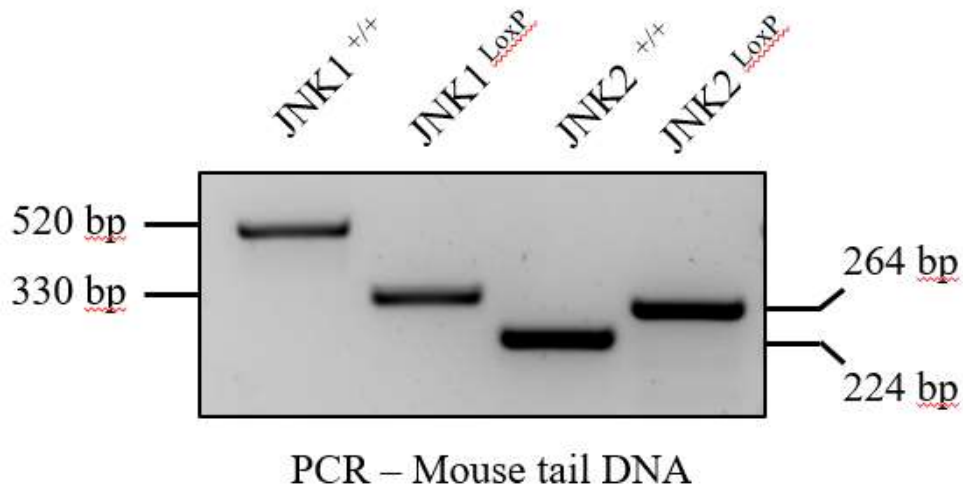
❖ **Central question** of this research is to: **Characterize the role of JNK in both gonadotropin synthesis and subsequently fertility in both males and females *in vivo***

Utilization of CRE/LOX Technology



- ❖ Have a specific Cre to only effect the gonadotrope (GRIC-Cre)
- ❖ Floxed JNK 1&2 contain LoxP on both ends of the gene
- ❖ Floxed JNK 1&2 function normally until combined with GRIC-Cre
- ❖ Once combined JNK 1/2 are ablated to achieve a double knockout (DKO)

