# Sample Scientific Paper

**Quantitative Assessment of Cell Populations in Testes from Neonatal Mice with Variable Y Gene Content and X Chromosome Dosage**

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## Personal Reflection

My continuation of INBRE over the course of the summer has been as fruitful as the spring semester. I originally started my research working with adult testes and the cells within. I learned lab skills, theories and facts about the sex chromosomes, and the processes of histology and its importance in basic research. This summer I furthered my research through an understanding of sexual differentiation and germline establishment prior to and including the first wave of spermatogenesis. My summer under the mentorship of Dr. Victor Ruthig and Dr. Monika Ward was educational and inspiring and I am thankful for their efforts in making my experience as great as it was.

## Introduction

Prior to the process of spermatogenesis, which produces spermatozoa (sperm), primordial germ cells (PGCs) go through prespermatogenesis to ultimately become spermatogonia, the adult population of male germ cells that enter spermatogenesis (*1*). Epiblast cells, derived from the embryonal ectoderm, differentiate into PGCs, multipotent cells capable of differentiating into either male spermatogonia or female oogonia (*1-3*). These primordial germ cells actively proliferate while migrating to the genital ridges and, in the male mouse, enter the newly formed testis cords around 10 – 11 days postcoitus (dpc). Now termed pro-spermatogonia, these cells undergo heavy mitotic proliferation and then arrest cell cycle at $G_1/G_0$ phase until birth or shortly after. At approximately 3 days postpartum (dpp) most pro-spermatogonia resume mitotic activity with a portion beginning the first wave of spermatogenesis between 3-6 dpp (*3-5*). This entire process is similar in human males (*6*). Located on the human and mouse Y chromosome are the genes necessary for proper male development and maintenance of fertility. One of these genes, *Sry*, plays an important role in the formation of the testis in mice and men, while in the mouse a second gene *Eif2s3y* is a critical factor in initiating spermatogenesis (*7, 8*). Our previous work with *Sry* and *Eif2s3y* has shown that these two genes make up the minimal Y gene contribution to produce a male mouse that can sire offspring using assisted reproduction technology (*9*). We have also demonstrated that *Eif2s3y*'s role in spermatogenesis can be substituted by transgenic overexpression of its X-linked homologue *Eif2s3x* (*10*). The goal of my project was to expand previous published results showing a lack of significant difference in spermatogenesis between normal adult XY males and males with a *Sry* deletion on the Y chromosome (*Tdym1*) rescued with transgenic addition of *Sry* ($XY^{Tdym1}Sry$) by investigating prespermatogenesis in neonates of the same genotypes (*11*). I also looked into the effects of variable doses of endogenous *Eif2s3y* and *Eif2s3x* on prespermatogenesis and the first wave. Thus XY and $XY^{Tdym1}Sry$ were also compared to males lacking a Y entirely with transgenic *Sry* in the context of one or two X chromosomes (XO*Sry* and XX*Sry*, respectively). These males therefore had variable doses of *Eif2s3x/y*: XY and $XY^{Tdym1}Sry$ (1 copy of *Eif2s3y* and *Eif2s3x*) XX*Sry* (2 copies of *Eif2s3x* and no *Eif2s3y*) and XO*Sry* (1 copy of *Eif2s3x* and no *Eif2s3y*).

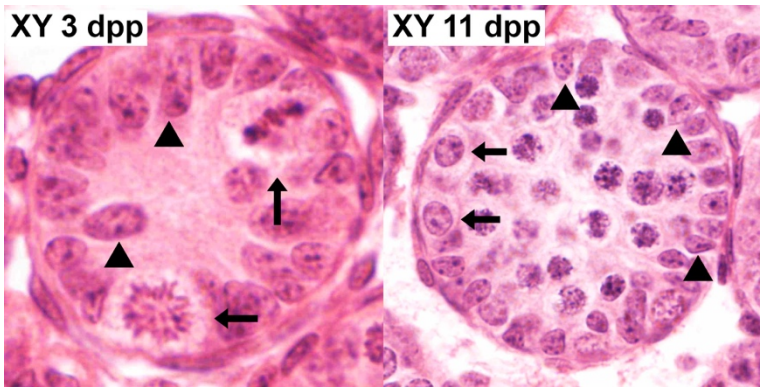


**Fig. 1.** XY male seminiferous Tubule at 3 days post partum (dpp (left) and 11dpp (right). Sertoli cells indicated by arrowheads; pro-spermatogonia, long arrows (left); spermatogonia, short arrows (right)

## Methods and Materials

Three cell populations, Sertoli cells, pro-spermatogonia, and spermatogonia were counted on testis sections stained with Hematoxylin and Eosin (H&E). Cells were identified by nuclear morphology, size, and location with respect to the basement membrane. Testicular cell counts were done on mice at 3 days post partum (dpp) and 11dpp (Fig. 1) to determine the efficiency of prespermatogenesis prior to the first wave of spermatogenesis between mice with *Sry* in different genetic contexts.

