Understanding the ramification of meiotic recombination variation in male sheep

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Authorization to Submit Thesis

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Abstract

Meiotic recombination, an important process during gamete formation, ensures proper chromosome segregation and contributes to genetic variation. It is clear from previous studies that at least one crossover (CO) per chromosome arm is necessary to avoid mis-segregation, and placement of CO is not random, showing preferences called “hotspots.” The total number of CO per spermatocyte differs between strains of mice, however this has not been evaluated in sheep. This study used a cytogenetic approach to quantify the number of CO per spermatocyte for three breeds of sheep: Suffolk, Icelandic, and Targhee. Further, the number and location of the CO were characterized for each chromosome pair within the spermatocytes. Significant differences in the number of CO per spermatocyte was identified between individuals within and across breeds ($P<0.01$). Additionally, a correlation was identified between the number of CO and length of the homologous chromosome pair, as well as distinct CO location patterns.

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Dedication

I would like to dedicate this thesis to my sister, parents, and grandparents for their unwavering encouragement and support that made this thesis possible.

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List of Abbreviations

ADB	Antibody dilution buffer
CO	Crossover
DNA	Deoxyribonucleic acid
E	Embryonic day (in mice)
EBV	Estimated breeding value
FISH	Fluorescence in-situ hybridization
GWAS	Genome-wide association study
NCO	Non-crossover
NHEJ	Non-homologous end joining
NSIP	National Sheep Improvement Program
PAR	Psuedoautosomal region
PGC	Primordial germ cell
SC	Synaptonemal complex
SMC	Structural maintenance of chromosomes
SNP	Single nucleotide polymorphism
SSC	Spermatogonial stem cells
QTL	Quantitative trait locus

Chapter 1: Literature Review

Introduction

Sheep production for meat and wool is an important agricultural industry across the world. In the United States, sheep numbers have decreased from 56.2 million in 1942 to just 5.32 million in 2016 (Lupton, 2008; USDA NASS, 2016). The number of sheep operations have also declined from 241,290 in 1965 to 88,338 in 2012 (Lupton, 2008; USDA NASS, 2014). Wool crop has decreased from 176 million kg in 1942 to 15.7 million kg in 2007 (Lupton, 2008). Similar to the cattle industry, lamb live weights at slaughter have increased from 36.3 kg to 62.6 kg (Lupton, 2008). Total lamb crops have also increased since 1925 from 87% to 100% in 2007, indicating a higher incidence of twins and more lambs raised per ewe (Lupton, 2008). Although sheep numbers have declined in the U.S., there is still a demand for meat and wool domestically. However, the U.S. will need to compete with larger lamb and wool producers, notably Australia and New Zealand. Therefore this, as well as other research, is critical to support the sustainability of U.S. sheep production through improvement of genetic selection tools.

Genomic selection strategies are not widely implemented in the sheep industry when compared to other livestock industries such as dairy and beef. Although cattle industries have capitalized on the use of estimated breeding values (EBVs) and genomically enhanced EBVs, the sheep industry is just now beginning to realize the utility of this tool. The National Sheep Improvement Program (NSIP) has spearheaded the implementation of better record keeping to identify superior breeding animals and use of genetic selection tools including EBVs on sheep operations. This program was started in

1987 and supported by both producers and the American Sheep Industry (Wilson and Morrical, 1991; Lupton, 2008). Currently, the NSIP allows input of traits such as birth weight, weaning weight, carcass traits, and breed specific production traits. As this program is more widely applied on sheep operations, selection for animals with desired traits using basic genetic tools will greatly accelerate improvement of product quality, yield, and uniformity of U.S. lamb and wool products.

Although some genetic selection tools are available, their use by many sheep producers is limited by factors including cost and phenotypic record keeping. For sheep producers, the benefit of implementing genetic tools have not yet been fully realized; this is partially due to the relatively high costs associated with using genetic tools. Lack of production record keeping can also limit the usefulness of these tools. In addition, the production practices such as fostering lambs without identifying parents can pose issues in selecting for offspring that inherited superior traits from parents. Additionally, turning over rams quickly and lack of artificial insemination use in a flock also limits the ability to build more accurate EBVs.

Regardless, some genetic resources are commercially available for the sheep industry including a few specific tests for genetic disorders. For example, single nucleotide polymorphism (SNP) markers for the disease traits scrapie and spider lamb are available for producers to determine if individuals in the flock have these detrimental mutations (Baylis and Goldman, 2004; Cockett et al., 1999; Cockett and Beever, 2001). Markers for callipyge, Booroola (fecundity), and Woodlands (fecundity) have also been identified in sheep (Frecking et al., 1998; Wilson et al., 2001; McNatty et al., 2007). Scrapie resistance is a trait selected for by producers, specifically the mutation at codon 171 with the “R”

allele denoting a less susceptible phenotype and the “Q” allele denoting a more susceptible phenotype (Baylis and Goldman, 2004). Scrapie is a prion disease that has previously devastated the sheep industry (Baylis and Goldman, 2004). Polymorphisms on codons 171, 154, and 136 of the prion gene *PRNP* were identified as mutations associated with disease susceptibility (Baylis and Goldman, 2004). Since then, sheep producers have capitalized on this information to select for less susceptible animals and participate in the USDA National Scrapie Eradication Program which monitors and prevents scrapie incidences. For many producers buying animals to add to a flock, the scrapie susceptibility genotypes are an important piece of information to consider.

The International Sheep Genomics Consortium consisting of members from across the world that are heavily involved in sheep genetics research have compiled various SNPs into a parentage panel to trace and/or confirm parentage of lambs to make accurate selection decisions based on correctly identified parents. Additionally, larger SNP panels are commercially available for genetic evaluations and research purposes, such as the Illumina Ovine50K BeadChip consisting of 54,241 SNPs and the Illumina High Density (HD) chip consisting of 603,350 SNPs. These panels include parentage markers as well as markers that are evenly spaced along the entire genome. Both the improvement of the genetic tools available and their implementation in selection decisions are important steps to accelerate genetic progress in the sheep industry.

In order to improve the accuracy of genetic selection within the sheep industry, it is important to understand how the genome and its associated traits are inherited. During formation of gametes, chromosomes must replicate, pair, recombine, and divide, resulting in unique genetic combinations that are passed on to the next generation. Understanding

the process of meiotic recombination during spermatogenesis, such as the number of times recombination occurs and where it occurs in sheep, will aid in improving genetic selection tools and accelerate genetic progress in the sheep industry. Additionally, this knowledge can be applied to other livestock industries to improve and accelerate genetic progress.

Spermatogenesis

Spermatogenesis is the generation of gametes in mammalian males, which is an essential process for the propagation of the next generation. In mice and cattle, the length of one complete cycle of spermatogenesis is 30-40 and 61 days, respectively; however, in sheep, spermatogenesis extends between 47-48 days (Ortavant, 1956; Cardoso and Queiroz, 1988; Franca et al., 2005; Zheng et al., 2006; Griswold, 2016). Spermatogenesis occurs within the seminiferous tubules of the testis (Russell et al., 1990; Jan et al., 2012). Inside the seminiferous tubules, germ cells develop near Sertoli cells, which help stabilize the environment for cell proliferation (Jan et al., 2012). Between the seminiferous tubules are Leydig cells, blood vessels, lymphatic vessels, and peritubular myoid cells, which aid in developmental signaling and transport of nutrients and hormones (i.e. testosterone) to the developing germ cells (Russell et al., 1990).

Development of Primordial Germ Cells

During development of the mammalian fetus, epiblast cells are fated to become primordial germ cells (PGCs) (Saitou, 2009; Richardson and Lehmann, 2010). In mice, the gestational period is approximately 20 days (Ohinata et al., 2005). It is hypothesized that a single layer of epiblast cells begins to express the gene *Prdm1*, a transcription regulator

required for PGC specification, on embryonic day 6 (E6) (Ohinata et al., 2005). Further, bone morphogenic proteins (BMPs) and the gene *Prdm1* expressed in the extraembryonic ectoderm are thought to be essential for PGC specification in mice and other mammals (Lawson et al., 1999; Yin et al., 2000; de Sousa Lopes et al., 2004).

Primordial germ cells (PGCs) migrate from the posterior primitive streak to the genital ridge to further develop and proliferate. In mice, PGCs migrate from the primitive streak to the allantois and embryonic endoderm at E7.5-8 and arrive in the hindgut by E9 (Anderson et al., 2000; Molyneaux et al., 2001). Migration is complete by E13.5 when PGCs arrive at the genital ridges and begin to proliferate (Tam et al., 1981). Migration of PGCs to the incorrect location or arrival at the incorrect time will result in apoptosis in these cells (Runyan et al., 2006). Interaction between ligands secreted by somatic cells including stem cell factor (STF) and stroma cell-derived factor 1 (SDF1), and their receptors expressed by PGCs, tyrosine kinase receptor c-KIT and G-protein coupled receptor CXCR4, aid in migration from the hindgut to the genital ridges to colonize and proliferate (Ara et al., 2003; Molyneaux et al., 2003; Runyan et al., 2006; Richardson et al., 2010). In addition, mammalian PGCs express adhesion molecule cadherin (CDH1) which help with migration (Bendel-Stenzel et al., 2000; Di Carlo and De Felici, 2000).

At the genital ridge, PGCs are now termed gonocytes and are surrounded by Sertoli cells. These gonocytes are subject to signals from the somatic cells that promote masculinization, and express the protein DAZL in response to these signals (Durcova-Hills and Capel, 2008; Gill et al., 2011). In mice, gonocytes enter quiescence in E15-16 until birth when they begin to move from the center of the tubule towards the basement membrane and transition into A spermatogonia (Sapsford, 1962; Kluin and de Rooij, 1981;

Vergouwen et al., 1991; Nagano et al., 2000). A balance between self-renewal and differentiation of A spermatogonia must occur, but the mechanism behind this is poorly understood (Buaas et al., 2004; Sada et al., 2009; Oatley et al., 2011). Genes including DNA binding 4, zinc finger and BTB domain containing 15 (ZBTB15), and nanos C2HC-type zinc finger 2 (NANOS2) are thought to be involved in the regulation of differentiation, cell renewal, and somatic cells of the testis (Oatley et al., 2011; Buaas et al., 2004; Sada et al., 2009; de Rooij, 2009).

Mitotic and Meiotic Phases of Spermatogenesis

During spermatogenesis, spermatogonial stem cells progress through differentiation to eventually develop into spermatozoa, as outlined in **Figure 1.1** (Russell et al., 1990; Jan et al., 2012). The phases of spermatogenesis can be classified as mitotic phase, meiotic phase, and spermiogenesis (Russell et al., 1990; Jan et al., 2012). During the mitosis phase, PGCs undergo DNA replication and cell division to produce type A daughter

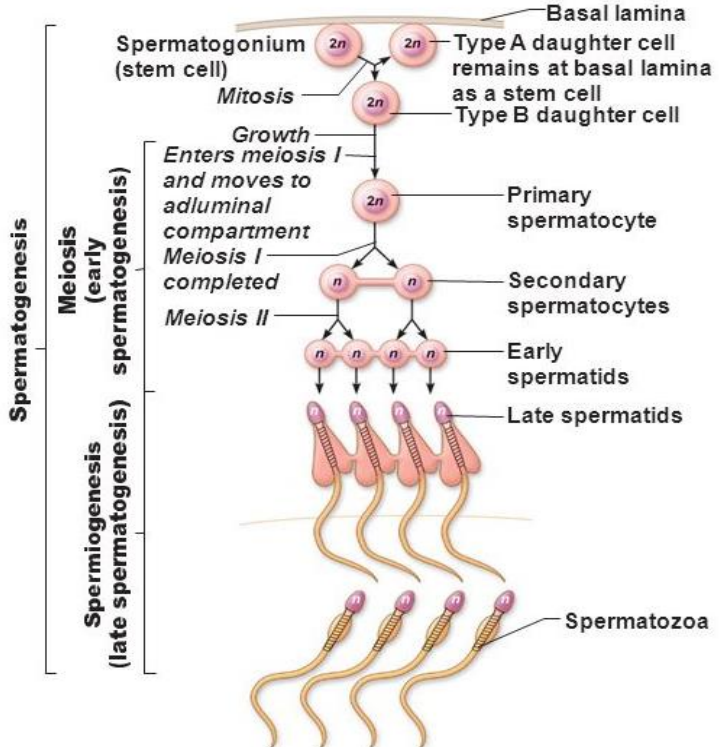


Figure 1.1: Spermatogenesis overview. This figure depicts the different stages of spermatogenesis from spermatogonium through spermatozoa (adapted from Pearson Education, copyright 2010).

cells. Most type A daughter cells will progress through the meiosis phase; however, some will continue to self-renew to allow for a continuous supply of germ cells. Cells that progress through meiosis will undergo both equational and subsequently reductional divisions, thereby reducing the diploid genome to haploid. During spermiogenesis, haploid round spermatids elongate and are released into the lumen of the seminiferous tubules as spermatids (Russell et al., 1990; Jan et al., 2012). Each step in this process can be observed in **Figure 1.1**.

Spermatogonial stem cells (SSCs) are located at the basement membrane of the seminiferous tubules (Jan et al., 2012). Single spermatogonia (As) first undergo mitotic divisions, and the resulting cells either divide again to either maintain the stem cell pool or differentiate into spermatogonia (de Rooij, 1998; Jan et al., 2012). Next, spermatogonia undergo multiple mitotic divisions. However, during this stage, cytokinesis is incomplete and spermatogonia remain connected by an intercellular bridge (Aponte et al., 2005). After the first incomplete mitotic division, spermatogonia are called Apr (A paired), and after a subsequent division are called Aal (A aligned) spermatogonia (Aponte et al, 2005). Cell differentiation is an organized and time regulated process, with differentiation of Aal spermatogonia and spermatogenic waves occurring continuously (Jan et al., 2012). As a result of the spermatogenic waves, cells in different stages of spermatogenesis can be visualized in a cross section of a seminiferous tubule (Leblond and Clermont, 1952; Oakberg, 1956).

In mice and rats, Aal differentiate into A1 spermatogonia without any further mitotic divisions, then continue to divide mitotically to A2, A3, and A4 stages (Aponte et al, 2005; Jan et al., 2012). The transition of Aal to A1 spermatogonia is hypothesized to be

dependent on retinoic acid, as vitamin A deficient mouse spermatogonia do not transition to the A1 stage (van Pelt and de Rooij, 1990; Gaemers et al., 1998). Next, A spermatogonia transition into B spermatogonia, divide, and enter meiosis, which will be discussed in detail in a subsequent section (Aponte et al, 2005; Jan et al., 2012; Handel and Schimenti, 2010).

Spermiogenesis

After meiosis is complete, round spermatids progress into spermiogenesis, which consists of four distinct phases: golgi, cap, acrosomal, and maturation. Through these phases, spermatids undergo morphological and cytological changes including the formation of a distinct head, midpiece, and tail region (Jan et al., 2012). The chromatin inside the nucleus undergoes remodeling, including condensation and cessation of transcription (Meistrich et al., 2003). The acrosome, a vesicle that contains enzymes necessary for penetration of the oocyte during fertilization, is formed at the top of the nucleus (Meistrich et al., 2003). Mitochondria migrate to the midpiece, as they are required to convert glucose to energy to aid in movement of the sperm (Sun and Yang, 2010). The flagellum, a structure that enables motility, develops and is composed of a microtubular structure called the axoneme (Fawcett, 1975).

Sertoli cells continue to be important contributors in this stage of spermatogenesis, as they provide junctions composed of integrins, laminins, cadherins, and catenin complexes that hold spermatids in place until spermiation (O'Donnell et al., 2011). After these morphological changes, elongated spermatids are then released into the lumen of the seminiferous tubule through a process termed spermiation (Jan et al., 2012). During

spermiation, the cytoplasm is removed by a residual body that is pinched off at spermiation and a cytoplasmic droplet is formed and migrates down the midpiece and is eventually sloughed from the end of the tail (Zheng et al., 2007).

The process of spermatogenesis is essential for fertility and reproductive efficiency of sheep and ultimately the economic viability of the sheep industry. Understanding the mechanism of spermatogenesis and the factors that affect this process will help us understand sperm abnormality and fertility, as well as how genetic material is passed on to the next generation.

Meiosis

Arguably one of the most important stages in spermatogenesis is meiosis. Without this essential process functioning properly, the formation of mature sperm would cease. Primary spermatocytes must first undergo DNA replication during a prolonged S-phase, followed by a highly regulated G2-phase and initiation of meiotic prophase I (Lin et al., 2008; Anderson et al., 2008). Transition to prophase I of meiosis depends on RNA binding protein DAZL, causing expression of stimulated by retinoic acid 8 (STRA8), initiating prophase I (Lin et al., 2008; Anderson et al., 2008).

After DNA replication, homologous chromosomes must pair and synapse, forming a synaptonemal complex, during the process of recombination (de Boer and Heyting, 2006; Costa and Cooke, 2007; Yang and Wang, 2009). Subsequent to recombination, the synaptonemal complex is degraded to allow for separation of homologous chromosomes (Yang and Wang, 2009). Recombination events are eventually resolved into chiasmata, which help maintain orientation of homologous chromosomes and provide the foundation

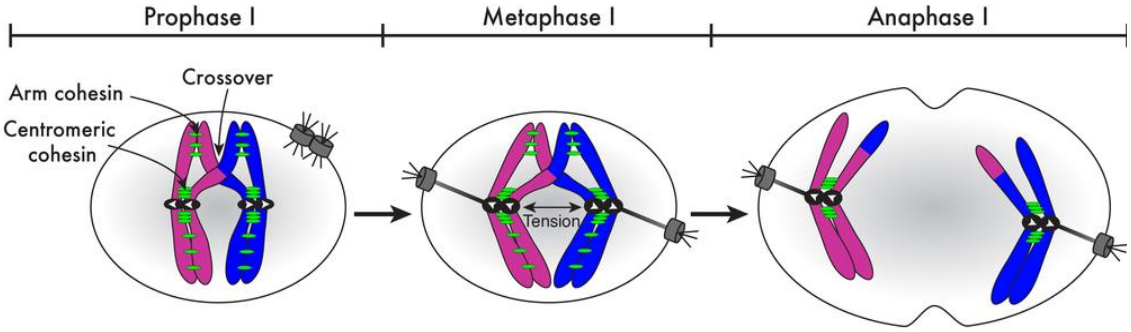


Figure 1.2: Chromosome segregation and equational meiotic division. This image depicts a recombination event (crossover), spindle attachment, and segregation of homologous aided by tension to the proper side of the cell to progress further into meiosis II (adapted from Miller et al., 2012).

for direct meiotic spindle attachment (Vogt et al., 2008; Holt and Jones, 2009). Following recombination, chromosomes arrange on the metaphase plate, attach to spindle poles, and undergo the first meiotic division (**Figure 1.2**) (Handel and Schimenti, 2010).

Homologous chromosomes are not syanpsed during S phase; however, syanpsis occurs during prophase I in the G2 stage of the cell cycle (Miller et al., 2013). Synapsis is guided by the cohesion complex, and homologous chromosome pairs are fully synapsed by pachytene stage of prophase of meiosis I (also termed prophase I). Prophase I of meiosis is the longest stage because of the process of homologous recombination (Handel and Schimenti, 2010). Cells then progress through the remainder of meiosis I by lining up on the metaphase plate and undergoing equational division (Anderson et al., 2008; Handel and Schimenti, 2010; Jan et al., 2012).

During metaphase I, spindles from opposite poles of the cell must attach to homologous chromosomes to segregate properly during the first meiotic division (Cleveland et al., 2003; Westermann et al., 2007; Winey et al., 1995). Spindle attachment of homologous chromosomes to opposite poles are guided by the cohesion complex (Bernard et al., 2001). Cohesins are established earlier during meiosis and are essential for

pairing of homologous chromosomes and orienting kinetochores (Miller et al., 2013; Murdoch et al., 2013). Physical connections of spindles to the homologous chromosomes, including attachment to chiasma and kinetochores, provide adequate resistance to pulling forces (Buonomo et al., 2000). Tension of the meiotic spindles is a major force that aids in achieving the desired outcome of proper chromosome segregation (Miller et al., 2013). Once each of the homologs attach to spindles from opposite poles, the kinetochore-spindle-chromosome interface exerts force to pull chromosomes apart (Miller et al., 2013).