Data: Confirmation of JNK 1/2 Ablation

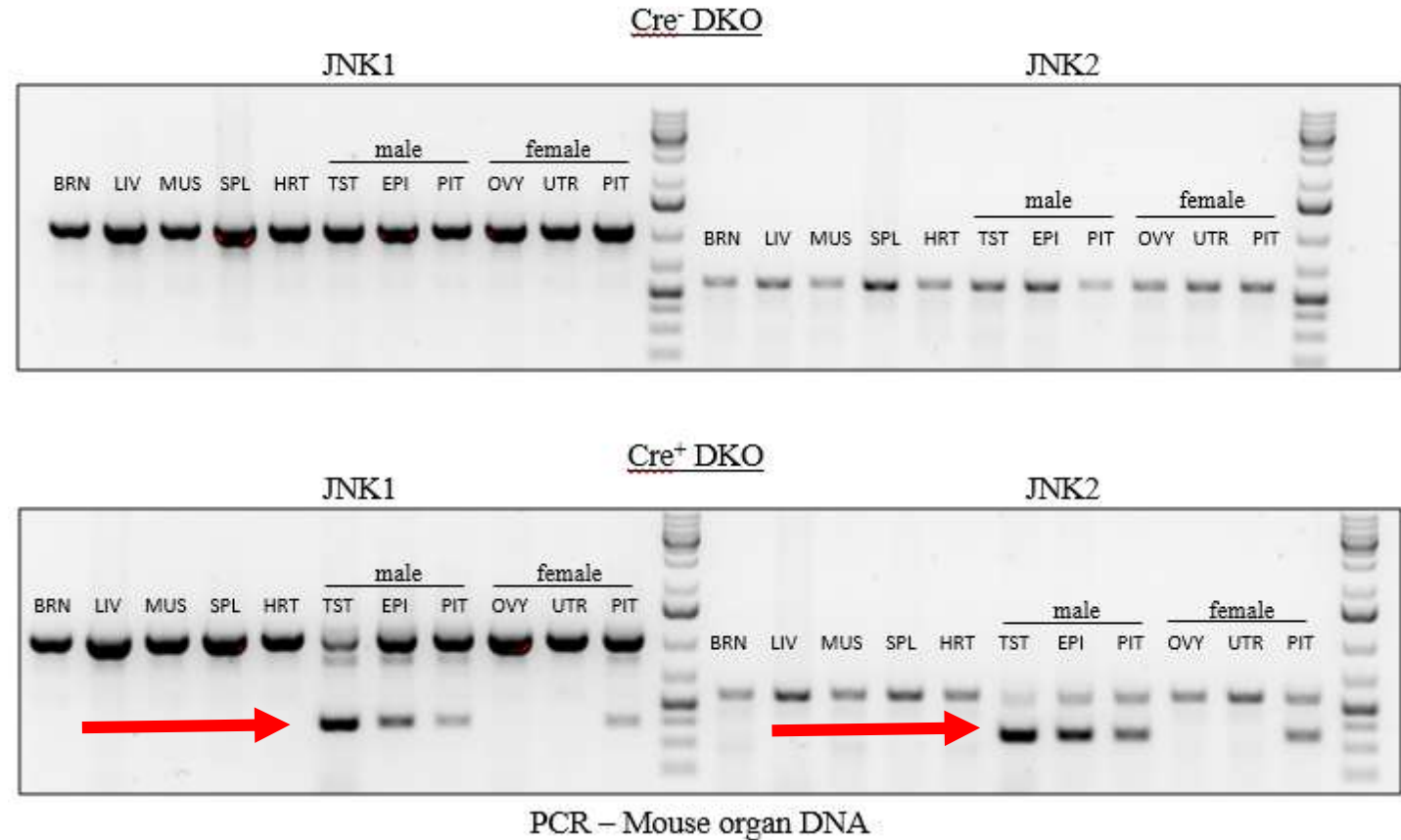


Legend:

+/+ = wild type mice

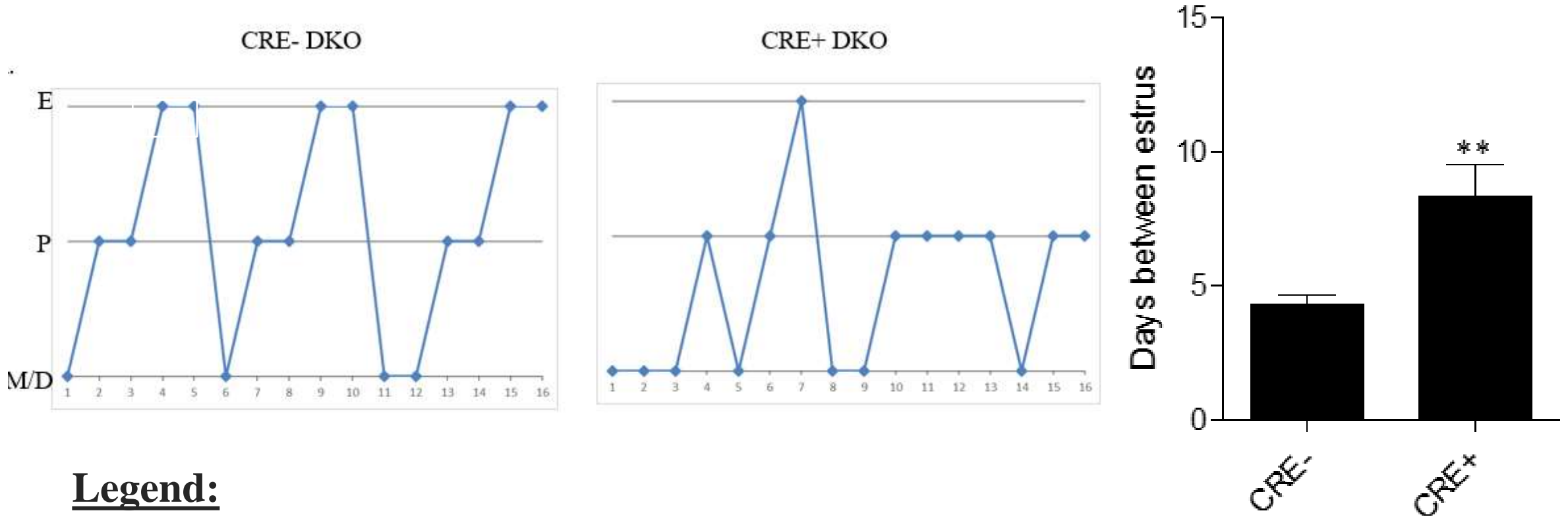
LoxP = deleted JNK

- ❖ Data above used to continue breeding, this data is confirmed by ear tagging mice and PCR.



