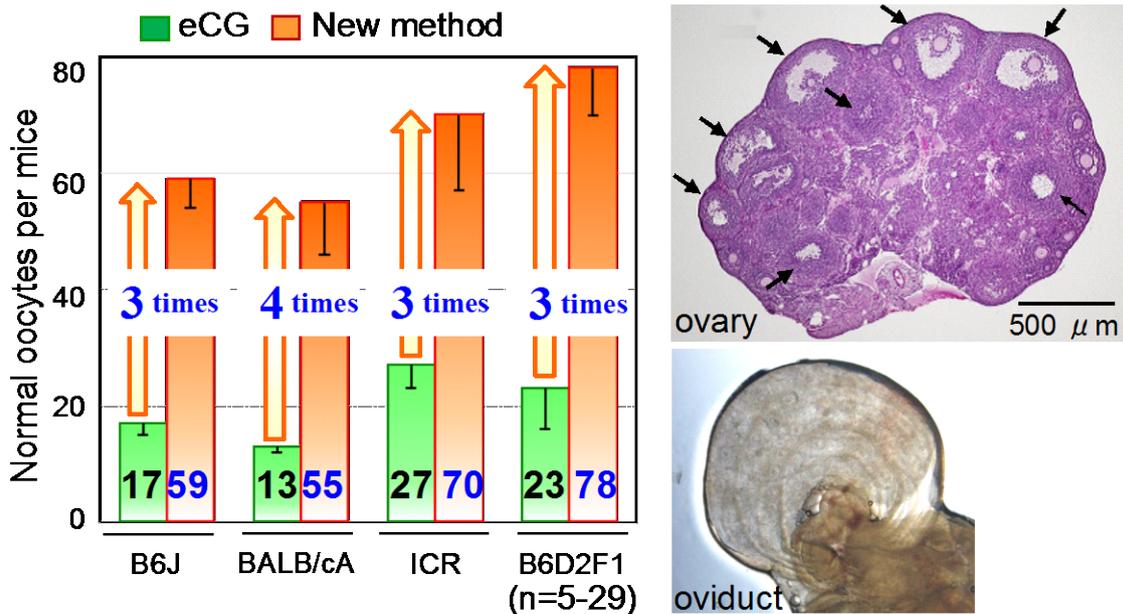


### 10. High-Yield Superovulation in Adult Mice

Three-fold increase by anti-Inhibin serum treatment combined with estrous cycle synchronization



Producing many mature oocytes is of great importance for assisted reproductive technologies. In mice, superovulation by consecutive injections of equine chorionic gonadotropin (eCG) and human chorionic gonadotropin (hCG) has been the gold standard for oocyte collection. However, the yield of mature oocytes by this regimen can fluctuate according to the stage of the estrous cycle, strain, and age. Therefore, we have developed a new high-yield superovulation protocol to collect higher numbers of oocytes from adult female mice of different strains and ages. We found that the estrous cycle of C57BL/6 (B6) female mice was synchronized to metestrus after two daily injections of progesterone. Meanwhile, we found that with the injection of anti-inhibin serum (AIS) instead of eCG, the mean number of ovulated oocytes almost doubled (21 vs. 41 per mouse). This was because AIS increased the endogenous levels of follicle-stimulating hormone (FSH). Then, we combined estrous cycle synchronization with two AIS injections and obtained 62 oocytes per mouse, about three times that with the eCG–hCG protocol. Importantly, this approach increased the proportion of mice that ovulated >25 oocytes from about 40% (eCG–hCG) to 90%. The same protocol was also effective in other inbred (BALB/cA), outbred (ICR), and hybrid (B6D2F1) strains. In addition, B6 female mice aged over 1 year ovulated 1.8-fold more oocytes by this protocol. Thus, estrous cycle synchronization followed by AIS–hCG yielded a broadly applicable, highly efficient superovulation. This protocol should promote the effective use of invaluable female mouse strains and decrease the numbers of animals euthanized.

[1] Hasegawa A, Mochida K, Inoue H, Noda Y, Endo T, Watanabe G, Ogura A. High-Yield Superovulation in Adult Mice by Anti-Inhibin Serum Treatment Combined with Estrous Cycle Synchronization. *Biol Reprod* 2015 Dec 2. pii: biolreprod.115.134023. [Epub ahead of print] PMID: 26632610