The loss of cohesion complexes that hold homologous chromosomes together during prophase I, and importantly, recombination, is essential for proper segregation (Miller et al., 2013). Cohesins distal to the site of recombination events must be cleaved to physically allow for spindle attachment (Buonomo et al., 2000). However, pericentric (in close proximity to the centromere) cohesions must be maintained to link sister chromatids together and facilitate segregation during meiosis II (Grinthal et al., 2010). A key factor in maintaining centromeric cohesion is a cohesion complex containing REC8 (Miller et al., 2013; Katis et al., 2004). The protein REC8 is known to be part of the kleisin family of structural maintenance of chromosome (SMC) proteins which aid in both holding together sister chromatids during meiosis and facilitating recombination of homologous chromosomes (Sandor et al., 2012). Meiocytes then progress through meiosis II and a reductional division, resulting in haploid spermatids (Anderson et al., 2008; Handel and Schimenti, 2010; Jan et al., 2012).

Meiosis is an essential process during proper gamete formation that allows for equational and reductional divisions of the genome from diploid to haploid. Homologous recombination during meiosis contributes to genetic variation and ensures proper

chromosome segregation. Furthermore, the combination of independent assortment and recombination of homologous chromosomes stabilize genetic variation.

Meiotic Recombination Mechanism

Meiotic recombination during gamete formation is an important contributor to genetic diversity in mammals. Understanding this process and how chromosomes recombine will contribute to more accurate genetic predictions to select for superior offspring.

Inadequate or misplaced meiotic recombination may result in improper segregation of chromosomes during meiosis. This is because the orientation of homologous chromosomes and spindle attachment while at the metaphase plate are dependent on meiotic recombination to ensure proper segregation (Hassold et al., 2007; Handel and Schimenti, 2010). Nondisjunction, or failure of homologous chromosomes to separate properly during meiosis, can lead to aneuploidy which is the leading cause of miscarriage and congenital birth defects in humans (Hassold and Hunt, 2001). Aneuploidy is characterized by an extra chromosome or lack of an entire chromosome. An example of survivable aneuploidy in humans is Down Syndrome, or trisomy 21, a condition which describes an extra whole or partial segment of chromosome 21 (Hassold et al., 2007). Although there are clear differences among organisms, many of the biological features of meiotic recombination are conserved including the pathway or processes, structures, proteins, and genes involved in the pathway (Hunter, 2003).

Synapsis of Homologous Chromosomes

Before recombination can occur during meiosis, homologous chromosomes must replicate, pair, and synapse. Replication occurs during a prolonged S-phase (Lin et al., 2008). Telomere tethering to the nuclear envelope aids in the correct pairing of homologs aided by the protein SUN1 (SUN domain-containing protein 1) (Baudat et al., 2013; Boateng et al., 2013). Next, these homologs need to be anchored together in order to exchange genetic material. A cohesion complex containing REC8, SMC1β, and SMC3 hold sister chromatids together and are hypothesized to play a role in guiding synapsis of homologous chromosomes and proper segregation (Murdoch et al., 2013). Both *Rec8* and *Smc1b* null (-/-) male mice exhibit impaired synapsis of homologous chromosomes and shortened synaptonemal complex phenotypes (Bannister et al., 2004; Revenkova et al., 2004; Xu et al., 2005). In addition, dosage of these cohesins in male mice has an effect on the number of spermatocytes that are able to properly synapse and progress past prophase I (Murdoch et al., 2013).

The synaptonemal complex (SC) is a protein complex that includes synaptonemal complex protein 1 (SYCP1) at the central zone, and synaptonemal complex protein 3 (SYCP3) and synaptonemal complex protein 2 (SYCP2), which occupy the lateral, or axial, elements of the complex (see **Figure 1.3**) (Baudat et al., 2013). The process of synapsis is a separate pathway from recombination, however recombination cannot occur if homologs are not properly synapsed. Synapsis begins in leptotene stage of prophase I with the formation of the SC guided by the cohesion complex (Miller et al., 2013; Baudat et al., 2013). In this stage, small sections of the protein complex start to form. As the cell progresses to zygotene stage, the complex continues to form and has the appearance of

"zipping" of paired chromosomes together (Zickler and Kleckner, 2015). Homologous chromosomes are completely synapsed with a complete SC by pachytene stage. During diplotene, the SC begins to separate and eventually degrade, except at chiasmata sites (Zickler and Kleckner, 2015).

DNA Double Strand Breaks and Intermediate Processing

Prior to synapsis in G2 and in leptotene, a meiotic topoisomerase-like protein (SPO11) catalyzes DNA double stranded breaks (de Massy, 2013; Zickler and Kleckner, 2015). The beta form of this enzyme generates most of the double stranded breaks; however, the alpha form is necessary for catalyzing double strand breaks of the pseudoautosomal region of the XY chromosome pairing in males (Bellani et al., 2010; Kauppi et al., 2011). This enzyme is thought to be widely conserved among mammalian species (Malik et al., 2007). In addition to SPO11, other proteins including MEI1 (MEI1-homolog), MEI4 (MEI4-homolog), and REC114 (REC114-homolog) are involved with double strand break formation, however their roles are poorly understood (Muyt et al., 2007; Kumar et al., 2010).

Subsequent to end maturation, double strand breaks are processed with the assistance of co-localized recombinases DNA meiotic recombinase 1 (DMC1) and RAD51 recombinase (RAD51) (Baudat et al., 2013). These recombinases can be visualized with immunofluorescence staining and previous research has used these proteins as markers for double stranded breaks (Baudat et al., 2013). In humans, between 200 and 400 DMC1 foci per cell are present (Baudat and de Massy, 2007). Histone variant H2AX is another marker for double strand break locations as it is phosphorylated by ATM kinase in order to trigger

responses to the break (Barchi et al., 2005; Bellani et al., 2005). The breast cancer protein BRCA2 (breast cancer 2, DNA repair associated) is also thought to assist the function of DMC1 and RAD51 (Sharan et al., 2004). In addition, DMC1 is enhanced by both HOP2 (homologous-pairing protein 2 homolog) and MND1 (meiotic nuclear division protein 1 homolog) during single strand end processing (Chi et al., 2007; Petukhova et al., 2005). At this stage, breaks are not committed to a particular pathway and can be resolved by either a crossover (CO) or a non-crossover (NCO).

Following end maturation and processing, a 3-prime overhang is formed and strand invasion and intermediate processing begins (Baudat et al., 2013). Several proteins help stabilize and/or process intermediates and may regulate commitment to CO or NCO pathways (see **Figure 1.3**). During intermediate processing, MSH4 (mutS protein homolog 4), MSH5 (mutS protein homolog 5), TEX11 (testis-expressed sequence 11), and RNF212 (ring finger protein 212) co-localize with RAD51 and DMC1 (Baudat et al., 2013). Ring finger protein 212 (RNF212) is thought to stabilize MSH4 and TEX11, and may be specific to eventual crossover formation (Cheng et al., 2006).

Recombination intermediates are then further processed and resolved as either a NCO or CO. Most double stranded DNA breaks are resolved into the NCO repair pathway as a gene conversion or non-homologous end joining (NHEJ). A gene conversion event does not involve an exchange of genetic material, but rather the repair of the broken strand of DNA using the other as a template, and in the process, may acquire some variation from the original sequence (Baudat et al., 2013).

Crossover Resolution

Few double stranded DNA breaks are resolved into CO. The majority of CO are subject to the principle of interference, which states that one CO cannot occur in too close of proximity with another (Holloway et al., 2008; Zickler and Kleckner, 2015). Crossovers susceptible to this phenomenon are widely spaced along chromosomes, and are hypothesized to be less likely to cause problems during segregation because of their placement (Holloway et al., 2008). In mice, approximately 90% of CO experience positive interference (Holloway et al., 2008). Intermediates committed to forming CO that experience positive interference will be processed into double Holliday junctions and further resolved into a CO (Baudat et al., 2013). Proteins in this pathway include mutL homolog 1 (MLH1), mutL homolog 3 (MLH3), and exonuclease 1 (EXO1) which are involved in resolving these double Holliday junctions into CO (see **Figure 1.3**) (Baker et al., 1996; Edelmann et al., 1996; Lipkin et al., 2002; Wei et al., 2003). The protein MLH1 is commonly used as a marker for visualizing CO in humans, mice, and other mammals (Hassold et al., 2007).

Approximately 10% of crossovers are resolved through an interference independent pathway in mice (Holloway et al., 2008). Intermediates processed into single Holliday junctions are resolved into CO with the assistance of the protein MUS81 structure-specific endonuclease subunit (MUS81) (Baudat et al., 2013). The CO pathway involving MUS81 is independent of both the MLH1/MLH3 and CO interference pathway (Holloway et al., 2008). In MLH1 and MLH3 null mice, not all CO events were eliminated, which suggested the existence of an alternative CO pathway independent of the MLH1/MLH3 pathway (Guillon et al., 2005; Svetlanov et al., 2008). The exact mechanism of this pathway

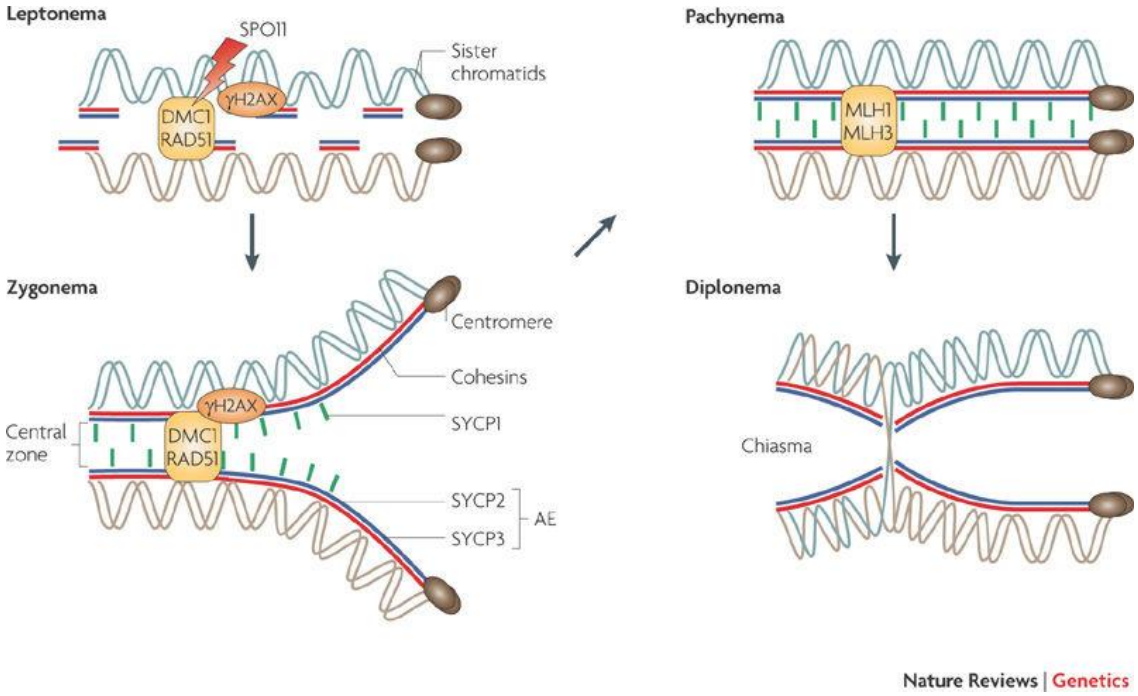


Figure 1.3: Synapsis and crossing over of homologous chromosomes. Depicts the process of synapsis in each stage of meiotic prophase I. In the zygotene picture, elements of the synaptonemal complex and other surrounding proteins are labeled. Proteins involved in recombination are also shown in each stage.

remains unclear, though there are a few proposed theories (Holloway et al., 2008). The protein MUS81 is proposed to interact earlier in the pathway, before RAD51 and DMC1, to cleave 3' flaps after DNA double strand breaks, as well as later in the pathway to resolve Holliday junctions (Hunter and Kleckner, 2001; Hollingsworth and Brill, 2004; Holloway et al., 2008). Reduced reproductive function, including testis size and sperm count, resulted from *MUS81* null male mice; however, this was not sufficient to severely reduce fertility (McPherson et al., 2004; Dendouga et al., 2005). Null *MUS81* mice also had more MLH1 and MLH3 in pachytene stage, likely resolving the CO that would have been part of the *MUS81* pathway (Holloway et al., 2008). These additional CO appeared to be interference independent even though MLH1 and MLH3 proteins were involved (Holloway et al., 2008).

After CO are established by either the interference dependent pathway with the aid of MLH1, MLH3, and EXO1, or the interference independent pathway with MUS81, chiasmata are formed and cells progress through the rest of meiosis. Defects in the meiotic recombination process can result in the arrest or delay in pachytene stage and therefore the progression from prophase I (Handel and Schimenti, 2010). Further, defects in chromosome pairing and failure to synapse can result in serious defects of the downstream products. A stringent checkpoint exists at pachytene stage in males to eliminate defective spermatocytes from progressing through spermatogenesis (Handel and Schimenti, 2010).

To reiterate, meiotic recombination is an important process in mammals that is essential for adequate formation of sperm and contributes to genetic diversity. In sheep, understanding the process of meiotic recombination will help to uncover the underlying biological and genetic mechanisms that control how often and where these recombination events occur. Because recombination is important to ensure proper chromosome segregation in meiosis, failure, inadequate, or misplaced recombination events can lead to nondisjunction and decreased fertility.

Global Recombination

Previous studies in mammals indicate that meiotic recombination is not a random process. At least one recombination event, or crossover (CO) per chromosome arm is necessary to avoid mis-segregation (Hassold et al., 2007). Understanding involvement of various genes in the recombination pathway can aid in prediction of the numbers and locations of recombination events in sheep. Additionally, males and females are dimorphic in most species as they do not exhibit the same number of CO (Matise et al., 2007;

Murdoch et al., 2010). For example, in humans and mice, females have greater numbers of CO per meiotic cell than males (Coop and Przeworski, 2007; Dumont and Payseur, 2011). However, according to linkage map lengths, sheep are hypothesized to exhibit opposite dimorphism than humans and mice, as males are predicted to have higher numbers of CO than females (Dumont and Payseur, 2011).

Meiotic recombination has been shown to be variable in mice and human models. Murdoch and associates reported that global recombination, or the total number of CO per meiotic cell, was significantly different between two strains of mice, CAST/EiJ and C57BL/6J ($P < 0.00001$) (Murdoch et al., 2010). When these two strains were crossed, F1 and F2 generations exhibited global recombination numbers that were different than the parental strains (Murdoch et al., 2010). An association study was performed using single nucleotide polymorphism (SNP) data, and multiple quantitative trait loci (QTLs) were associated on chromosomes 2, 3, 4, 15, 17, and X that cumulatively explained approximately 50% of the phenotypic variation (Murdoch et al., 2010). Further investigation of QTL locations revealed that the gene *Prdm9*, a known player in the recombination pathway on chromosome 17, was not associated with global recombination numbers (Murdoch et al. 2010). However, the QTL on the X chromosome in close proximity to *Tex11*, which is known to be essential for male meiosis and is involved in recombination, was highly associated (Murdoch et al. 2010, Yang et al. 2008, Zheng et al. 2010). This study provides strong evidence that the complexity of meiotic recombination and the many loci that may contribute to CO numbers.

Interestingly, the number of double strand DNA breaks is unlikely to be directly responsible for overall differences in CO numbers between strains of mice (Keeney et al.,

1997). In male mice, heterozygous *Spo11* null (+/-) animals exhibited a decreased amount of DNA double strand breaks, however the number of MLH1 foci remained consistent with wild type mice (Keeney et al., 1997; Baier et al., 2014). This study implies that the determinant for the number of CO per spermatocyte is established before DNA double strand breaks occur.

The gene ring finger 212 (*RNF212*) has been associated with global recombination numbers in mice and humans (Kong et al., 2008; Chowdhury et al. 2009, Baudat et al., 2010; Berg et al., 2010; Parvanov et al., 2010). In mice, males heterozygous for null (+/-) *Rnf212* are still sufficiently fertile, however they had significantly fewer MLH1 foci compared to wild type males ($P < 0.001$) (Reynolds et al., 2013). Additionally, the number of MSH4 foci was significantly decreased in heterozygous *Rnf212* null male mice ($P < 0.05$) (Reynolds et al., 2013).

In Holstein-Friesian cattle, associations with number of CO derived from SNP and pedigree data in males were observed with variants in both *RNF212* and REC8 meiotic recombination protein (*REC8*) (Sandor et al., 2012). Another study in Holstein cattle using SNP and pedigree data identified regions near complexin 1 (*CPLX1*), REC114 meiotic recombination protein (*REC114*), and NIMA related kinase 9 (*NEK9*) that associated with number of CO in males and females (Ma et al., 2015). The gene *REC114* is known to be involved with DNA double strand break formation during meiosis (Muyt et al., 2007; Kumar et al., 2010). In Soay sheep, a SNP at the end of OAR6 near *RNF212* and *CPLX1* (s74824.1) was associated with number of CO identified by a similar pedigree and SNP method in females, but not males (Johnston et al., 2016).

In addition to the influence of various loci, chromatin compaction and length of the synaptonemal complex is related to global recombination numbers. In mice, strain-specific differences in both synaptonemal complex length and DNA loop size was characterized using fluorescence in-situ hybridization (FISH) probes and measuring the width of DNA loops (Baier et al., 2014). Interestingly, CO numbers increased with synaptonemal complex length in male mice, implying that more CO occur on longer homologous chromosome pairs (Baier et al., 2014). A similar result was found in other species including cattle, goats, and sheep (Frolich et al., 2015). Additionally, chromatin loops size was inversely related to CO number in mice, implying that compaction may contribute to CO number early in meiosis (Baier et al., 2014).

Elucidating genetic control of meiotic recombination and understanding chromatin packaging will aid in increasing the accuracy of genetic predictions. Genes that are associated with the number of CO in sheep and other livestock species could be used to predict the number of CO in individual animals. The ability to both understand and predict the process of meiotic recombination based on influences such as chromatin packaging and genetic variation will increase the accuracy of genetic tools and selection strategies used within livestock industries.

Hotspot Utilization and Regulation

Some crossovers exhibit distinct location preferences which are termed “hotspots.” This non-random characteristic of recombination events is fascinating and increases the predictability of recombination. In addition, CO experience interference in that one CO cannot occur in too close of proximity with another (Zickler and Kleckner, 2015).

Understanding how hotspots are regulated and where they occur will contribute to making genetic predictions more accurate in sheep as well as other livestock species, enabling us to essentially predict where homologs are exchanging genetic material. This basic understanding of the non-random characteristics of recombination is strong evidence that there is some biological and mechanistic control over the location and frequency of hotspot occurrence.

One essential CO location that is arguably considered a hotspot in mammalian species is located on the pseudoautosomal region (PAR) of the X and Y chromosomes in spermatocytes (Hinch et al., 2014). In mice, previous studies have identified two additional hotspots, *Psmb9* on chromosome 17 and *Hlx1* on chromosome 1, both of which are mediated by the gene *Prdm9* (Baudat et al., 2010). In humans, there are population specific hotspots, and in mice, there are strain specific hotspots (Baudat et al., 2010). Hotspots are rarely conserved between species, which is evident with hotspot differences in chimpanzees and humans, despite over 99% DNA sequence similarity between these species (Winckler et al., 2005). Differences in the distributions of hotspots occur in both human and mouse species (Baudat et al., 2010; Parvanov et al., 2010). In sheep, specific hotspots apart from the PAR have yet to be identified.