Red arrows indicate presence of the deleted form of JNK 1/2 when Cre is present

Data: Estrous Cycling



Legend:

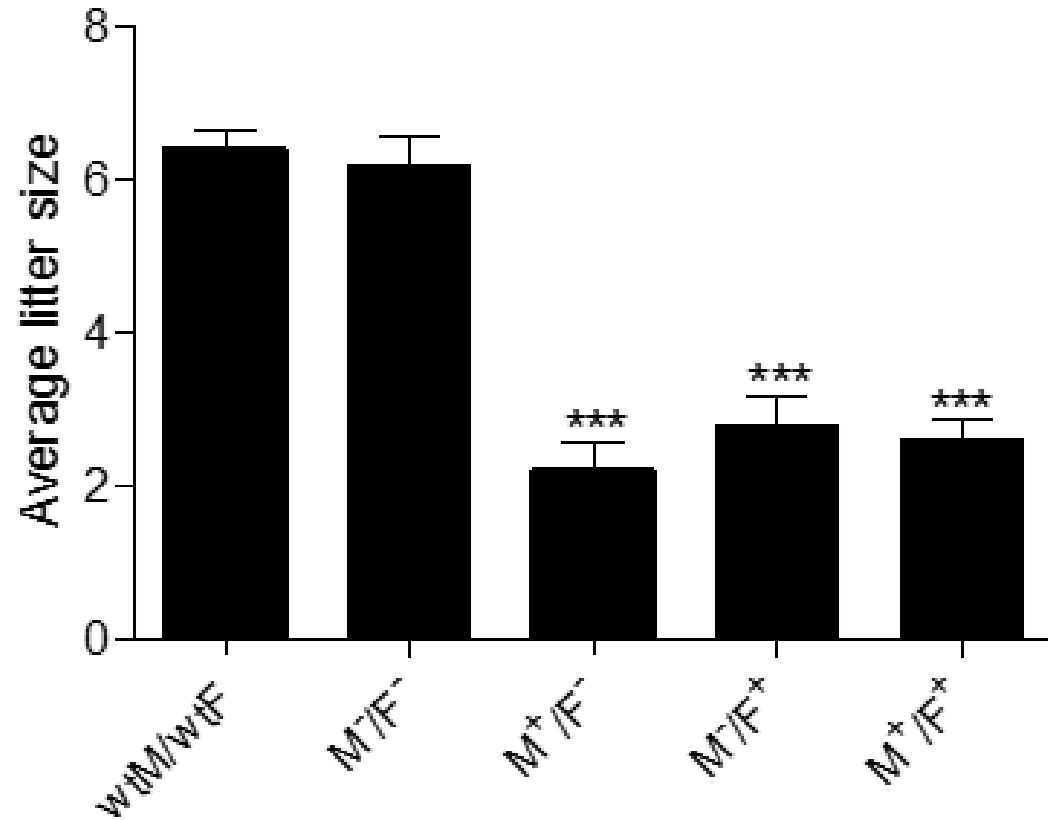
E: Estrus

P: Proestrus

M/D: Metestrus/Diestrus

- ❖ Estrous cycling is completed by vaginal swabbing of mice. Classification of stage in cycle obtained with staining and histological evaluation with a microscope.
- ❖ Increased days between estrus seen with mutated mice (CRE +)

Data: Litter Size



Legend:

wtM/wtF: Both wild type

M-/F-: Both Cre -

M+/F-: Male Cre + and Female Cre -

M-/F+: Male Cre - and Female Cre +

M+/F+: Both Cre +

❖ Smaller litter sizes are seen when a Cre + mice is involved—independent of sex

Summary and Future Directions

- ❖ Gonadotrope specific DKO of JNK 1/2 is possible with Cre/Lox technology
- ❖ Conditionally knocking out JNK 1/2 leads abnormal estrous cycling
- ❖ Both males and females are subfertile

❖ Future directions:

- ❖ Histological examination of gonads in both sexes
- ❖ Sperm counts and corpus luteal counts
- ❖ Quantify serum LH and FSH
- ❖ Quantify GnRH receptor, FSH β , and LH β mRNA levels
- ❖ Gonadectomize both males and females and examine reproductive tract
- ❖ Profile known immediate early gene expression downstream of JNK (c-Jun)

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