The counts were done on 60 tubules per mouse (n=3 males, for each genotype at each time point). Student's T-test was used to determine significance.

Results

At 3 dpp Sertoli cell number was decreased in XO*Sry* when compared to XY$^{Tdym1}$*Sry* and XX*Sry* (Fig. 2, P<0.05). The numbers of germ cell were similar in all genotypes except for XX*Sry* which was lower than XY and XY$^{Tdym1}$*Sry* (Fig. 2, P<0.05). Germ cell/Sertoli cell ratio (GC/SC) comparisons showed decrease in XX*Sry* males compared to all other genotypes (Fig. 2, P<0.05). At 11 dpp both XO*Sry* and XX*Sry* were significantly different from other genotypes, and from each other, demonstrating significantly higher numbers of Sertoli cells, lower numbers of germ cells, and lower GC/SC ratios (Fig. 2, P<0.05), with XX*Sry* being more affected. At both 3dpp and 11dpp XY and XY$^{Tdym1}$*Sry* failed to exhibit any significant differences (Sertoli cell, P>0.3; germ cell, P>0.8). XY and XY$^{Tdym1}$*Sry* had also similar GC/SC ratios at 3 and 11 dpp (P>0.9 and P>0.7, respectively).

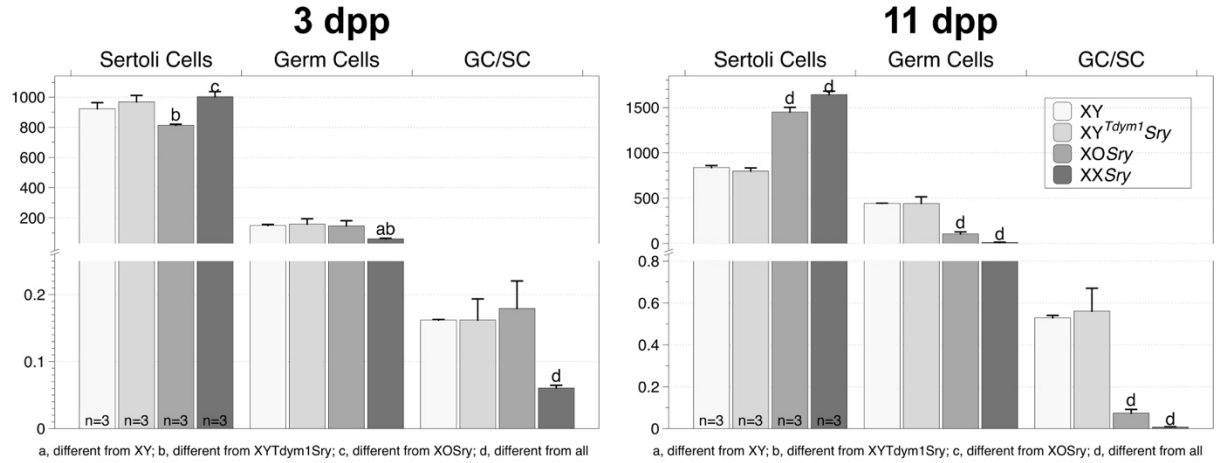


**Fig. 2.** Cell counts and germ cell ratios (GC/SC) of XY mice (control) and mice with limited Y gene content.

Quantitative assessment of pre-spermatogenesis and the first wave of spermatogenesis verified previous published work showing that that there are no significant differences between XY and XY$^{Tdym1}$*Sry* neonates, and that both are equally efficient in driving spermatogenesis. The data further showed that XO*Sry* mice are just as efficient as XY and XY$^{Tdym1}$*Sry* at 3 dpp but do not experience expected substantial increase in germ cells at 11 dpp. XX*Sry*, however, demonstrated a significant decrease in efficiency of the first wave of spermatogenesis at both 3 dpp and 11 dpp as compared to all other genotypes. The differences between XO*Sry* and XX*Sry* might be due to varying *Eif2s3x* dosage (1 and 2 copies, respectively).

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