The gene that is thought to mediate most hotspots is *PRDM9*, which is expressed exclusively in meiotic cells (Baudat et al., 2013). Histone methyl transferase family member *PRDM9* contains multiple domains including KRAB, SSXRD, PR/SET, and zinc finger domains (Baudat et al., 2013). The PR/SET domain is responsible for the methyl transferase activity as this protein trimethylates histone H3 on the fourth lysine (H3K4me3) when bound (**Figure 1.4**) (Baudat 2013). After trimethylation of H3K4, the

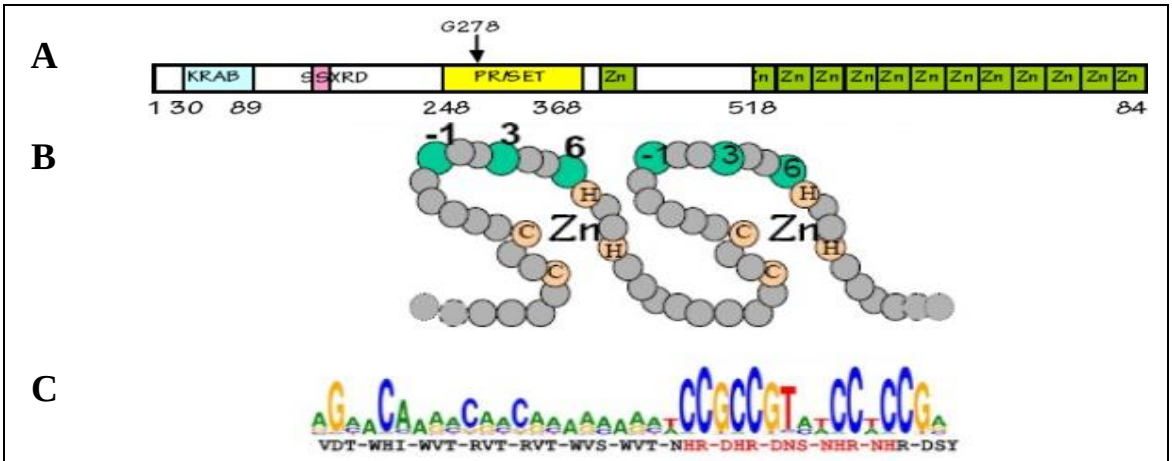


Figure 1.4: Structure of the *PRDM9* gene in mice. *PRDM9* is a histone methyl transferase family member with the A) PR/SET domain responsible for methyl transferase activity and zinc finger repeat region responsible for recognition and binding to specific DNA motifs, and B) the amino acid structure of zinc fingers. Regions -1, 3, and 6 are involved with DNA motif recognition and show the most sequence variation in mice. C) A depiction of the binding specificity of *PRDM9* zinc fingers to a DNA motif (adapted from Kumar and de Massy, 2010).

SPO11 enzyme complex is recruited to induce a DNA double strand break (Hayashi et al., 2005; Wu et al., 2013; Baudat et al. 2013). However, the zinc finger domain consisting of a minisatellite 84 base pair repeat region, can vary both in sequence and number of finger repeats (Baudat et al. 2013). The amino acid structure of the zinc finger domain, specifically positions -1, 2, 3, and 6 relative to the start of the alpha helix, recognize and bind to specific DNA motifs and determine binding specificity (see **Figure 1.4**) (Baudat et al., 2013).

The gene *PRDM9* is expected to specify almost all hotspots in humans except the PAR on sex chromosomes (Baudat et al., 2010; Berg et al., 2010). In *Prdm9* null (-/-) mice, spermatocytes fail to progress through meiosis, or more specifically, prophase I (Hayashi et al., 2005). Interestingly, canines lack a functional *PRDM9*, however this species still has CO events and may have hotspots (Muños-Fuentes et al., 2011). This mechanism is not as well understood in canines.

The zinc finger array of *PRDM9* is thought to be undergoing positive selection, however the KRAB box component of this gene retains a conserved function (Oliver et al., 2009; Birtle and Ponting, 2006). As hotspots mediated by *PRDM9* eventually erode, new variants in the zinc finger array are introduced to replace DNA binding motifs that have been broken down (Baker et al., 2015). This process has been termed the recombination "hotspot paradox" (Baker et al., 2015; Boulton et al., 1997; Myers et al., 2010).

Additionally, *PRDM9* is considered the "speciation gene" because of the rapidly evolving zinc finger array that can shift genome wide hotspot positions, potentially causing incompatibility between different genetic backgrounds (Dumont and Payseur, 2011). In a previous study in male mice, the resultant protein from a *Prdm9* allele derived from C57BL/7 (*Mus musculus musculus*) mice had decreased or lost activity when placed in CAST/EiJ (*Mus musculus castaneous*) mice (Dumont and Payseur, 2011). In mice, *Mus musculus domesticus* and *Mus musculus musculus* hybrids exhibited male-specific sterility that resulted from incompatibility of the zinc finger array of *Prdm9* and failure of meiotic prophase (Mihola et al., 2009). The phenotype of sterile hybrid males is similar to *Prdm9* null (-/-) males: defective synapsis and unrepaired recombination intermediates (Bhattacharyya et al., 2013). Interestingly, inserting a human allele of (humanizing) *Prdm9* in *Mus musculus domesticus* and *Mus musculus musculus* hybrids restored fertility in males (Davies et al., 2016). This information suggests that *PRDM9* and its role in the hotspot pathway has a strong influence on progression of the meiotic recombination process.

The binding specificity of *PRDM9* to DNA motifs in various mammalian species is key to understanding where hotspots occur and how to predict them. In sheep, specific

hotspots are not well understood, however several research groups have begun to characterize *PRDM9* in different breeds of sheep from India as well as the United States. Understanding the predictability of hotspot locations and how hotspots differ within and between sheep will increase accuracy of genetic predictions.

Meiotic Recombination in Livestock

Our understanding of meiotic recombination in livestock is arguably lacking comparatively with humans and mice. Given the economic importance of livestock to the U.S., improving our understanding of meiotic recombination will advance reproduction and increase the accuracy of genetic predictions.

In addition to their importance to animal agriculture, sheep provide an interesting animal model to study meiotic recombination. Generally, mammalian females exhibit higher recombination than males (Lenormand and Dutheil, 2005). However, males are thought to have higher recombination than females in sheep based on linkage map length (Maddox et al., 2001; Johnston et al., 2016). One theory suggests that it is important for females to have higher recombination to prevent aneuploidy, as oocytes undergo a long period of arrest in diplotene (Koehler et al., 1996; Morelli and Cohen, 2005; Nagaoka et al., 2012). Research in meiotic recombination in sheep may help elucidate how this pathway differs in males in females, and answer important biological questions as they relate to sheep and other livestock species.

Recombination Characterization Using Cytogenetics

Little research has been conducted thus far on meiotic recombination in sheep, especially using a cytogenetic approach, as most studies have employed the use of SNP data combined with pedigree information. One study conducted by Frolich and associates briefly characterized recombination in male cattle (n=4, Czech spotted breed), sheep (n=11, mixed breeds), and goats (n=8, pygmy breed) using a cytogenetic approach. Significant differences in the average number of crossovers (CO) represented by MLH1 foci was found between sheep, goats, and cattle species, as well as males within the same species ($P < 0.01$) (Frolich et al., 2015). Sheep exhibited higher numbers of MLH1 foci than goats and cattle, and cattle had 33.1% lower number of foci than sheep (Frolich et al., 2015). Species differences in number of MLH1 foci were attributed to environment and environmental stress, as higher numbers of CO may help in a species' ability to respond to the changing environment by creating a greater number of new allele combinations (Butlin, 2005).

Differences in synaptonemal complex (SC) length was also identified between species, with sheep having significantly longer SC's than cattle by 7.5% ($P < 0.01$) (Frolich et al., 2015). This difference could be attributed by variation in chromatin packaging or genome size (Kleckner et al., 2003; Kauppi et al., 2012). Centromere activity is hypothesized to suppress CO formation along with DNA methylation and packaging of chromatin (Smagulova et al., 2011). No CO were observed in close proximity to the centromere in the centromere in sheep, cattle, or goats characterized (Frolich et al., 2015).

In addition, there was a significant correlation between individual SC length and number of CO on that SC ($P < 0.01$) (Frolich et al., 2015). As SC length increased, the

number of CO on that SC also increased, however, no significant correlation was found between total CO and total SC length (Frolich et al., 2015). Interestingly, almost all SC's had at least one MLH1 foci in sheep, goats, and cattle (Frolich et al., 2015). The "recombination density" calculation was made by dividing the number of MLH1 foci by the total autosomal SC length was also compared between species (Frolich et al., 2015). The highest density of recombination was observed in sheep, and the lowest observed in goats (Frolich et al., 2015). Goats have a longer total SC length than both sheep and cattle, although cattle have the lowest number of CO of all three species (Frolich et al., 2015).

Genetic Association Studies with Global Recombination

Genetic associations with meiotic recombination in sheep were identified by Johnston and associates in a study consisting of Soay sheep, a once-domestic breed of sheep currently living unmanaged on the St. Kilda archipelago in Scotland (Clutton-Brock et al., 2004; Johnston et al., 2016). The number of recombination events was determined using a sub-pedigree analysis and SNP data obtained from the Ovine50K chip. The length of the sex-average linkage map was reported as 3304 cM; with the male map (3748 cM) longer than the female map (2860 cM) (Johnston et al., 2016). In addition, the ratio of crossovers per length of chromosome was higher on smaller chromosomes, indicating that the one obligatory CO per homologous chromosome pair was met (Johnston et al., 2016). The number of CO was also positively associated with GC content in sheep (Johnston et al., 2016). The sex averaged heritability of global recombination numbers was reported as 0.15 in sheep; with sex-specific heritability in females higher (0.16) than males (0.12) (Johnston et al., 2016).

In sheep, associations with global recombination was found most significantly in a region on OAR6 containing the genes *RNF212* and *CPLX1* (Johnston et al., 2016). Although this was significantly associated in both sexes, further examination via sex-specific models revealed that this association was significant in females but not in males (Johnston et al., 2016). The SNP in close proximity to *RNF212* and *CPLX1* explained 8.02% of the phenotypic variance and 46.7% of the additive genetic variance in females, but did not contribute in males (Johnston et al., 2016). This region on OAR6 was examined and found to be in high linkage disequilibrium ($r^2 > 0.8$) (Johnston et al., 2016). Additionally, a regional association approach identified a region on OAR7 in a 1.09-Mb segment containing *RNF212B* and *REC8* (Johnston et al., 2016). Both *RNF212B* and *REC8* are genes of interest in the recombination pathway, and explained 4.12% of the phenotypic variance and 26.2% of the additive genetic variance in both sexes (Johnston et al., 2016). However, this association did not hold up in sex-specific multiple testing (Johnston et al., 2016). Additional SNPs were highly associated with recombination on OAR3 near the genes *LRRTM4* (leucine-rich repeat transmembrane neuronal 4), *WDR61* (WD repeat domain 61), and *RPL10* (ribosomal protein L10) which warrant further investigation (Johnston et al., 2016).

Characterization of PRDM9

Hotspot mediator gene *PRDM9* was characterized by Ahlawat and associates in cattle (n=280 animals), goats (n=183 animals), and sheep (n=250 animals) from India (Ahlawat et al., 2016a). In sheep, cattle, and goats, the zinc finger repeats have been reported to be 84 bp or 28 amino acids long (Ahlawat et al., 2016a). The zinc finger

domain of *PRDM9* varies in both number of repeats (size) and nucleotide sequence (Ahlawat et al., 2016a). The *PRDM9* paralog on ovine chromosome 1 (OAR1) is thought to be the location of the functional *PRDM9* in ruminant species (Ahlawat et al., 2016a; Ahlawat et al., 2016b). After PCR amplification of the zinc finger array and gel electrophoresis, two alleles were identified by size in the 25 breeds of sheep examined (Ahlawat et al., 2016a). The D allele was most commonly occurring (estimated size of 9 fingers), and the C allele was less common (estimated size of 8 fingers) (Ahlawat et al., 2016a). Most sheep exhibited the DD genotype, but 8.8% of sheep in 9 breeds exhibited the CD genotype and no sheep exhibited CC genotype (Ahlawat et al., 2016a).

When *PRDM9* was sequenced in sheep, the first zinc was found to be highly conserved in all breeds (Ahlawat et al., 2016a). There were 52 different zinc finger haplotypes identified in the sheep examined, with greater non-synonymous variability in -5, -1, 3, and 6 residue positions relative to the alpha helix structure of the protein (Ahlawat et al., 2016a). Some haplotypes were conserved; however, several were only seen in single samples (Ahlawat et al., 2016a). In total, the sheep examined displayed 26 unique alleles by sequence (Ahlawat et al., 2016a). Finally, a dN/dS analysis revealed that residues -1, 3, 4, and 6 were under positive selection in sheep (Ahlawat et al., 2016a).

Although some research on meiotic recombination has been conducted in sheep, knowledge of this process remains arguably lacking compared with other mammalian species. This presents opportunity for continued investigation of the recombination process in sheep as well as other economically important livestock species in order to increase accuracy of genetic predictions.

Summary

Although sheep numbers and operations have declined over the past few decades, demand for lamb and wool products still exists in the United States. Therefore, the desire to produce superior products will motivate the advancement of the sheep industry. Utilization of genetic tools to select superior offspring will contribute to a more productive, efficient, and sustainable industry. Currently, the use of estimated breeding values (EBVs) is starting to increase. Understanding how the process of homologous recombination occurs, and how genetic material is exchanged and passed to subsequent generations, will improve EBVs. Specific information regarding where and how many times homologous chromosomes recombine will be valuable information for directly improving the accuracy of genetic predictions. This knowledge will benefit the sheep industry as well as other livestock industries by accelerating genetic progress in achieving producer production goals. While the sheep industry is still lagging behind other livestock industries such as the cattle industry for implementation of genetic tools in selection and culling decisions, improvement of these tools, decreasing the cost, and increasing availability will accelerate genetic progress.

Chapter 2: Understanding Meiotic Recombination in Rams

Introduction

Meiotic recombination is an important process during gametogenesis that ensures proper chromosome segregation and contributes to genetic variation. It is clear from previous studies in mammalian species that recombination is not random, and at least one recombination event or crossover (CO) per chromosome arm is necessary for proper chromosome segregation (Vogt et al., 2008; Holt and Jones, 2009; Handel and Schimenti, 2010). Inadequate CO may result in improper segregation of chromosomes during meiosis, as CO facilitate the orientation and connection of homologous chromosomes for proper spindle attachment (Hassold et al., 2007; Handel and Schimenti, 2010). Nondisjunction, or failure of homologous chromosomes to separate properly during meiosis, can lead to aneuploidy which is the leading cause of miscarriage and congenital birth defects in humans (Hassold et al., 2007).

During prophase I, homologous chromosomes pair, aided by telomere tethering, and synapse together (Baudat et al., 2013; Boateng et al., 2013). The synaptonemal complex (SC) is a protein complex that includes synaptonemal complex protein 1 (SYCP1) in the central zone, and synaptonemal complex protein 3 (SYCP3) and synaptonemal complex protein 2 (SYCP2), which occupy the lateral, or axial, elements of the complex (Baudat et al., 2013). By pachytene stage, homologous chromosomes are fully synapsed with the SC fully formed (Zickler and Kleckner, 2015). The SC anchors homologous chromosomes together, an important process in order for recombination to occur.

Double strand breaks created in the DNA prior to synapsis are either repaired through the non-crossover (NCO) or the CO pathway. The resolution of breaks into a CO is aided by mutL homolog 1 (MLH1) (Baudat et al., 2013). The location of these COs are not random as some COs have been known to exhibit distinct location preferences which have been termed "hotspots" (Baudat et al., 2010; Parvanov et al., 2010; Smagulova et al., 2011; Baker et al., 2015). Additionally, COs experience interference, in that one CO cannot occur in too close of proximity with another (Zickler and Kleckner, 2015).

The total number of COs that occur in a cell is known to differ between strains of mice, however this has not been evaluated fully in livestock. We hypothesize that the number of COs per spermatocyte differ between individual rams within a breed as well as across breeds of sheep. Additionally, we hypothesize that the length of homologous chromosome pair is correlated with the number of CO, and CO exhibit location patterns that are conserved between individual rams and across breeds of sheep. In this study, CO were quantified and their locations on the SC characterized in males from different breeds of sheep. To accomplish this, we used a cytogenetic approach to directly examine CO which has advantages in that a reference genome or large number of progeny are not necessary to fully visualize CO.

Materials and Methods

Animals

Testicular tissue samples were collected post mortem from sexually mature Suffolk (n = 17), Targhee (n = 5), and Icelandic (n = 5) sheep. The Suffolk and Targhee rams were raised at the University of Idaho Sheep Center and sold by private contract to individuals

for harvest and meat consumption. The samples for the Icelandic rams were acquired from a flock that was processed through a local abattoir.

Sample preparation

Testicles were collected immediately postmortem and transported on ice to the laboratory for surface spread preparation (Anderson et al., 1999). Testicular tissue was dissected into approximately one-gram pieces and incubated for 45 minutes in a hypotonic buffer containing 30mM Tris, 50mM sucrose, 17 mM sodium citrate, 5 mM EDTA, 2.5 mM DTT, and 0.5 mM PMSF. Small sections (approximately 3 mm) of seminiferous tubule were cut and cells were removed gently to make a slurry in 100mM sucrose solution. Cell slurry (10 µl) was spread on microscope slides and fixed with 1% paraformaldehyde. Slides were placed in a humid chamber overnight to dry and incubated for 2.5 minutes in a bath of Kodak photoflo (1:200 dilution). Slides were stained immediately or frozen at -20ºC for future immunofluorescence staining.

Immunofluorescent staining

Immunofluorescent staining was performed to identify mutL homolog 1 (MLH1) and synaptonemal complex protein 3 (SYCP3) proteins as well as the centromere. Slides were immersed in a solution containing 1% Normal Donkey Serum, 3 mg/mL BSA, 0.005% Triton X-100, PBS (ADB) for 1 hour at room temperature. The polyclonal rabbit anti-mouse MLH1 antibody (Calbiochem, PC56-100UG) was diluted 1:60 in ADB and the human anti-centromere antibody (Antibodies Inc., Davis, CA, 15-234-0001) was diluted 1:100 in ADB and 60 µl applied to each slide. A cover slip was placed on the slide and

sealed with rubber cement. Slides were incubated in a humid chamber at 37ºC for 12-14 hours. After the removal of the coverslip, slides were washed twice (30 minutes and 1 hour) in ADB. AlexaFluor 488-conjugated donkey anti-rabbit secondary antibody (Jackson ImmunoResearch, Baltimore, MD, 711-005-152) was diluted 1:60 and donkey anti-human AMCA secondary antibody (Jackson Immunoresearch, 705-005-149) was diluted 1:100 in ADB and placed on the slides. Slides were covered with a cover slip, sealed with rubber cement, and incubated in a humid chamber at 37ºC for 4 hours. Slides were then washed in ADB for 30 minutes and 1 hour. The polyclonal rabbit anti-human SYCP3 antibody (Abcam, San Francisco, CA, ab15093) was diluted 1:100 in ADB and 60 µl placed on slides. Parafilm cover slips were applied and slides were incubated in a humid chamber for 2 hours at 37ºC. Slides were washed twice (30 minutes and 1 hour) in ADB. Rhodamine donkey anti-rabbit secondary antibody (Santa Cruz Biotechnology, sc-516249) was diluted 1:150 in ADB and 60 µl placed on slides. Parafilm cover slips were applied and slides were incubated for 1 hour in a humid chamber at 37ºC. Slides were then placed in three consecutive PBS washes (30 min, 30 min, and 1 hour). Lastly, 20 µl of ProLong® Gold Antifade Mountant (Fisher Scientific, P36930) was applied to slides and sealed with a cover slip. Slides were stored at 4ºC.

Analysis

A Leica DM6 B fluorescence microscope with appropriate filters (405, GFP, and Y3 cubes) were used for imaging. Pachytene stage cells (meiotic cells with fully synapsed homologous chromosomes) were identified and images were captured with an Andor Zyla sCMOS camera (Oxford Instruments). Only the images of pachytene stage cells with no

obstructions or defects were used in the subsequent dataset. In total, MLH1 foci was quantified in 2,758 spermatocytes in Suffolk (1,647 cells), Targhee (530 cells), and Icelandic (581 cells) rams by two separate scorers blinded to the results of the other using the Leica LASX software version 3.0.2. Weak fluorescence signal and too many cells clustering together and overlapping were reasons not to include spermatocytes from the final dataset.

Synaptonemal complex (SC) length was measured from the end to the first MLH1 foci, then measured between subsequent MLH1 foci, and finally from the final MLH1 foci to the distal end of the SC. Measurements were made in Leica LASX version 3.0.2 and ImageJ version 1.51 software, and recorded in μm. In total 270 cells (10 cells from each ram) were measured. The total length of each individual SC was determined by adding measurements between MLH1 foci. To compare MLH1 foci locations, SCs were sorted by size: the three metacentric homologous chromosome pairs, 4 large SCs, 5 small SCs, and 13 SCs that occupied an intermediate group. The total SC length per spermatocyte was calculated by adding all SC measurements in each spermatocyte. Locations of MLH1 foci on SCs were compared between breeds and individuals by calculating the foci location as a percent of the total SC length beginning from the shortest end to MLH1 foci distance.

After checking the data for normality using a histogram of residuals, differences in number of MLH1 foci per spermatocyte were examined across and within breeds using a one-way ANOVA and subsequent Tukey-Kramer multiple comparison test to account for differences in the number of spermatocytes per ram or breed. Statistical analyses were completed in R version 3.3.3 using the stats and psych packages. Differences between individual rams was examined by ANOVA using ram as the dependent variable and

average number of MLH1 foci per ram as the independent variable. Differences between breeds were examined with ANOVA using breed as the dependent variable and average number of MLH1 foci per breed as the independent variable. Significance was identified when $P < 0.05$.

Pearson's correlations and regression coefficients were calculated between the number of MLH1 foci and individual as well as total SC length. The percent of SCs with 1 – 9 MLH1 foci were calculated and compared between breeds. Additionally, differences in MLH1 foci locations on SCs between breeds and individuals were examined using a one-way ANOVA in R version 3.3.3 using the stats package, location as the independent variable, and ram or breed as the dependent variable.

Results

To investigate meiotic recombination characteristics in rams, a cytogenetic approach was used to directly characterize CO numbers and locations. In total, 2,758 spermatocytes and over 170,000 CO were examined. Testicular tissue samples were collected from 27 sexually mature rams from Icelandic, Suffolk, and Targhee breeds.

The number of CO per spermatocyte was determined by counting the number of MLH1 foci, a mismatch repair protein, in each spermatocyte. The total number of MLH1 was counted by two individuals, blinded to the score of the other, and the values were averaged. The focus on the XY chromosome were excluded from the total count. These counts were then compared within and between Icelandic, Suffolk, and Targhee breeds.

MLH1 counts in individual rams within each breed

Statistical differences in number of MLH1 foci were determined between individual rams in Suffolk, Targhee, and Icelandic breeds. The number of MLH1 foci in individual spermatocytes for each animal are plotted in **Figure 2.1** with a red bar denoting animal average. Within the Suffolk breed, 10 rams had no statistical difference in the number of MLH1 counts and the mean MLH1 number in these animals was consistent with the overall breed mean (x = 61.0 MLH1 per spermatocyte). However, four Suffolk rams exhibited a significantly ($P < 0.05$) greater number of MLH1 counts while three rams exhibited a significantly ($P < 0.05$) fewer number of MLH1 counts than the breed mean. Targhee and Icelandic breeds exhibited similar trends as Suffolk, where the majority of the individual rams do not have a significantly different number of MLH1 from their respective breed mean (66.3 and 63.8). In both the Targhee and the Icelandic breeds, one ram had a significantly greater ($P < 0.05$) and one ram had a significantly fewer ($P < 0.05$) number of MLH1 counts compared to their respective breed mean. In summary, the majority of individual rams within a breed (Suffolk, Icelandic, and Targhee) exhibited similar MLH1 counts, however a few rams did have significantly different MLH1 counts compared to their respective breed mean.

MLH1 counts in different ram breeds

The total number of MLH1quanitfied per spermatocyte for individual rams were plotted in **Figure 2.2** by breed. Each dot represents an individual spermatocyte and the red bar denotes the breed mean. Targhee rams had a significantly ($P < 0.01$) greater number of MLH1 counts than Icelandic and Suffolk rams. Icelandic rams also had a significantly ($P <$

0.01) greater number of foci than Suffolk rams. Targhee rams exhibited the greatest number of mean MLH1 counts (66.3 ± 0.26 SE), in comparison to that of Icelandic rams, which were intermediate (63.8±0.27 SE), and Suffolk rams (61.0±0.15 SE) which exhibited the least number of mean MLH1 counts. In summary, all three breeds were significantly different ($P < 0.01$) when compared to each other.

Synaptonemal complex length and number of MLH1 foci

To further characterize meiotic recombination in sheep spermatocytes, we examined the relationship between the length of the synaptonemal complex (SC) and number of recombination events for each homologous chromosome pairing. In total, SC lengths were measured in 270 spermatocytes from each Suffolk (n=170 spermatocytes, 10 spermatocytes from each of 17 rams), Icelandic (n=50 spermatocytes, 10 spermatocytes from each of 5 rams), and Targhee (n=50 spermatocytes, 10 spermatocytes from each of 5 rams) rams. These data for all spermatocytes were then plotted with the SC length on the x-axis and number of MLH1 foci on the y-axis (**Figure 2.3**) to examine the relationship between the length of the SC and the number of CO.

A positive Pearson correlation was observed between the length of the individual SC and the number of MLH1 counts on that SC. A similar correlation was identified for all three breeds. Spermatocytes for rams in the Targhee breed exhibited the strongest correlation ($r^2 = 0.77$), followed by the Icelandic ($r^2 = 0.67$) and then Suffolk ($r^2 = 0.66$). Based on these results, longer SCs are more likely to exhibit a greater number of MLH1 counts, and conversely, shorter SCs are more likely to exhibit fewer MLH1 counts. The

number of MLH1 foci per spermatocyte had no correlation with the total SC length per spermatocyte (average $r^2 = 0.06$; data not shown).

Next, we wanted to determine how many MLH1 events occurred for each SC in the spermatocytes examined. The percent of SCs with 1, 2, 3, and up to the maximum observed of 9 foci was calculated and compared in **Figure 2.4** for each breed. Targhee rams had the greatest percent of SCs with three and greater MLH1 counts (46%) compared to Icelandic (38%) or Suffolk (31%) rams. The greatest number of MLH1 observed on any one individual SC was 9, and occurred in one Suffolk and five Targhee rams. Suffolk rams had the greatest percent of SC's with 1 or 2 MLH1 counts (69%), followed by Icelandic rams (62%), and Targhee rams (54%). Synaptonemal complexes without at least one CO were rarely observed in all rams. In summary, there is a correlation between SC length and number of MLH1 on that SC.

MLH1 Foci Locations on the SC

In addition to measuring the length of each SC, the location of each MLH1 foci was recorded to examine and compare where MLH1 foci occur. Using the same spermatocytes as described above for SC measurements, locations of MLH1 foci were measured (in µm) for each individual SC. Distances between each focus was measured for each SC and cumulative lengths as well as total length were calculated. For each spermatocyte, SC measurements were sorted by length into four categories derived by considering chromosome type and SC lengths: large metacentric (3 distinctly longest SC's), large acrocentric (4 SC's), medium acrocentric (13 SC's), and small acrocentric (6 shortest SC's).

To examine how the locations of MLH1 foci are distributed on the SC, we plotted the MLH1 locations as a percentage of the total length of the SC in **Figure 2.5.** Locations of MLH1 foci exhibited distinct patterns and preferences for 2 and 3 numbers of foci, as displayed in **Figure 2.5**. Synaptonemal complexes with. 1 and 4 foci also displayed location patterns and are included in **Supplemental Material**. There was no significant difference in location preference observed between individuals within a breed or across breeds. In summary, MLH1 foci exhibit distinct relative location preferences that are conserved between Suffolk, Icelandic, and Targhee breeds as well as individual rams.

Discussion

Our understanding of meiotic recombination in sheep is lacking comparatively with humans and mice. Given the economic importance of sheep in the U.S. and abroad, improving our understanding of meiotic recombination in sheep will advance reproduction and genetic prediction in this important species. Characterizing meiotic recombination in sheep will enhance accurate identification of animals with desirable traits resulting in more informative breeding programs. Overall, this knowledge will contribute to increased production and sustainability of the sheep industry.

Characterizing meiotic recombination using a cytogenetic approach offers many advantages. First, recombination can be visualized without parent offspring trios or a large number of offspring. Second, this method is independent of a reference genome. Defects in meiosis and recombination such as lack of crossing over or improper pairing of chromosomes can be observed in pachytene stage primary spermatocytes using this cytogenetic method. Presence of these issues can lead to downstream fertility consequences such as decreased sperm count (Hassold et al., 2007). Of the sheep

spermatocytes included in this dataset, we rarely observed a homologous chromosome pair without a crossover. In addition, defects including asynapsis was almost never observed.

Although differences in total number of crossovers (CO) per spermatocyte have been observed in strains of mice, this has not been thoroughly evaluated in livestock species (Murdoch et al., 2010). We hypothesized that the number of CO per spermatocyte differs between breeds of sheep as well as between individual rams. We sampled close to 3,000 spermatocytes in three breeds: Suffolk, Targhee, and Icelandic. Interestingly, the average number of CO significantly ($P < 0.01$) different in all three breeds. Suffolk rams exhibited the lowest average number of CO, while Targhee rams displayed the highest. Homologous chromosome pairs on average exhibited greater than the obligatory one CO per chromosome arm, with breed CO averages of 2.1, 2.2, and 2.3 CO per chromosome arm in Suffolk, Icelandic, and Targhee rams, respectively. These results are pertinent because they not only raise the evolutionary question of why recombination numbers differ, but they also have the potential to be incorporated into breed specific genetic predictions to aid in selection decisions in the sheep industry.

In addition to variation across breeds, we found significant variation between individual rams within a breed ($P < 0.05$). Most rams exhibited approximately the same average number of crossovers per spermatocyte as breed average, however a few rams had significantly higher or lower averages ($P < 0.05$). Variation between individual rams provides an opportunity for selection within a breed if a desire exists to increase or decrease CO numbers. Greater CO numbers will increase the species' ability to respond to a changing environment as a higher number of novel gene combinations provide a greater chance of adaptation and survival (Korol and Iliadi, 1994; Battagin et al., 2016). A lower

number of CO could lead to greater predictability and uniformity of offspring (Korol and Iliadi, 1994; Battagin et al., 2016). However, recombination has a relatively low heritability of 0.15 as previously reported (Johnston et al., 2016). In addition, a previous study involving modeling recombination in cattle and observing how selection can influence recombination in livestock identified that selection would be more difficult because of low heritability and generation time it would take to achieve results in a reasonable amount of time (Battagin et al., 2016).

Within spermatocytes, the length of the synaptonemal complex (SC) was correlated with the number of MLH1 foci on that SC. This correlation was observed in all three ram breeds. Proportionally, Suffolk rams had the greatest SCs with 1 or 2 CO, and Targhee rams had the fewest. Targhee rams had up to 9 CO on one SC, and a much greater proportion of SCs that had four or greater CO on them when compared to Suffolk and Icelandic rams. Since Targhee rams have the greatest mean number of CO per spermatocyte, this result is not surprising. Icelandic rams had intermediate number of CO per SC to Suffolk and Targhee rams. Interestingly, 70% of SCs in all three breeds exhibited 2 or 3 CO. Because these MLH1 foci were shown to have distinct location patterns, the predictability of CO occurrence in chromosome pairs with 2 or 3 CO has the potential to be high in these three breeds of sheep.

The length of the SC is indicative of the homologous chromosome pair length, and as the homologous chromosome pair length increases, the more crossovers are predicted to be observed on the pair. These results are consistent with a previous study in sheep as well as cattle and goats (Frolich et al., 2015). However, SC length is somewhat indicative of chromatin packaging and these lengths can vary depending on the exact time point of

pachytene stage that spermatocytes are found in. Not all SC lengths, including the total SC length per spermatocyte, were identical within the same animal. When examined, the total length of the SC within each spermatocyte did not correlate with the total number of CO in all three ram breeds. Understanding this correlation between length of homologous chromosome pair and number of CO is beneficial to implement in genetic predictions, as CO number per chromosome predictions could be made based on length.

To investigate location preferences of CO in sheep, the location of CO on similar sizes of SCs were measured. The SCs were grouped to compare according to CO location based on length as well as number of CO on that SC. The three distinctly longest SCs are undoubtedly the three metacentric chromosomes in sheep, and the remaining chromosomes are acrocentric grouped by largest, smallest, and others. Acrocentric chromosomes were placed into three groups by size. Within groups, CO occurred in the same approximate locations and did not vary significantly between individual rams or breeds. This suggests that crossover locations relative to the length of the SC are conserved across animals in this study. Homologous chromosome pairs with 1, 2, and 3 CO had very distinct and visible location patterns. As the number of CO increased to 4 and greater per SC, patterns in locations were still present, however less clear. This preference as well as the spacing of CO is likely due to interfere, in which one CO cannot occur into close of proximity to another. Crossovers were not observed directly next to each other, therefore supporting the hypothesis that interference is acting between these CO and impeding adjacent placement.

This information has immense potential to contribute to increasing the accuracy of genetic predictions. These data support the hypothesis that MLH1 foci differences exist within and between breeds of male sheep. Additionally, MLH1 foci numbers compared to

SC length as well as foci location as a percent of the total SC length suggests that chromosomes have specific CO location preferences. Moving forward, characterizing CO locations on specific chromosomes could contribute directly to improving the accuracy of genetic selection tools by chromosome, especially if these locations are conserved between breeds. This will in turn enhance the accuracy of genetic predictions in sheep.

Conclusion

Meiotic recombination is an important process in mammals that is essential for adequate formation of sperm and contributes to genetic diversity. In sheep, understanding the process of meiotic recombination will aid in uncovering the underlying biological and genetic mechanisms that control how often and where these recombination events occur. This will improve the accuracy of genetic predictions by the opportunity to implement information on how chromosomes recombine and how traits are likely going to be inherited in subsequent generations of lambs.

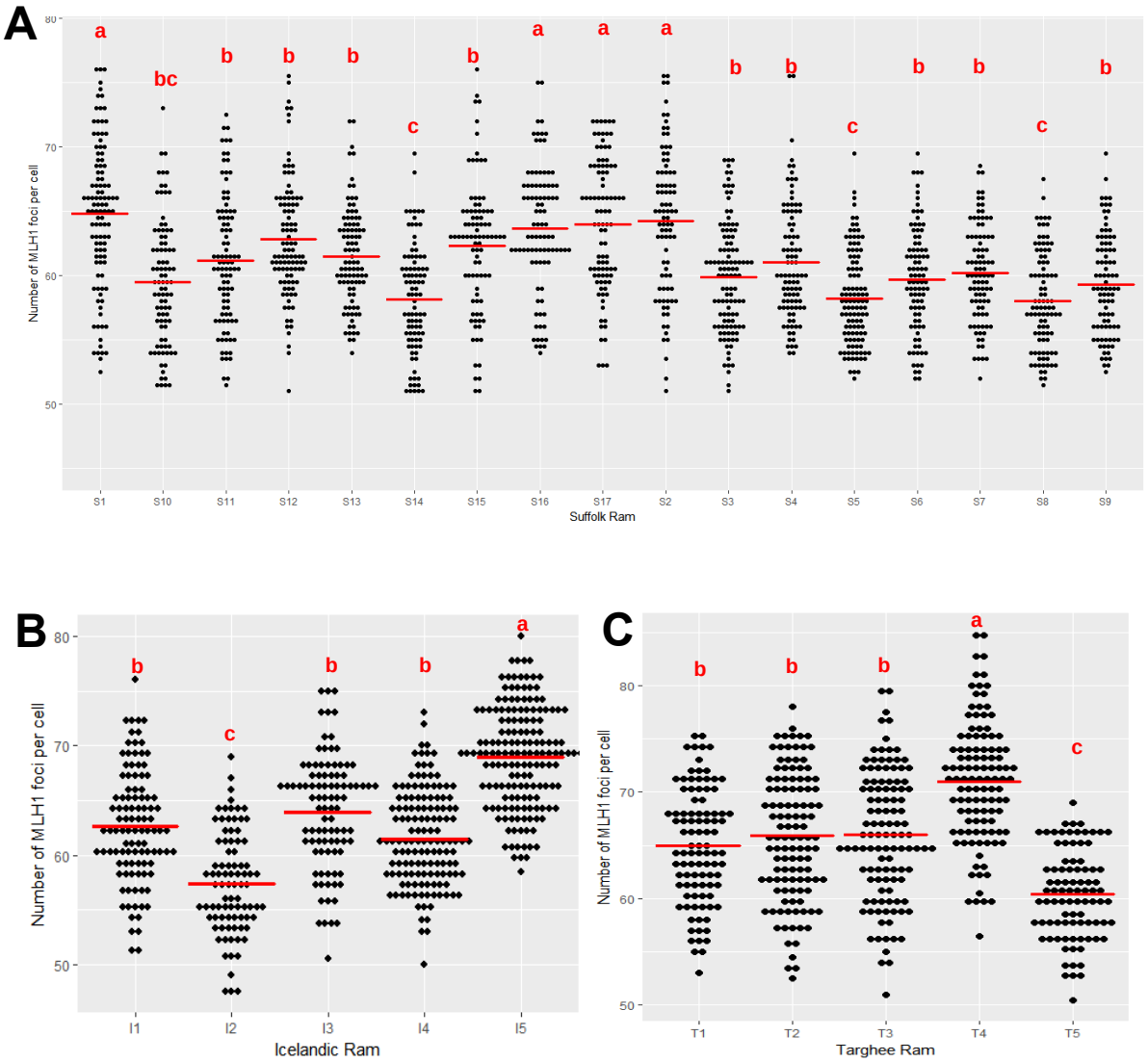


Figure 2.1: Meiotic crossover averages in individual rams. Each dot represents the number of MLH1 from individual pachytene cells per animal in A) Suffolk, B) Icelandic, and C) Targhee rams, red bars denote mean, and superscripts [a, b, c] denote significant differences ($p < 0.01$).

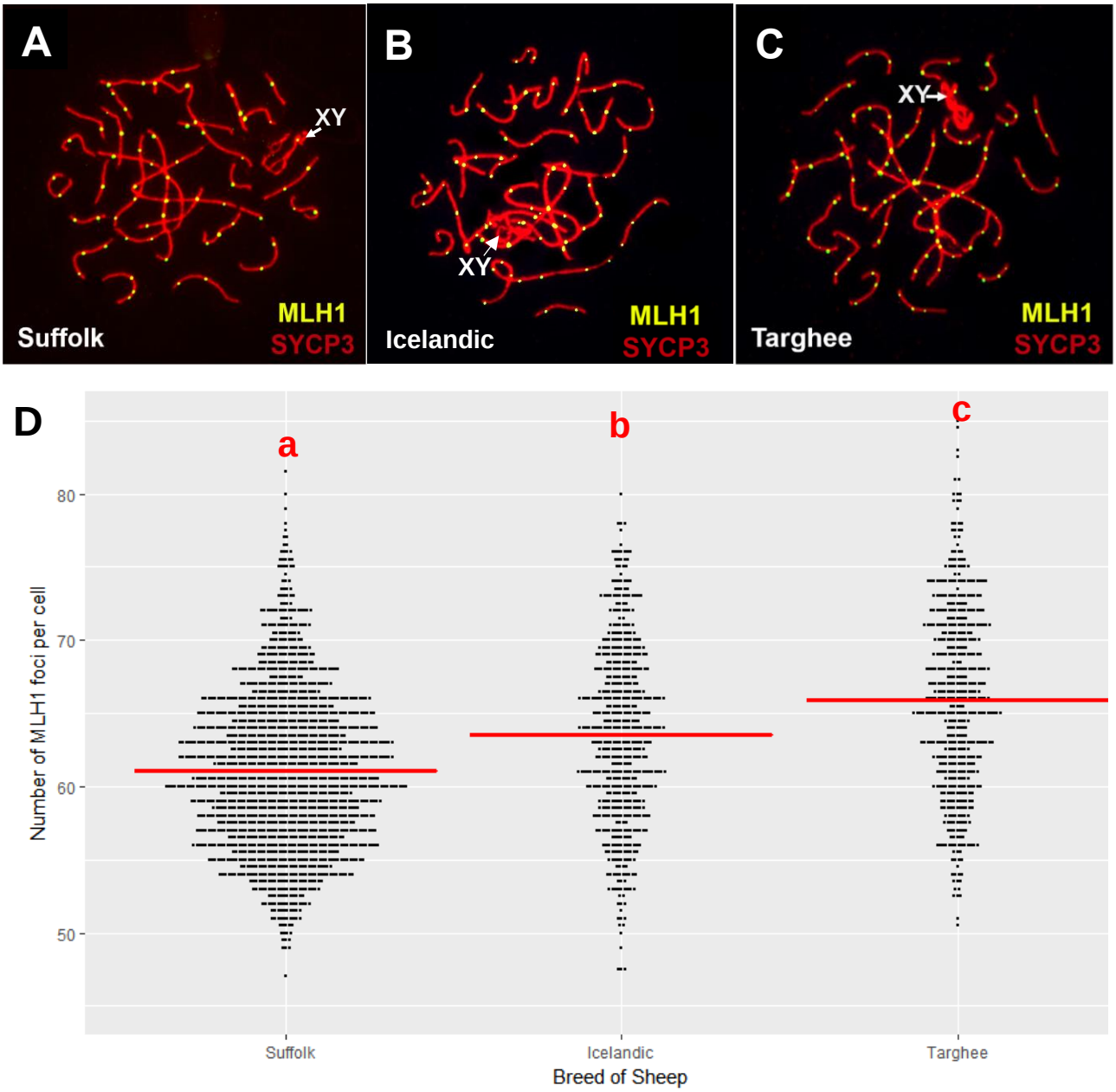


Figure 2.2: MLH1 counts in rams. Representative images of immunofluorescent staining of pachytene stage primary spermatocytes for MLH1 and SYCP3 from A) Suffolk ram (61 MLH1 foci) spermatocyte, B) Icelandic ram (70 MLH1 foci) spermatocyte, and C) Targhee ram (78 MLH1 foci) spermatocyte. D) Graph of number of MLH1 foci in Suffolk, Icelandic, and Targhee rams. Each dot represents the number of crossover events from individual pachytene cells, red bars denote mean, and superscripts $^{a, b, c}$ denote significant differences ($p < 0.01$).

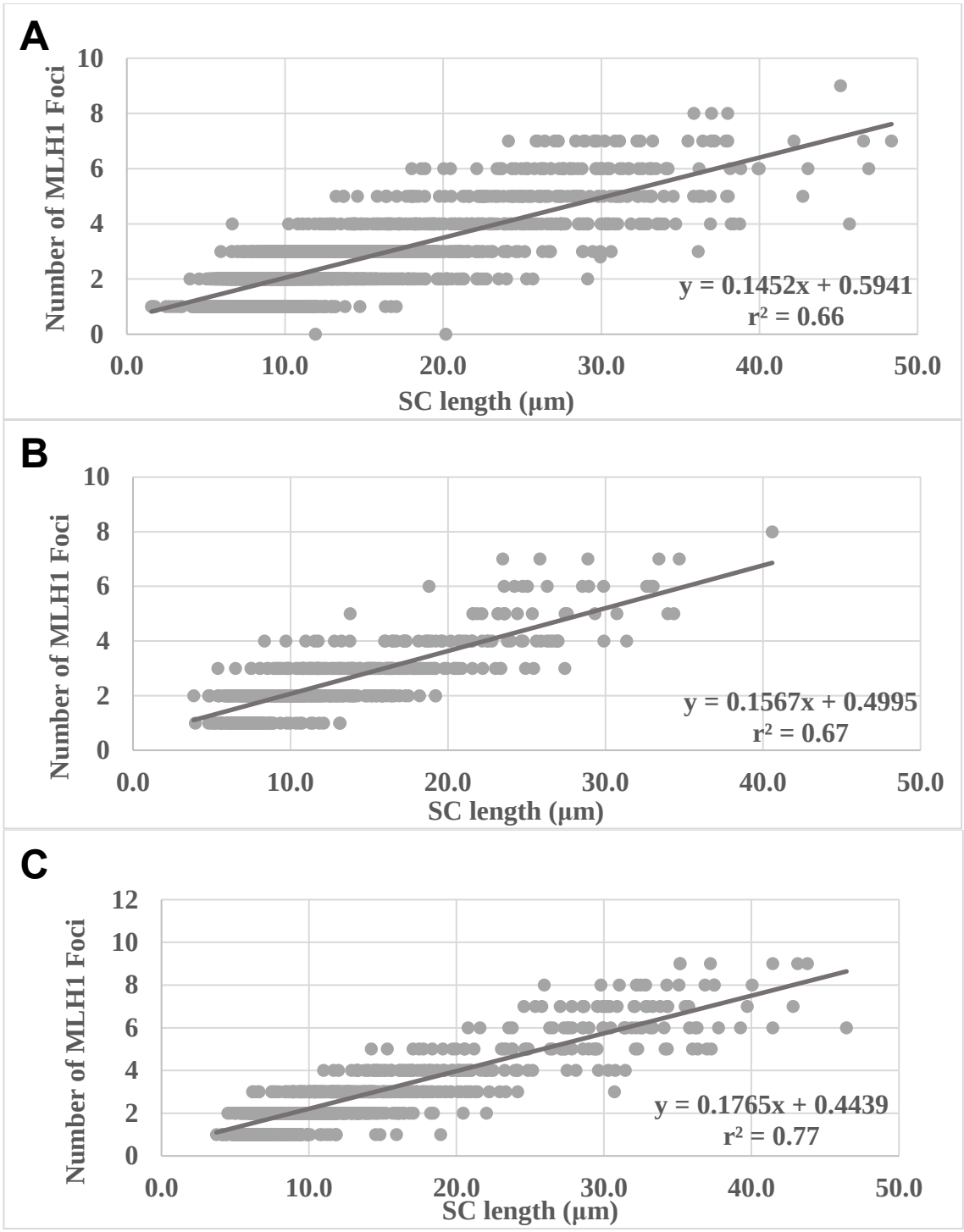


Figure 2.3: MLH1 foci number for each SC in A) Suffolk (170 cells, 4,420 SCs), B) Icelandic (50 cells, 130 SCs), and C) Targhee (50 cells, 130 SCs) rams. Each dot represents an individual SC. As the length of the SC increases, the more MLH1 foci are expected to occur on that SC. Pearson correlations and regression coefficients for each breed are shown.

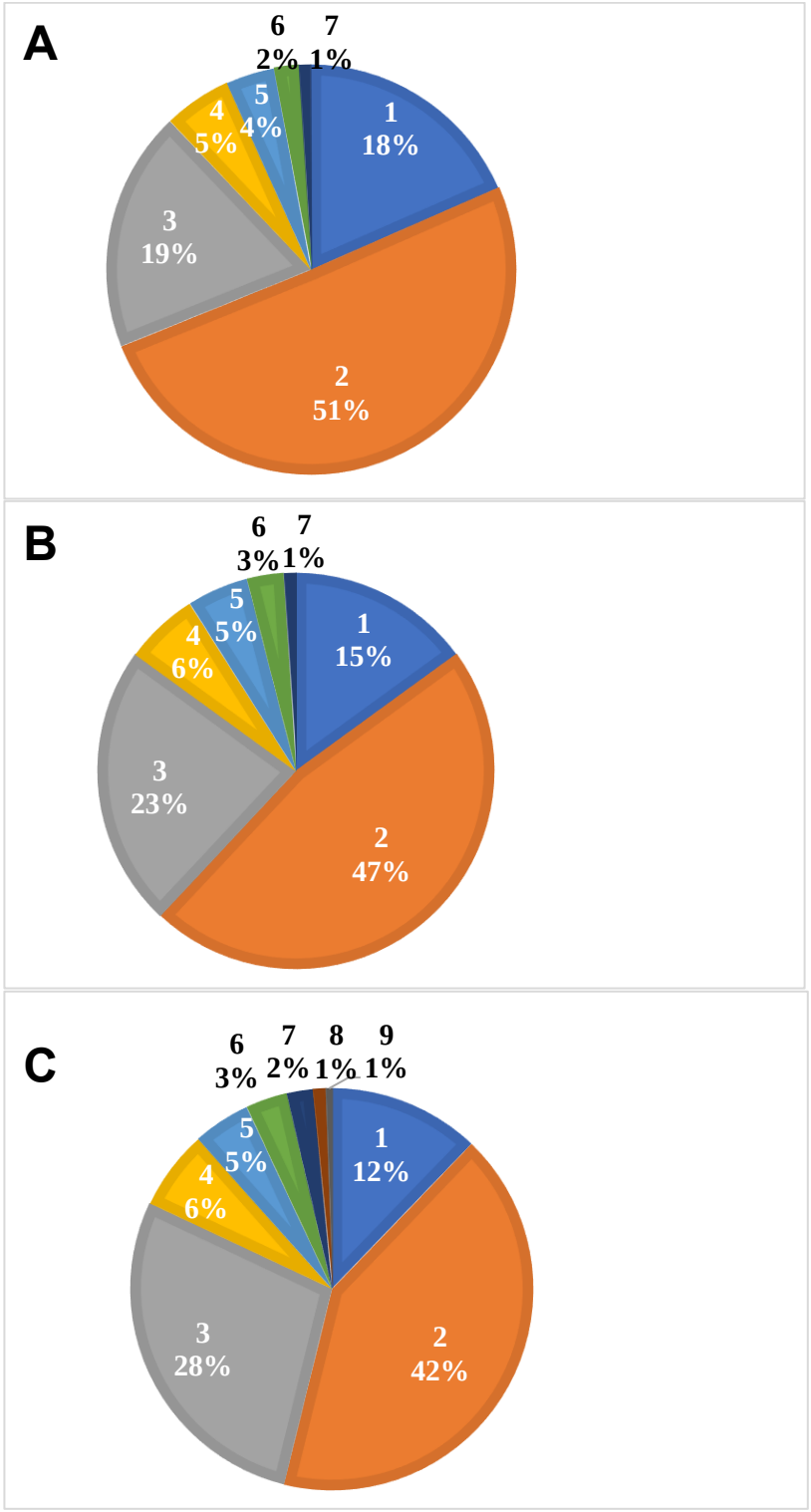


Figure 2.4: Percent of SC's with 1-9 MLH1 foci in A) Suffolk, B) Icelandic, and C) Targhee rams. In all three breeds, 70% of SCs have 2 or 3 MLH1 foci. Targhee rams have the highest percentage of SCs with 7-9 MLH1 foci. Suffolk have the greatest percent of SCs with 1 MLH1 focus.

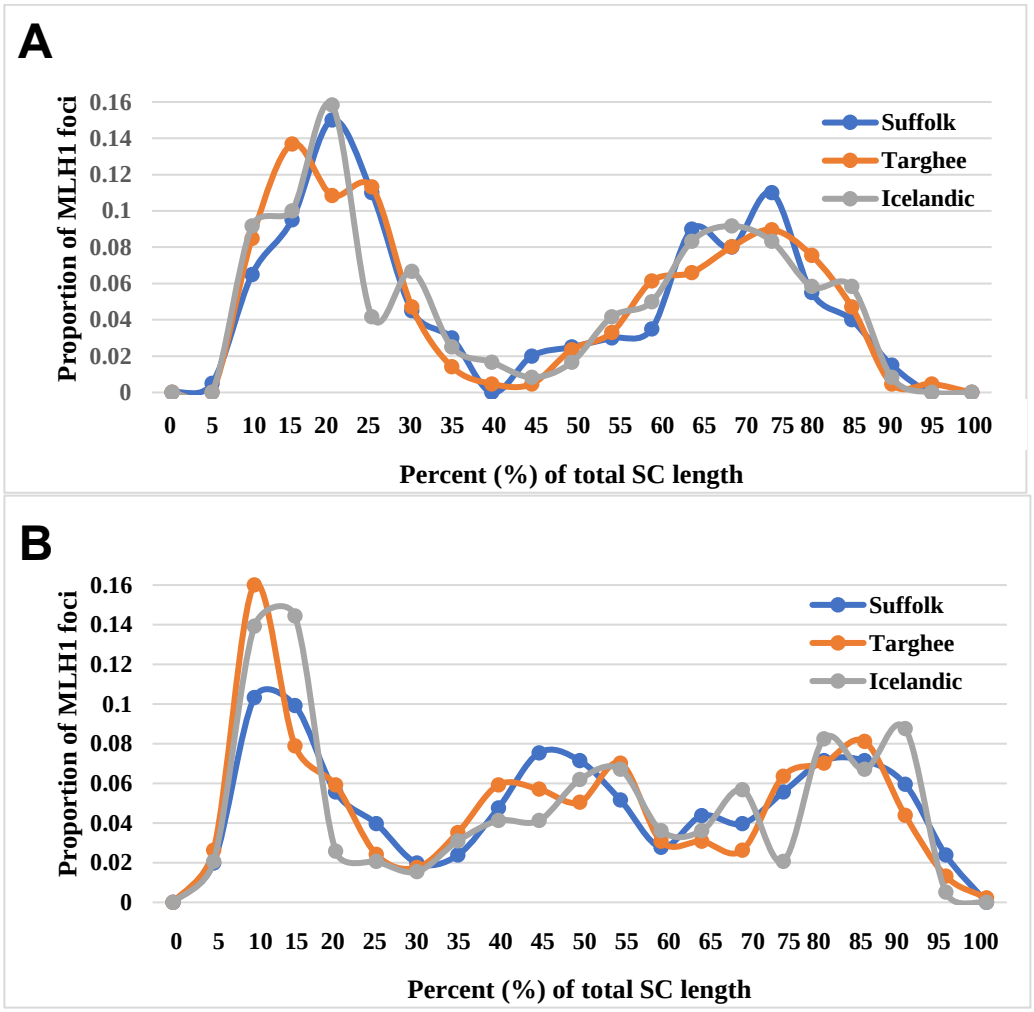


Figure 2.5: Locations of MLH1 foci on SCs in Suffolk, Icelandic, and Targhee rams in SC's with A) 2 MLH1 foci and B) 3 MLH1 foci. Distances are expressed as the percent of the SC length. MLH1 foci exhibit distinct location patterns with 2 and 3 MLH1 foci.

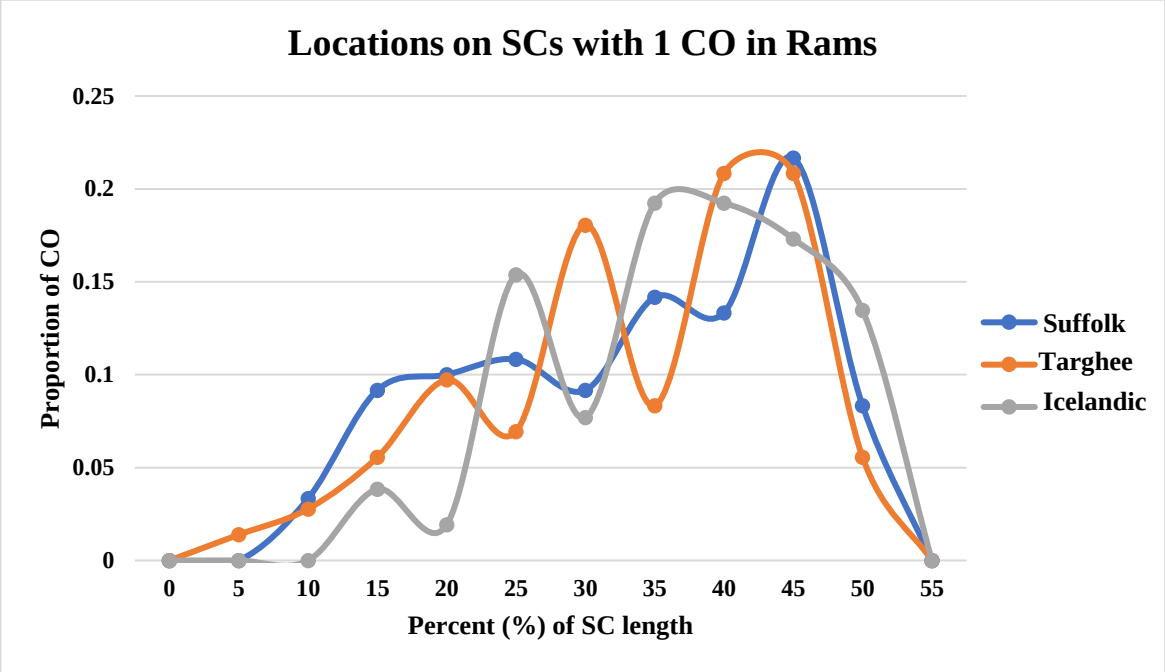
Supplemental Material

Supplemental Table 1: MLH1 Foci in Suffolk Rams

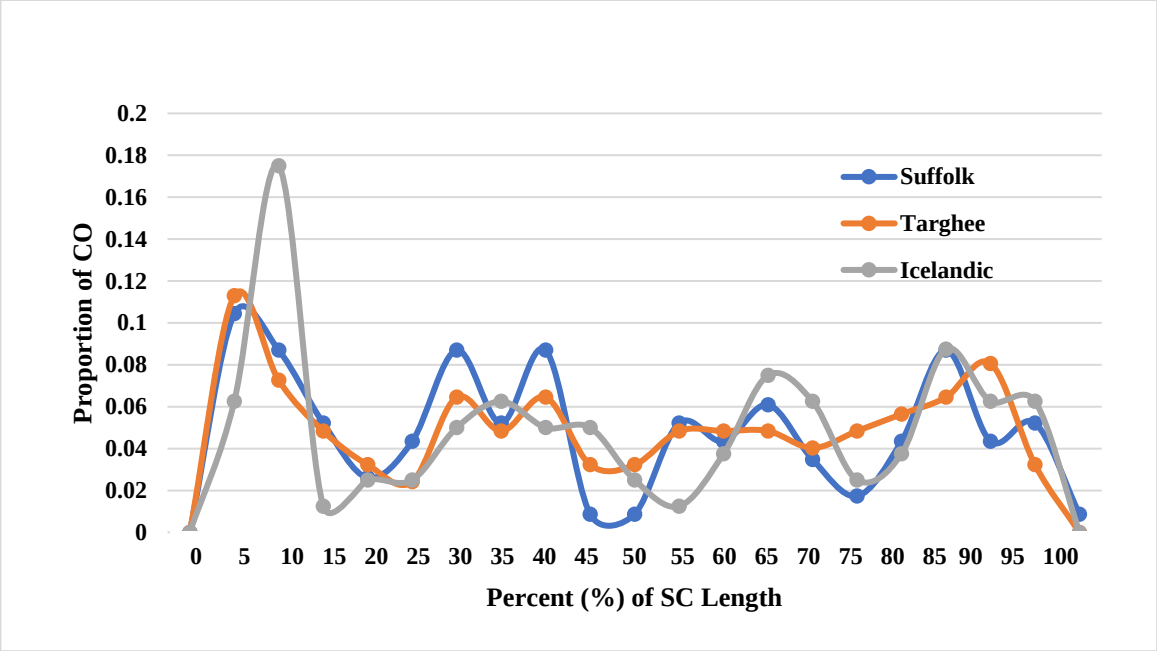
Suffolk Ram	No. Cells	Average MLH1 Foci	Standard Deviation
S1	110	64.9	6
S2	91	64.2	6.4
S3	110	59.9	4.3
S4	97	60.8	5.1
S5	112	58	3.8
S6	92	59.7	4.1
S7	92	59.9	3.8
S8	95	58	3.9
S9	94	60.3	8.7
S10	91	59.4	4.9
S11	95	61.2	5.1
S12	102	62.7	4.9
S13	95	61.4	4
S14	93	58.1	4.2
S15	92	62.8	5.8
S16	92	63.4	5.4
S17	94	64.2	5.3

Icelandic Ram	No. Cells	Average MLH1 Foci	Standard Deviation
I1	104	62.6	5.2
I2	86	57.4	4.7
I3	94	64	5.2
I4	138	61.5	4.3
I5	159	69	4.6

Targhee Ram	No. Cells	Average MLH1 Foci	Standard Deviation
T1	96	65	5.3
T2	114	65.9	6.1
T3	112	66	6
T4	111	71	5.8
T5	97	60.3	4.2



Supplemental Figure 1: MLH1 focus location on SCs with 1 focus. Each set of colored bars is one breed of sheep. MLH1 foci locations are visually shifted between these breeds, however this was not statistically significant.



Supplemental Figure 2: MLH1 foci locations on SCs with 4 foci. MLH1 foci in Suffolk, Targhee, and Icelandic rams exhibit distinct location preferences on SCs with 4 foci.

References

1. Ahlawat, S., P. Sharma, R. Sharma, R. Arora, N.K. Verma, B. Brahma, P. Mishra, and S. De. 2016 (a). Evidence of positive selection and concerted evolution in the rapidly evolving PRDM9 zinc finger domain in goats and sheep. Anim Genet. 47: 740-751.

2. Ahlawat, S., S. De, P. Sharma, R. Sharma, R. Arora, R.S. Kataria, T.K. Datta, and R.K. Singh. 2016 (b). Evolutionary dynamics of meiotic recombination hotspots regulator PRDM9 in bovids. Mol Genet Genomics. 292: 117-131.

3. Anderson, E.L., A.E. Baltus, H.L. Roepers-Gajadien, T. Hassold, D.G. de Rooij, A.M. van Pelt, and D.C. Page. 2008. Stra8 and its inducer, retinoic acid, regulate meiotic initiation in both spermatogenesis and oogenesis in mice. Proc Natl Acad Sci U.S.A. 105: 14976-14980.

4. Anderson, L.K., A. Reeves, L.M. Webb, and T. Ashley. 1999. Distribution of crossing over on mouse synaptonemal complexes using immunofluorescent localization of MLH1 protein. Genetics. 151(4): 1569-1579.

5. Anderson, R., T.K. Copeland, H. Scholer, J. Heasman, and C. Wylie. 2000. The onset of germ cell migration in the mouse embryo. Mech Dev. 91: 61-68.

6. Aponte, P.M., M.P. van Bragt, D.G. de Rooij, and A.M. van Pelt. 2005. Spermatogonial stem cells: characteristics and experimental possibilities. APMIS. 113: 727-742.

7. Ara, T., Y. Nakamura, T. Egawa, T. Sugiyama, K. Abe, T. Kishimoto, Y. Matsui, and T. Nagasawa. 2003. Impaired colonization of the gonads by primordial germ cells in mice lacking a chemokine, stromal cell-derived factor-1 (SDF-1). Proc Natl Acad Sci U.S.A. 100: 5319-5323.

8. Baier, B., P. Hunt, K.W. Broman, and T. Hassold. 2014. Variation in genome-wide levels of meiotic recombination is established at the onset of prophase in mammalian males. PLoS Genet. 10(1).

9. Baker, C.L., S. Kajita, M. Walker, R.L. Saxl, N. Raghupathy, K. Choi, P.M. Petkov, and K. Paigen. 2015. PRDM9 Drives Evolutionary Erosion of Hotspots in Mus musculus through Haplotype-Specific Initiation of Meiotic Recombination. PLoS Genet. 11(1).

10. Baker, S.M., A.W Plug, T.A. Prolla, C.E. Bronner, A.C. Harris, Z. Yao, D.M. Christie, C. Monell, N. Arnheim, A. Bradley, T. Ashley, and R.M. Liskay. 1996. Involvement of mouse Mlh1 in DNA mismatch repair and meiotic crossing over. Nat Genet. 13(3): 336-342.

11. Bannister, L.A., L.G. Reinholdt, R.J. Munroe, and J.C. Schimenti. 2004. Positional cloning and characterization of mouse mei8, a disrupted allele of the meiotic cohesin Rec8. Genesis 40: 184–194.

12. Barchi, M., S. Mahadevaiah, M.D. Giacomo, F. Baudat, D.G. de Rooij, P.S. Burgoyne, M. Jasin, and S. Keeney. Surveillance of different recombination defects in mouse spermatocytes yields distinct responses despite elimination at an identical developmental stage. 2005. Mol Cel Biol. 25(16): 7203-7215.

13. Baudat, F. and B. de Massy. Regulating double-stranded DNA break repair towards crossover or non-crossover during mammalian meiosis. 2007. Chromosome Res. 15: 565-577.

14. Battagin, M., G. Gorjanc, A.M. Faux, S.E. Johnston, and J.M. Hickey. 2016. Effect of manipulating recombination rates on response to selection in livestock breeding programs. Genet Sel Evol. 48: 44.

15. Baudat, F., J. Buard, C. Grey, A. Fledel-Alon, C. Ober, M. Przeworski, G. Coop, and B. de Massy. 2010. PRDM9 is a major determinant of meiotic recombination hotspots in humans and mice. Science. 327: 836–840.

16. Baudat, F., Y. Imai, and B. de Massy. Meiotic recombination in mammals: localization and regulation. 2013. Nat Rev Genet. 14.

17. Baylis, M., and W. Goldmann. 2004. The genetics of scrapie in sheep and goats. Curr Mol Med. 4: 385-396.

18. Bellani, M.A., P.J. Romanienko, D.A. Cairatti, and R.D. Camerino-Otero. SPO11 is required for sex-body formation, and Spo11 heterozygosity rescues the prophase arrest of Atm-/- spermatocytes. 2005. J. Cell Sci. 188: 3233-3245.

19. Bellani, M. A., K. A. Boateng, D. McLeod, and R.D. Camerini-Otero. The expression profile of the major mouse SPO11 isoforms indicates that SPO11β introduces double strand breaks and suggests that SPO11α has an additional role in prophase in both spermatocytes and oocytes. 2010. Mol. Cell. Biol. 30: 4391–4403.

20. Bendel-Stenzel, M.R., M. Gomperts, R. Anderson, J. Heasman, and C. Wylie. 2000. The role of cadherins during primordial germ cell migration and early gonad formation in the mouse. Mech Dev. 91: 143-152.

21. Berg, I.L., R. Neumann, K.W. Lam, S. Sarbajna, L. Odenthal-Hesse, C.A. May, and A.J. Jeffreys. 2010. PRDM9 variation strongly influences recombination hot-spot activity and meiotic instability in humans. Nat Genet. 42(10): 859–863.

22. Bernard P, Maure JF, Partridge JF, Genier S, Javerzat JP, Allshire RC. Requirement of heterochromatin for cohesion at centromeres. Science. 2001;294:2539–2542.

23. Bhattacharyya, T., S. Gregorova, O. Mihola, M. Anger, J. Sebestova, P. Denny, P. Simecek, and J. Forejt. 2013. Mechanistic basis of infertility of mouse intersubspecific hybrids. Proc. Natl Acad. Sci. USA. 110(6): E468–E477.

24. Birtle, Z. and C.P Ponting. 2006. Meisetz and the birth of the KRAB motif. Bioinformatics. 22(23): 2841–2845.

25. Boateng, K.A., M.A. Bellani, I.V. Gregoretti, F. Pratto, and R.D. Camerini-Otero. 2013. Homologous pairing preceding SPO11-mediated double-strand breaks in mice. Dev. Cell. 24: 196-205.

26. Buaas, F.W., A.L. Kirsh, M. Sharma. D.J. McLean, J.L. Morris, M.D. Griswold, D.G. de Rooij, and R.E. Braun. 2004. Plzf is required in adult male germ cells for stem cell self-renewal. Nat Genet. 36: 647-652.

27. Buonomo SB, Clyne RK, Fuchs J, Loidl J, Uhlmann F, Nasmyth K. Disjunction of homologous chromosomes in meiosis I depends on proteolytic cleavage of the meiotic cohesin Rec8 by separin. Cell. 2000;103:387–398.

28. Butlin, R.K. 2005. Recombination and speciation. Mol Ecol. 14(9): 2621-2635.

29. Cardoso, F.M. and G.F. Queiroz. 1988. Duration of the cycle of the seminiferous epithelium and daily sperm production of Brazilian hairy rams. Anim Reprod Sci. 17: 77-84.

30. Cheng, C.H., Y.H. Lo, S.S. Liang, S.C. Ti, F.M. Lin, C.H Yeh, H.Y. Huang, and T.F. Wang. 2006. SUMO modifications control assembly of synaptonemal complex and polycomplex in meiosis of Saccharomyces cerevisiae. 20(15): 2067-2081.

31. Chi, P., J. San Filippo, M.G. Sehorn, G.V. Petukhova, and P. Sung. 2007. Bipartite stimulatory action of the Hop1-Mnd1 complex on the Rad51 recombinase. Genes Dev. 21: 1747-1757.

32. Chowdhury, R., P.R. Bois, E. Feingold, S.L. Sherman, and V.G. Cheung. 2009. Genetic analysis of variation in human meiotic recombination. PLoS Genet. 5.

33. Cleveland DW, Mao Y, Sullivan KF. Centromeres and kinetochores: from epigenetics to mitotic checkpoint signaling. Cell. 2003;112:407–421.

34. Clutton-Brock, T., J. Pemberton, T.N. Coulson, I.R. Stevenson, and A.D.C. MacColl. 2004. Soay sheep: dynamics and selection in an island population. Cambridge University Press. 2: 17-51.

35. Cockett, N.E., T.L. Shay, J.E. Beever, D. Nielsen, J. Albresten, M. Georges, K. Peterson, A. Stephens, W. Vernon, O. Timofeevskaia, S. South, J. Mork, A. Maciulis,

and T.D. Bunch. 1999. Localization of the locus causing spider lamb syndrome to the distal end of ovine chromosome 6. Mamm Genome. 10: 35-38.

36. Cockett, N. and J. Beever. 2001. Screening for the molecular defect causing spider lamb syndrome in sheep. US Patent No. 6,306,591 Bl.

37. Coop G. and M. Przeworski. 2007. An evolutionary view of human recombination. Nat Rev Genet. 8: 23-34.

38. Costa, Y. and H.J. Cooke. 2007. Dissecting the mammalian synaptonemal complex using targeted mutations. Chromosome Res. 15: 579-589.

39. Davies, B., E. Hatton, N. Altemose, J.G. Hussin, F. Pratto, G. Zhang, A.G. Hinch, D. Moralli, D. Biggs, R. Diaz, C. Preee, R. Li, E. Bitoun, K. Brick, C.M. Green, R.D. Camerini-Otero, S.R. Myers, and P. Donnelly. 2016. Re-engineering the zinc fingers of PRDM9 reverses hybrid sterility in mice. Nature. 530(7589): 171-176.

40. de Boer, E. and C. Heyting. 2006. The diverse roles of transverse filaments of synaptonemal complexes in meiosis. Chromosoma. 115: 220-234.

41. de Massy, B. Initiation of meiotic recombination: how and where? 2013. Annu Rev Genet.

42. de Rooij, D.G. 1998. Stem cells in the testis. Int J Exp Pathol. 79: 67-80.

43. de Rooij, D.G. 2009. The spermatogonial stem cell niche. Microsc Res Tech. 72: 580-585.

44. de Sousa Lopes, S.M., B.A. Roelen, R.M. Monteiro, R. Emmens, H.Y. Lin, E. Li, K.A. Lawson, and C.L. Mummery. 2004. BMP signaling mediated by ALK2 in the visceral endoderm is necessary for the generation of primordial germ cells in the mouse embryo. Genes Dev. 18: 1838-1849.

45. Dendouga N., H. Gao, D. Moechars, M. Janicot, J. Vialard, and C.H. McGowan. 2005. Disruption of murine Mus81 increases genomic instability and DNA damage sensitivity but does not promote tumorigenesis. Mol Cell Biol. 25: 7569–7579.

46. Di Carlo, A., and M. De Felici. 2000. A role for E-cadherin in mouse primordial germ cell development. Dev Biol. 226: 209-219.

47. Dumont B.L., and B.A. Payseur. 2011. Genetic analysis of genome-scale recombination rate evolution in house mice. PLoS Genet. 7(6).

48. Durcova-Hills, G. and B. Capel. 2008. Development of germ cells in the mouse. Curr Top Dev Biol. 83: 185-212.

49. Edelmann, W., P.E. Cohen, M. Kane, K. Lau, B. Morrow, S. Bennett, A. Umar, T. Kunkel, G. Cattoretti, R. Chaganti, J.W. Pollard, R.D. Kolodner, and R. Kucherlapati. 1996. Cell. 85(7): 1125-1134.

50. Fawcett, D.W. 1975. The mammalian spermatozoon. Dev Biol. 44: 394-436.

51. Fledel-Alon, A., E.M. Leffler, Y. Guan, M. Stephens, G. Coop, and M. Przeworski. 2011. Variation in human recombination rates and its genetic determinants. PloS One. 6.

52. Franca, L.R., G.F. Avelar, and F.F.L. Almeida. 2005. Spermatogenesis and sperm transit through the epididymis in mammals with emphasis on pigs. Theriogenology. 63(2): 300-318.

53. Freking, B.A., J.W. Keele, C.W. Beattie, S.M. Kappes, T.P.L. Smith, T.S. Sonstegard, M.K. Nielsen, and K.A. Leymaster. 1998. Evaluation of the ovine callipyge locus: I. Relative chromosomal position and gene action. J Anim Sci. 76: 2062-2071.

54. Frolich, J., M. Vozdova, S. Kubickova, H. Cernohorska, H. Sebestova, and J. Rubes. 2015. Variation of meiotic recombination rates and MLH1 foci distribution in spermatocytes of cattle, sheep, and goats. Cytogenet Genome Res. 146(3): 211-221.

55. Fullerton S., A. Bernardo Carvalho, and A.G. Clark. 2001. Local rates of recombination are positively correlated with GC content in the human genome. Mol Biol Evol. 18(6): 1139-1142.

56. Gaemers, I.C., E. Sonneveld, A.M. van Pelt, B.H. Schrans, A.P. Themmen, P.T. van der Saag, and D.G. de Rooij. 1998. The effect of 9-cis-retinoic acid on proliferation and differentiation of a spermatogonia and retinoid receptor gene expression in the vitamin A-deficient mouse testis. Endocrinology. 139: 4269-4276.

57. Gill, M.E., Y.C. Hu, Y. Lin, and D.C. Page. 2011. Licensing of gametogenesis, dependent on RNA binding protein DAZL, as a gateway to sexual differentiation of fetal germ cells. Proc Natl Acad Sci U.S.A. 108: 7443-7448.

58. Grinthal A, Adamovic I, Weiner B, Karplus M, Kleckner N. PR65, the HEAT-repeat scaffold of phosphatase PP2A, is an elastic connector that links force and catalysis. Proc Natl Acad Sci U S A. 2010;107:2467–2472.

59. Griswold, M.D. 2016. Spermatogenesis: the commitment to meiosis. Physiol Rev. 96(1):1-17.

60. Guillon H., F. Baudat, C. Grey, R.M. Liskay, and B. de Massy. 2005. Crossover and noncrossover pathways in mouse meiosis. Mol Cell. 20: 563–573.

61. Handel, M.A. and J.C. Schimenti. 2010. Genetics of mammalian meiosis: regulation, dynamics and impact on fertility. Nat Rev Genet. 11(2):124-136.

62. Hassold, T. and P. Hunt. 2001. To err (meiotically) is human: the genesis of human aneuploidy. Nat Rev Genet. 2: 280-291.

63. Hassold, T., H. Hall, and P. Hunt. 2007. The origin of human aneuploidy: where we have been, where we are going. Hum Mol Genet. 16:2 03-208.

64. Hayashi, K., K. Yoshida, and Y. Matsui. 2005. A histone H3 methyltransferase controls epigenetic events required for meiotic prophase. Nature. 438: 374–378

65. Hinch, A.G., N. Altermose, N. Noor, P. Donnelly, and S.R. Meyers. 2014. Recombination in the human pseudoautosomal region PAR1. PLoS Genet. 10(7): e10045033.

66. Holloway, J.K., J. Booth, W. Edelmann, C.H. McGowan, and P.E. Cohen. 2008. MUS81 generates a subset of MLH1-MLH3-independent crossovers in mammalian meiosis. PLoS Genet. 4(9).

67. Holt, J.E. and K.T. Jones. 2009. Control of homologous chromosome division in the mammalian oocyte. Mol Hum Reprod. 15: 139-147.

68. Hunter, N. Synaptonemal complexities and commonalities. 2003. Mol Cell. 12: 533-535.

69. Jan, S.Z., G. Hamer, S. Repping, D.G. de Rooij, A.M.M. van Pelt, and T.L. Vomer. 2012. Molecular control of rodent spermatogenesis. Biochim Biophys Acta. 1822: 1838-1850.

70. Johnston, S.E., C. Berenos, J. Slate, and J.M. Pemberton. 2016. Conserved genetic architecture underlying individual recombination rate variation in a wild population of Soay sheep. Genet. 203: 583-598.

71. Katis VL, Galova M, Rabitsch KP, Gregan J, Nasmyth K. Maintenance of cohesin at centromeres after meiosis I in budding yeast requires a kinetochore-associated protein related to MEI-S332. Curr Biol. 2004;14:560–572.

72. Kauppi, L., M. Jasin, and S. Keeney. 2012. The tricky path to recombining X and Y chromosomes in meiosis. Ann NY Acad Sci. 1267: 18-23.

73. Kauppi, L., M. Barchi, F. Baudat, P.J. Romanienko, S. Keeney, M. Jasin. Distinct properties of the XY pseudoautosomal region crucial for male meiosis. 2011. Science. 331: 916–920.

74. Keeney, S., C.N. Giroux, and N. Kleckner. 1997. Meiosis-specific DNA double-strand breaks are catalyzed by Spo11, a member of a widely conserved protein family. Cell. 88(3): 375-384.

75. Kierszenbaum, A.L. and L.L. Tres. 2004. The acrosome-acroplaxome-manchette complex and the shaping of the spermatid head. Arch Histol Cytol. 67: 271-284.

76. Kleckner, N., A. Storlazzi, and D. Zickler. 2003. Coordinate variation in meiotic pachytene SC length and total crossover/chiasma frequency under conditions of constant DNA length. Trends Genet. 19: 623-628.

77. Kluin, P.M. and D.G. de Rooij. 1981. A comparison between the morphology and cell kinetics of gonocytes and adult type undifferentiated spermatogonia in the mouse. Int J Androl. 4: 475-493.

78. Koehler, K.E., R.S. Hawley, S. Sherman, and T. Hassold, 1996. Recombination and nondisjunction in humans and flies. Hum Mol Genet. 5: 1495-1504.

79. Korol, A.B and K.G. Iliad. 1994. Increased recombination frequencies resulting from directional selection for geotaxis in Drosophila. Heredity. 72: 64-68.

80. Kumar, R. and B. de Massy. 2010. Initiation of Meiotic Recombination in Mammals. Genes. 1(3): 521-549.

81. Kumar, R., H.M. Bourbon, and B. de Massy. Functional conservation of Mei4 for meiotic DNA double-strand break formation from yeasts to mice. 2010. Genes Dev. 24: 1266-1280.

82. Lawson, K.A., N.R. Dunn, B.A. Roelen, L.M. Zeinstra, A.M. Davis, C.V. Wright, J.P. Korving, and B.L. Hogan. 1999. Bmp4 is required for the generation of primordial germ cells in the mouse embryo. Genes Dev. 13: 424-436.

83. Leblond, C.P. and Y. Clermont. 1952. Definition of the stages of the cycle of the seminiferous epithelium in the rat. Ann N.Y. Acad. Sci. 55: 548-573.

84. Lenormand, T., and J. Dutheil. 2005. Recombination difference between sexes: a role for haploid selection. PLoS Biol. 3: e63.

85. Lin, Y., M.E. Gill, J. Koubova, and D.C. Page. 2008. Germ cell-intrinsic and -extrinsic factors govern meiotic initiation in mouse embryos. Science. 322: 1685-1687.

86. Lipkin, S.M., P.B. Moens, V. Wang, M. Lenzi, D. Shanmugarajah, A. Gilgeous, J. Thomas, J. Cheng, J.W. Touchman, E.D. Green, P. Schwartzberg, F.S. Collins, and P.E. Cohen. 2002. Meiotic arrest and aneuploidy in MLH3-deficient mice. Nat Genet. 31(4): 385-390.

87. Lupton, C.J. 2008. Impacts of animal science research on United States sheep production and predictions for the future. J Anim Sci. 86(11): 3252-3274.

88. Ma, L., J.R. O'Connell, P.M. VanRaden, B. Shen, A. Padhi, C. Sun, D.M. Bickhart, J.B. Cole, D.J. Null, G.E. Liu, Y. Da, and G.R. Wiggans. 2015. Cattle sex-specific recombination and genetic control from a large pedigree analysis. PLoS Genet. 11(11).

89. Malik, S. B., M.A. Ramesh, A.M Hulstrand, and J.M. Logsdon, Jr. Protist homologs of the meiotic Spo11 gene and topoisomerase VI reveal an evolutionary history of gene duplication and lineage-specific loss. 2007. Mol. Biol. Evol. 24: 2827–2841.

90. Matise, T.C., F. Chen, W. Chen, F.M. De La Vega, M. Hansen, C. He, F.C. Hyland, G.C. Kennedy, X. Kong, S.S. Murray, J.S. Ziegle, W.C. Stewart, and S. Buyske. 2007. A second-generation combined linkage physical map of the human genome. Genome Res. 17(12): 1783-1786.

91. McNatty, K.P., N.L. Hudson, L. Whiting, K.L. Reader, S. Lun, A. Western, D.A. Heath, P. Smith, L.G. Moore, and J.L. Juengel. 2007. The effects of immunizing sheep with different BMP15 or GDF9 peptide sequences on ovarian follicular activity and ovulation rate. Biol Reprod. 76: 552-560.

92. McPherson J.P., B. Lemmers, R. Chahwan, A. Pamidi, E. Migon, E. Matysiak-Zablocki, M.E. Moynahan, J. Essers, K. Hanada, A. Poonepalli, O. Sanchez-Sweatman, R. Khokha, R. Kanaar, M. Jasin, M.P. Hande, and R. Hakem. 2004. Involvement of mammalian Mus81 in genome integrity and tumor suppression. Science. 304: 1822–1826.

93. Meistrich, M.L., B. Mohapatra, C.R. Shirley, and M. Zhao. 2003. Roles of transition nuclear proteins in spermiogenesis. Chromosoma. 111: 483-488.

94. Mihola, O., Z. Trachtulec, C. Vlcek, J.C. Schimenti, and J.A. Forejt. 2009. A mouse speciation gene encodes a meiotic histone H3 methyltransferase. Science. 323: 373–375.

95. Miller, M.P, A. Amon, and E. Unal. 2013. Meiosis I: when chromosomes undergo extreme makeover. Curr Opin Cell Biol. 25(6).

96. Molyneaux, K.A., H. Zinszner, P.S. Kunwar, K. Schaible, J. Stebler, M.J. Sunshine, W. O'Brien, E. Raz, D. Littman, C. Wylie, and R. Lehmann. 2003. The chemokine SDF1/CXCL12 and its receptor CXCR4 regulate mouse germ cell migration and survival. Development. 130: 4279-4286.

97. Molyneaux, K.A., J. Stallock, K. Schaible, and C. Wylie. 2001. Time-lapse analysis of living mouse germ cell migration. Dev Biol. 240: 488-498.

98. Morelli, M.A., and P.E. Cohen. 2005. Not all germ cells are created equal: aspects of sexual dimorphism in mammalian meiosis. Reproduction. 130: 761-781.

99. Muñoz-Fuentes, V., A. Di Rienzo, and C. Vilá. 2011. Prdm9, a major determinant of meiotic recombination hotspots, is not functional in dogs and their wild relatives, wolves and coyotes. PLoS One. 6(11).

100. Murdoch, B., N. Owen, M. Stevense, H. Smith, S. Nagaoka, T. Hassold, M. McKay, H. Xu, J. Fu, E. Revenkova, R. Jessberger, and P. Hunt. 2013. Altered cohesin gene dosage affects mammalian meiotic chromosome structure and behavior. PLoS Genet. 9(2).

101. Murdoch, B., N. Owen, S. Shirley, S. Crumb, K.W. Broman, and T. Hassold. 2010. Multiple loci contribute to genome-wide recombination levels in male mice. Mamm Genome. 21(11-12): 550-555.

102. Muyt, A.D., D. Vezon, G. Gendrot, J.L. Gallois, R. Stevens, and M. Grelon. AtPRD1 is required for meiotic double strand break formation in Arabidopsis thaliana. 2007. EMBO J. 26(18): 4126-4137.

103. Nagano R., S. Tabata, Y. Nakanishi, S. Ohsako, M. Kurohmaru, and Y. Hayashi. 2000. Reproliferation and relocation of mouse male germ cells (gonocytes) during prespermatogenesis. Anat Rec. 258: 210-220.

104. Nagaoka, S.I., T.J. Hassold, and P.A. Hunt. 2012. Human aneuploidy: mechanisms and new insights into an age-old problem. Nat Rev Genet. 13: 493-504.

105. O'Donnell, L., P.K. Nicholls, M.K. O'Bryan, R.I. McLachlan, and P.G. Stanton. 2011. Spermiation: the process of sperm release. Spermatogenesis. 1: 14-35.

106. Oakberg, E.F. Duration of spermatogenesis in the mouse and timing of stages of the cycle of the seminiferous epithelium. 1956. Am J Anat. 99: 507-516.

107. Oatley, M.J., A.V. Kaucher, K.E. Racicot, and J.M. Oatley. 2011. Inhibitor of DNA binding 4 is expressed selectively by single spermatogonia in the male germline and regulates the self-renewal of spermatogonial stem cells in mice. Biol Reprod. 85: 347-356.

108. Ohinata, Y., B. Payer, D. O'Carroll, K. Ancelin, Y. Ono, M. Sano, S.C. Barton, T. Obukhanych, M. Nussenzweig, A. Tarakhovsky, M. Saitou, and M.A. Surani. 2005. Blimp1 is a critical determinant of the germ cell lineage in mice. Nature. 436: 207-213.

109. Oliver, P.L., L. Goodstadt, J.J. Bayes, Z. Birtle, K.C. Roach, N. Phadnis, S.A. Beatson, G. Lunter, H.S. Malik, and C.P. Ponting. 2009. Accelerated evolution of the Prdm9 speciation gene across diverse metazoan taxa. PLoS Genet. 5(12).

110. Ortavant, R. 1956. Action of duration of light on spermatogenic processes in rams. C R Seances Soc Biol Fil. 150: 471-474.

111. Parvanov, E.D., P.M. Petkov, and K. Paigen. 2010. Prdm9 controls activation of mammalian recombination hotspots. Science. 327: 835.

112. Petukhova, G.V., R.J. Pezza, F. Vanevski, M. Ploquin, J.Y. Masson, and R.D. Camerini-Otero. 2005. The Hop2 and Mnd1 proteins act in concert with Rad51 and Dmc1 in meiotic recombination. Nat Struct Mol Biol. 12(5): 449-453.

113. Revenkova, E., M. Eijpe, C. Heyting, C.A. Hodges, P.A. Hunt, B. Liebe, H. Scherthan, and R. Jessberger. 2004. Cohesin SMC1 beta is required for meiotic chromosome dynamics, sister chromatid cohesion and DNA recombination. Nat Cell Biol. 6: 555–562.

114. Reynolds, A., Q. Huanyu, Y. Yang, J.K. Chen, N. Jackson, K. Biswas, J.K. Jolloway, F. Baudat, B. de Massy, J. Wang, C. Hoog, P.E. Cohen, N. Hunter. 2013. RNF212 is a dosage-sensitive regulator of crossing-over during mammalian meiosis. Nat Genet. 45(3): 269-278.

115. Richardson, B.E. and R. Lehmann. 2010. Mechanisms guiding primordial germ cell migration: strategies from different organisms. Nat Rev Mol Cell Biol. 11: 37-49.

116. Runyan, C., K. Schaible, K. Molyneaux, Z. Wang, L. Levin, and C. Wylie. 2006. Steel factor controls midline cell death of primordial germ cells and is essential for their normal proliferation and migration. Development. 133: 4861-4869.

117. Russell, E.R., A.P.S. Hikim, and E.D. Clegg. 1990. Histological and histopathological evaluation of the testis. Cache River Press.

118. Sada, A., A. Suzuki, H. Suzuki, and Y. Saga. 2009. The RNA-binding protein NANOS2 is required to maintain murine spermatogonial stem cells. Science. 325: 1394-1398.

119. Saitou, M. 2009. Specification of the germ cell lineage in mice. Front Biosci. 14: 1068-1087.

120. Sandor, C., L. Wanbo, W. Coppieters, T. Druet, C. Charlier, and M. Georges. 2012. Genetic variants in REC8, RNF212, and PRDM9 influence male recombination in cattle. PLoS Genet. 8(7).

121. Sapsford, C.S. 1962. Changes in the cells of the sex cords and seminiferous tubules during the development of the testis of the rat and mouse. Aust J Zool. 10: 178-192.

122. Sharan, S.K., A. Pyle, V. Coppola, J. Babus, S. Swaminathan, J. Benedict, D. Swing, B.K. Martin, L. Tessarollo, J.P. Evans, J.A. Flaws, and M.A. Handel. 2004. BRCA2

deficiency in mice leads to meiotic impairment and infertility. Development. 13(1): 131-142.

123. Smagulova, F., I.V. Gregoretti, K. Brick, P. Khil, R.D. Camerini-Otero, and G.V. Petukhova. 2011. Genome-wide analysis reveals novel molecular features of mouse recombination hotspots. Nature. 472: 375-378.

124. Sun, X. and W. Yang. 2010. Mitochondria: transportation, distribution and function during spermiogenesis. Adv Biosci Biotechnol. 1: 97-109.

125. Svetlanov A., F. Baudat, P.E. Cohen, and B. de Massy. 2008. Distinct functions of MLH3 at recombination hot spots in the mouse. Genetics. 178: 1937–1945.

126. Tam., P.P., and M.H. Snow. 1981. Proliferation and migration of primordial germ cells during compensatory growth in mouse embryos. J Embryol Exp Morphol. 64: 133-147.

127. United States Department of Agriculture (USDA) National Agricultural Statistics Service (NASS) 2012 Census of Agriculture, Final Data Release, May 2, 2014.

128. United States Department of Agriculture (USDA) National Agricultural Statistics Service (NASS) Agricultural Statistics Board, Sheep and Goats release, January 29, 2016.

129. Van Pelt, A.M. and D.G. de Rooij. 1990. Synchronization of the seminiferous epithelium after vitamin A replacement in vitamin A-deficient mice. Biol Reprod. 43: 363-367.

130. Vergouwen, R.P., S.G. Jacobs, R. Huiskamp, J.A. Davids, and D.G. de Rooij. 1991. Proliferative activity of gonocytes, Sertoli cells and interstitial cells during testicular development in mice. J Reprod Fertil. 93: 233-243.

131. Vogt, E., M. Kirsch-Volders, J. Parry, and U. Eichenlaub-Ritter. 2008. Spindle formation, chromosome segregation and the spindle checkpoint in mammalian oocytes and susceptibility to meiotic error. Mutat Res. 651: 14-29.

132. Wei, K., A.B. Clark, E. Wong, M.F. Kane, D.J. Mazur, T. Parris, N.K. Kolas, R. Russell, H. Hou Jr., B. Kneitz, G. Yang, T.A. Kunkel, R.D. Kolodner, P.E. Cohen, and W. Edelmann. 2003. Inactivation of Exonuclease 1 in mice results in DNA mismatch repair defects, increased cancer susceptibility, and male and female sterility. Genes Dev. 17(5): 603-614.

133. Westermann S, Drubin DG, Barnes G. 2007. Structures and functions of yeast kinetochore complexes. Annu Rev Biochem. 76:563–591.

134. Wilson, D.E. and D.G. Morrical. 1991. The National Sheep Improvement Program: a review. J Anim Sci. 69: 3872-3881.

135. Wilson, T., X.Y. Wu, J.L. Juengel, I.K. Ross, J.M. Lumsden, E.A. Lord, K.G. Dodds, G.A. Walling, J.C. McEwan, A.R. O'Connell, K.P. McNatty, and G.W. Montgomery. 2001. Highly prolific Booroola sheep have a mutation in the intracellular kinase domain of bone morphogenic protein 1B receptor (ALK-6) that is expressed in both oocytes and granulosa cells. Biol Reprod. 64: 1225-1235.

136. Winckler, W., S.R. Myers, D.J. Richter, R.C. Onofrio, G.J. McDonald, R.E. Bontrop, G.A. McVean, S.B Gabriel, D. Reich, P. Donnelly, and D. Altschuler. 2005. Comparison of fine-scale recombination rates in humans and chimpanzees. Science. 308(5718): 107-111.

137. Winey M, Mamay CL, O'Toole ET, Mastronarde DN, Giddings TH, Jr, McDonald KL, McIntosh JR. Three-dimensional ultrastructural analysis of the Saccharomyces cerevisiae mitotic spindle. J Cell Biol. 1995;129:1601–1615.

138. Wu, H., N. Mathioudakis, B. Diagouraga, A. Dong, L. Dombrovski, F. Baudat, S. Cusack, B. de Massy, and J. Kadlec. 2013. Molecular basis for the regulation of the H3K4 methyltransferase activity of PRDM9. Cell Rep. 5(1): 13-20.

139. Xu, H., M.D. Beasley, W.D. Warren, G.T. van der Horst, and M.J. McKay. 2005. Absence of mouse REC8 cohesin promotes synapsis of sister chromatids in meiosis. Dev Cell. 8: 949–961.

140. Yang F., K. Gell, G.W. van der Heijden, S. Eckardt, N.A. Leu, D.C. Page, R. Benavente, C. Her, C. Hoog, K.J. McLaughlin, and P.J. Wang. 2008. Meiotic failure in male mice lacking an X-linked factor. Genes Dev. 22(5): 682-691.

141. Yang, F. and P.J. Wang. 2009. The mammalian synaptonemal complex: a scaffold and beyond. Genome Dyn. 5: 69-80.

142. Ying, Y., X.M. Liu, A. Marble, K.A. Lawson, and G.Q. Zhao. 2000. Requirement of Bmp8b for the generation of primordial germ cells in the mouse. Mol Endocrinol. 14: 1053-1063.

143. Zheng, H., C.J. Stratton, K. Morozumi, J. Jin, R. Yanagimachi, and W. Yan. 2007. Lack of Spem1 causes aberrant cytoplasm removal, sperm deformation, and male infertility. Proc Natl Acad Sci U.S.A. 104: 6852-6857.

144. Zheng, K., F. Yang, and P.J. Wang. 2010. Regulation of male fertility by X-linked genes. J Androl. 21: 79-85.

145. Zheng, W., G.F. Avelar, R. Rathi, L.R. Franca, and I. 2006. Dobrinski. The length of the spermatogenic cycle is conserved in porcine and ovine testis xenografts. J Androl. 27(4): 527-533.

146. Zickler, D. and N. Kleckner. 2015. Recombination, pairing, and synapsis of homologs during meiosis. Cold Spring Harb Perspect Biol. 7(6).

Future Directions

Continuing to build on the research discussed in this project will lead to a better understanding of the mechanism of meiotic recombination. Understanding this important process in sheep, as well as other livestock species, will ultimately lead to an enhanced ability to predict how traits are passed through generations of animals. The improvement of genetic predictions will aid the industry in more accurate selection for animals exhibiting desirable traits, which will support the sustainability of livestock production.

Moving forward with research, I would like to investigate multiple hypotheses including potential genetic mechanisms behind CO numbers and locations, male and female CO differences, hotspot utilization and recognition, and CO numbers and locations in cattle. First, I would like to examine the hypothesized genetic basis for differences in CO numbers as previously identified in mice, humans, and cattle. To accomplish this, I will conduct a genome-wide association study (GWAS) with single-nucleotide polymorphism (SNP) data from these rams, as well as rams acquired in the future, using the number of COs per spermatocyte as the phenotype to potentially identify alleles or genotypes associated with greater or fewer CO numbers in rams.

Additionally, I would like to investigate sexual dimorphism in sheep. Rams are hypothesized to have a greater number of CO than ewes, which is interesting because it opposes trends in other mammals including mice and humans (typically females have higher numbers of CO than males). By studying sheep, we may be able to gain a unique perspective and greater insight into the mechanisms behind sex differences in CO numbers. To address the sexual dimorphism hypothesis, I will use a cytogenetic approach to quantify

CO and identify locations in developing oocytes and compare these data to males. In addition, I would conduct a genome-wide association study in females to potentially identify allelic and genotypic associations with CO numbers.

I am also interested in investigating recombination hotspots in sheep. Ultimately, hotspots are predictable, and the ability to predict the location of these exchanges of genetic material can contribute to the accuracy of genetic predictions. The gene *PRDM9* mediates hotspot utilization in mammals, and both variation in sequence as well as length in the zinc finger array of this gene contributes to altered DNA motif recognition and binding. I would like to identify sequence variation and zinc finger array size differences in sheep to relate to DNA binding motifs and ultimately hotspot locations in sheep.

And lastly, I would like to quantify CO numbers and characterize locations using cytogenetics in male and female cattle. This will be valuable in enhancing genetic predictions in the beef and dairy industries. Characterizing recombination using a cytogenetic approach has advantages to previous work done with linkage mapping and large pedigree datasets in that it does not require large pedigree datasets or a reference genome. In summary, understanding meiotic recombination in livestock species will enhance genetic predictions and contribute to the sustainability of animal production.

Appendix A: MLH1 Count Data in Rams

Appendix A Table 1: MLH1 Counts in Suffolk Rams

Suffolk Ram	MLH1	Suffolk Ram	MLH1	Suffolk Ram	MLH1	Suffolk Ram	MLH1	Suffolk Ram	MLH1	Suffolk Ram	MLH1	Suffolk Ram	MLH1	Suffolk Ram	MLH1	Suffolk Ram	MLH1
S1	74	S2	65	S3	64	S4	54.5	S5	49.5	S6	61.5	S7	53.5	S8	62.5	S9	54
S1	66	S2	66.5	S3	59	S4	60	S5	54.5	S6	55	S7	64.5	S8	58	S9	52.5
S1	68.5	S2	69	S3	56.5	S4	54	S5	55	S6	57.5	S7	64.5	S8	58	S9	56
S1	62.5	S2	75.5	S3	54.5	S4	54.5	S5	57	S6	60	S7	56	S8	60	S9	59
S1	61.5	S2	68	S3	61	S4	57.5	S5	54.5	S6	53	S7	63	S8	61	S9	66
S1	63	S2	65.5	S3	69	S4	55	S5	57	S6	52	S7	61	S8	57	S9	65.5
S1	71	S2	66.5	S3	61	S4	63	S5	54	S6	64	S7	55.5	S8	62	S9	63
S1	70	S2	67	S3	69	S4	65	S5	57	S6	55	S7	64.5	S8	57.5	S9	56
S1	56	S2	52	S3	55.5	S4	59	S5	60	S6	58.5	S7	58.5	S8	53	S9	54.5
S1	65.5	S2	63	S3	67	S4	63	S5	62.5	S6	57	S7	63	S8	52	S9	60
S1	64	S2	63.5	S3	66.5	S4	57.5	S5	55.5	S6	56	S7	63	S8	54	S9	55
S1	69.5	S2	69.5	S3	66.5	S4	55	S5	55.5	S6	59	S7	64.5	S8	61.5	S9	53.5
S1	69.5	S2	65.5	S3	64	S4	58	S5	52.5	S6	61.5	S7	62.5	S8	57	S9	53
S1	58	S2	66.5	S3	61	S4	56	S5	54	S6	68	S7	62	S8	51.5	S9	62.5
S1	63	S2	73.5	S3	61	S4	58	S5	55.5	S6	60.5	S7	55	S8	60	S9	63
S1	67.5	S2	64.5	S3	68.5	S4	59	S5	57	S6	67	S7	64.5	S8	59	S9	58
S1	58	S2	75	S3	60	S4	58.5	S5	52	S6	55.5	S7	55	S8	50	S9	56
S1	70	S2	71.5	S3	64.5	S4	54.5	S5	58	S6	57.5	S7	65.5	S8	55	S9	57.5
S1	72	S2	53.5	S3	67	S4	62	S5	56	S6	59.5	S7	58	S8	53	S9	60
S1	67	S2	62	S3	67	S4	64	S5	57.5	S6	54	S7	61	S8	54	S9	66
S1	64.5	S2	81.5	S3	66	S4	65	S5	53.5	S6	62	S7	59	S8	57.5	S9	64.5
S1	66.5	S2	75.5	S3	62	S4	68.5	S5	53.5	S6	58.5	S7	65	S8	58	S9	61
S1	65.5	S2	71	S3	63.5	S4	59	S5	54	S6	59.5	S7	56	S8	58	S9	67.5
S1	76	S2	64.5	S3	61	S4	62	S5	49.5	S6	61.5	S7	65.5	S8	60	S9	61
S1	60	S2	72.5	S3	61	S4	68.5	S5	54	S6	58.5	S7	64	S8	61	S9	59
S1	67	S2	71.5	S3	55.5	S4	63	S5	56.5	S6	61.5	S7	60.5	S8	54	S9	56.5
S1	73	S2	78	S3	68	S4	56.5	S5	62	S6	64.5	S7	58	S8	57	S9	61.5
S1	52.5	S2	63.5	S3	58	S4	60	S5	58	S6	60	S7	61	S8	64	S9	62
S1	65	S2	66.5	S3	58	S4	65.5	S5	57.5	S6	63	S7	58	S8	59.5	S9	53.5

S1	64	S2	58	S3	63.5	S4	58	S5	52.5	S6	61.5	S7	59.5	S8	59	S9	58
S1	64	S2	64	S3	60	S4	59.5	S5	54	S6	62	S7	60.5	S8	64	S9	56
S1	61	S2	60	S3	57	S4	60	S5	56	S6	58.5	S7	60	S8	53	S9	62
S1	75	S2	67	S3	57	S4	60	S5	59	S6	56	S7	68.5	S8	55.5	S9	58.5
S1	57.5	S2	49	S3	62	S4	66	S5	58	S6	60.5	S7	59.5	S8	56.5	S9	62
S1	64	S2	59	S3	61	S4	59	S5	63	S6	65.5	S7	64.5	S8	52.5	S9	63.5
S1	71	S2	55	S3	55	S4	69	S5	62.5	S6	62	S7	61.5	S8	64.5	S9	57
S1	61	S2	58	S3	55	S4	61	S5	58.5	S6	60.5	S7	57.5	S8	57.5	S9	60
S1	54	S2	55.5	S3	58	S4	65.5	S5	56.5	S6	59.5	S7	60	S8	60	S9	62
S1	71	S2	58	S3	57	S4	54	S5	57.5	S6	60.5	S7	66.5	S8	57.5	S9	58
S1	66	S2	63	S3	56	S4	67	S5	61	S6	61	S7	61.5	S8	54	S9	55
S1	66	S2	57	S3	57	S4	60	S5	64	S6	66.5	S7	63	S8	57	S9	60.5
S1	54	S2	65	S3	56	S4	59	S5	55.5	S6	60	S7	63	S8	53.5	S9	57
S1	57	S2	64	S3	51.5	S4	65	S5	59	S6	59	S7	53.5	S8	55.5	S9	55
S1	74	S2	58	S3	53	S4	56	S5	64.5	S6	56.5	S7	56	S8	57	S9	59
S1	63	S2	75	S3	60.5	S4	65	S5	53.5	S6	58	S7	58.5	S8	62	S9	64
S1	64	S2	63	S3	61	S4	55.5	S5	58	S6	58	S7	59.5	S8	53.5	S9	63
S1	50	S2	60	S3	61	S4	61	S5	60.5	S6	59.5	S7	60	S8	57.5	S9	58.5
S1	62	S2	64	S3	57	S4	59.5	S5	54	S6	65	S7	56	S8	62.5	S9	61
S1	53.5	S2	68	S3	56.5	S4	57.5	S5	58	S6	56	S7	54.5	S8	53	S9	54.5
S1	66	S2	63	S3	61.5	S4	56	S5	57	S6	57	S7	55.5	S8	56.5	S9	57
S1	66	S2	51	S3	58	S4	57	S5	63.5	S6	65	S7	59	S8	54	S9	65.5
S1	61	S2	50	S3	62	S4	63.5	S5	61	S6	52.5	S7	62	S8	52.5	S9	55
S1	59	S2	65	S3	57.5	S4	58	S5	58.5	S6	69.5	S7	58	S8	57	S9	60
S1	71	S2	70	S3	56	S4	64	S5	55	S6	59	S7	59	S8	60.5	S9	56
S1	65	S2	64	S3	56.5	S4	62	S5	58.5	S6	59	S7	58	S8	53	S9	57
S1	73	S2	67	S3	55.5	S4	57	S5	56.5	S6	54	S7	57	S8	61.5	S9	59
S1	56	S2	56	S3	64	S4	68	S5	54.5	S6	66	S7	59	S8	55.5	S9	61
S1	65	S2	57	S3	55	S4	55.5	S5	54	S6	57.5	S7	66.5	S8	56	S9	59
S1	72	S2	59	S3	65	S4	60	S5	56	S6	56.5	S7	57.5	S8	53.5	S9	65
S1	77.5	S2	63.5	S3	67.5	S4	57.5	S5	55	S6	63	S7	61	S8	62	S9	58.5
S1	66	S2	72.5	S3	64	S4	58.5	S5	63.5	S6	59	S7	57	S8	54	S9	55
S1	67	S2	68.5	S3	55	S4	61	S5	63.5	S6	53	S7	59	S8	53	S9	63
S1	70.5	S2	65	S3	54	S4	67.5	S5	59	S6	63.5	S7	58.5	S8	62.5	S9	63.5
S1	76	S2	68	S3	68	S4	61.5	S5	55.5	S6	53	S7	66	S8	63	S9	54
S1	59	S2	69.5	S3	65	S4	70.5	S5	69.5	S6	62.5	S7	68	S8	58	S9	59.5

S1	67.5	S2	65	S3	60	S4	65.5	S5	52.5	S6	61.5	S7	60	S8	56	S9	61
S1	65.5	S2	58	S3	60.5	S4	62	S5	54.5	S6	64	S7	55.5	S8	57.5	S9	59
S1	65	S2	56	S3	58	S4	58.5	S5	55	S6	64	S7	65	S8	60	S9	65
S1	70	S2	69	S3	56	S4	56	S5	61.5	S6	59	S7	62	S8	66	S9	58.5
S1	69	S2	63	S3	56	S4	50	S5	55	S6	63.5	S7	53.5	S8	64.5	S9	112
S1	61.5	S2	58	S3	56	S4	63	S5	59	S6	68	S7	52	S8	61	S9	113
S1	61.5	S2	60	S3	54.5	S4	60	S5	57.5	S6	58	S7	54.5	S8	67.5	S9	55
S1	63	S2	65.5	S3	63.5	S4	75.5	S5	58	S6	59.5	S7	57	S8	58.5	S9	60
S1	72	S2	71	S3	60	S4	80	S5	58.5	S6	57.5	S7	60.5	S8	63	S9	54.5
S1	68.5	S2	59	S3	60.5	S4	65	S5	63	S6	54.5	S7	59.5	S8	63	S9	69.5
S1	73	S2	70	S3	62	S4	67.5	S5	57.5	S6	54	S7	62	S8	64.5	S9	62
S1	74.5	S2	68	S3	55.5	S4	61.5	S5	53.5	S6	63.5	S7	53.5	S8	62.5	S9	50.5
S1	64.5	S2	61	S3	58	S4	66.5	S5	53.5	S6	54	S7	56	S8	62	S9	62.5
S1	76	S2	55	S3	53	S4	65.5	S5	62	S6	64	S7	57	S8	55	S9	59
S1	68	S2	61	S3	69	S4	57.5	S5	59	S6	54.5	S7	61	S8	64.5	S9	66
S1	62	S2	60.5	S3	61	S4	58	S5	58.5	S6	68	S7	60	S8	60	S9	65.5
S1	60	S2	59	S3	59	S4	57.5	S5	56.5	S6	61	S7	59	S8	53	S9	63
S1	65	S2	57.5	S3	51	S4	49.5	S5	62.5	S6	62	S7	63	S8	52	S9	56
S1	69	S2	68	S3	58.5	S4	59.5	S5	60	S6	54	S7	56	S8	64	S9	54.5
S1	66	S2	66.5	S3	56	S4	64.5	S5	62	S6	62	S7	64	S8	55	S9	60
S1	68.5	S2	70	S3	52.5	S4	62.5	S5	65.5	S6	52.5	S7	60	S8	58.5	S9	55
S1	66	S2	67	S3	53.5	S4	56.5	S5	61.5	S6	65	S7	61	S8	57	S9	56.5
S1	66	S2	57.5	S3	59	S4	67.5	S5	53.5	S6	60	S7	66	S8	56	S9	56
S1	59	S2	67	S3	58	S4	61.5	S5	58	S6	62.5	S7	60	S8	57	S9	63.5
S1	68	S2	65	S3	63.5	S4	62	S5	63	S6	60.5	S7	63	S8	54	S9	58
S1	65.5	S2	72	S3	60	S4	58.5	S5	60	S6	56	S7	68	S8	57	S9	50.5
S1	59			S3	62.5	S4	56	S5	55.5	S6	52	S7	61	S8	60	S9	62.5
S1	62.5			S3	63	S4	60	S5	63					S8	62.5	S9	53.5
S1	67			S3	62.5	S4	75.5	S5	61.5					S8	55.5	S9	53
S1	62.5			S3	55.5	S4	65.5	S5	60.5					S8	55.5		
S1	63			S3	61	S4	57.5	S5	54								
S1	77			S3	63	S4	59.5	S5	59								
S1	67			S3	60.5			S5	66								
S1	65			S3	58			S5	66.5								
S1	54			S3	59			S5	64.5								
S1	54			S3	56			S5	57.5								

S1	62			S3	60			S5	58.5								
S1	55			S3	62			S5	58.5								
S1	56			S3	61			S5	57								
S1	54.5			S3	55			S5	57								
S1	72			S3	64			S5	62								
S1	65			S3	63			S5	60.5								
S1	58.5			S3	53			S5	63								
S1	66.5			S3	58.5			S5	60.5								
S1	56			S3	60.5			S5	56								
								S5	56								
								S5	64								
Suffolk Ram	MLH1	Suffolk Ram	MLH1	Suffolk Ram	MLH1	Suffolk Ram	MLH1	Suffolk Ram	MLH1	Suffolk Ram	MLH1	Suffolk Ram	MLH1	Suffolk Ram	MLH1		
S10	60.5	S11	55.5	S12	58.5	S13	55	S14	55	S15	64	S16	58	S17	67.5		
S10	58.5	S11	58	S12	59.5	S13	57	S14	69.5	S15	63	S16	49	S17	64		
S10	55	S11	56.5	S12	56.5	S13	56.5	S14	61	S15	66	S16	63	S17	56		
S10	60	S11	61.5	S12	60.5	S13	64	S14	61.5	S15	60	S16	62	S17	57.5		
S10	54.5	S11	54	S12	59	S13	58.5	S14	60.5	S15	63	S16	75	S17	56		
S10	69.5	S11	56.5	S12	47	S13	55	S14	59.5	S15	64	S16	61	S17	58.5		
S10	62	S11	60	S12	65.5	S13	60	S14	68	S15	62.5	S16	64	S17	70.5		
S10	54	S11	59.5	S12	60.5	S13	60.5	S14	61.5	S15	60	S16	65.5	S17	60		
S10	57.5	S11	65	S12	57.5	S13	57.5	S14	56	S15	69	S16	65	S17	61		
S10	54	S11	57	S12	57.5	S13	62	S14	59.5	S15	64	S16	70.5	S17	63.5		
S10	58.5	S11	60.5	S12	66	S13	58.5	S14	59	S15	63	S16	66	S17	64		
S10	56.5	S11	61.5	S12	64.5	S13	57.5	S14	53.5	S15	56	S16	72	S17	65		
S10	56	S11	53.5	S12	57.5	S13	63	S14	58.5	S15	56	S16	63	S17	59		
S10	63.5	S11	56.5	S12	64	S13	60.5	S14	60	S15	63	S16	66	S17	55		
S10	58	S11	51.5	S12	58.5	S13	62	S14	58.5	S15	60	S16	67	S17	53		
S10	50.5	S11	55.5	S12	56.5	S13	64.5	S14	64.5	S15	63.5	S16	62	S17	67		
S10	62.5	S11	53.5	S12	61	S13	60.5	S14	59.5	S15	74	S16	56	S17	55		
S10	60.5	S11	63	S12	61	S13	64.5	S14	60.5	S15	69.5	S16	62	S17	58		
S10	60.5	S11	60.5	S12	61	S13	67	S14	60.5	S15	58.5	S16	68	S17	59		
S10	63	S11	58.5	S12	65	S13	62.5	S14	63	S15	64.5	S16	54.5	S17	62.5		
S10	51.5	S11	59	S12	62.5	S13	61.5	S14	57	S15	64	S16	71	S17	69		
S10	67	S11	61.5	S12	66	S13	64	S14	55	S15	62.5	S16	62	S17	60		

S10	62	S11	60.5	S12	68.5	S13	57.5	S14	60	S15	55	S16	55	S17	70		
S10	59.5	S11	68	S12	65.5	S13	65.5	S14	56	S15	76.5	S16	57	S17	79		
S10	60.5	S11	56.5	S12	60	S13	64	S14	56.5	S15	57	S16	67	S17	59.5		
S10	58.5	S11	59	S12	56	S13	59.5	S14	65	S15	69	S16	68	S17	63.5		
S10	61	S11	64	S12	61.5	S13	59.5	S14	56.5	S15	63	S16	66	S17	71		
S10	61	S11	57	S12	62	S13	65	S14	64	S15	64	S16	61	S17	62.5		
S10	57.5	S11	68	S12	60.5	S13	63.5	S14	65	S15	65	S16	64	S17	56.5		
S10	54	S11	71.5	S12	61	S13	65	S14	61.5	S15	64.5	S16	63	S17	53		
S10	60.5	S11	59.5	S12	60.5	S13	55.5	S14	55.5	S15	56.5	S16	59	S17	58.5		
S10	62.5	S11	52	S12	60.5	S13	59.5	S14	61.5	S15	51	S16	67	S17	69		
S10	64	S11	58	S12	59.5	S13	67.5	S14	61.5	S15	56.5	S16	62	S17	61		
S10	62	S11	60	S12	62	S13	66	S14	58.5	S15	52	S16	56	S17	72		
S10	66.5	S11	60.5	S12	63	S13	60.5	S14	55.5	S15	66	S16	62	S17	68.5		
S10	57	S11	72.5	S12	58.5	S13	67	S14	60.5	S15	61	S16	68	S17	72		
S10	55	S11	60.5	S12	62.5	S13	64.5	S14	56.5	S15	65	S16	54.5	S17	71		
S10	54.5	S11	52	S12	61	S13	70	S14	55.5	S15	73.5	S16	71	S17	68.5		
S10	63.5	S11	62	S12	61.5	S13	59.5	S14	52	S15	65	S16	62	S17	66		
S10	58	S11	62.5	S12	60.5	S13	60	S14	53.5	S15	60	S16	55	S17	66		
S10	62	S11	55	S12	64	S13	60	S14	50.5	S15	69	S16	57	S17	60.5		
S10	59.5	S11	66	S12	61.5	S13	65	S14	58	S15	62	S16	67	S17	66		
S10	59	S11	64.5	S12	69.5	S13	66	S14	51.5	S15	65.5	S16	68	S17	60.5		
S10	59.5	S11	61.5	S12	61	S13	56	S14	56	S15	62	S16	66	S17	68		
S10	64	S11	61	S12	59	S13	59.5	S14	52	S15	58	S16	61	S17	64		
S10	54	S11	63.5	S12	65	S13	60	S14	64	S15	53	S16	64	S17	60		
S10	59	S11	65	S12	54	S13	61	S14	60.5	S15	63	S16	63	S17	70		
S10	63.5	S11	69	S12	66	S13	55.5	S14	51.5	S15	61.5	S16	59	S17	59.5		
S10	61	S11	65.5	S12	63.5	S13	66	S14	56.5	S15	55	S16	54.5	S17	65		
S10	68	S11	55	S12	67	S13	56	S14	57	S15	76.5	S16	71	S17	61.5		
S10	66.5	S11	53.5	S12	66	S13	60.5	S14	59.5	S15	57	S16	62	S17	56.5		
S10	73	S11	65	S12	60.5	S13	63	S14	60.5	S15	69	S16	55	S17	53		
S10	68	S11	56.5	S12	66.5	S13	63	S14	51	S15	63	S16	57	S17	58.5		
S10	53	S11	54	S12	64	S13	62.5	S14	52	S15	64	S16	67	S17	69		
S10	59.5	S11	64.5	S12	65.5	S13	60	S14	54.5	S15	65	S16	65	S17	61		
S10	51.5	S11	70.5	S12	61.5	S13	61	S14	57	S15	64.5	S16	70.5	S17	72		
S10	56.5	S11	61.5	S12	61	S13	59.5	S14	54	S15	56.5	S16	66	S17	68.5		
S10	57	S11	58.5	S12	58.5	S13	59.5	S14	60	S15	51	S16	72	S17	72		

S10	64.5	S11	55	S12	61.5	S13	64	S14	54	S15	56.5	S16	63	S17	71		
S10	63.5	S11	69.5	S12	73	S13	55.5	S14	51.5	S15	52	S16	66	S17	68.5		
S10	52.5	S11	61	S12	63.5	S13	61	S14	60	S15	66	S16	67	S17	66		
S10	52	S11	63.5	S12	68	S13	63.5	S14	55.5	S15	61	S16	62	S17	66		
S10	66.5	S11	66.5	S12	62.5	S13	58	S14	65	S15	65	S16	49	S17	60.5		
S10	66.5	S11	64.5	S12	63.5	S13	61	S14	57.5	S15	73.5	S16	63	S17	66		
S10	54.5	S11	57.5	S12	73	S13	63	S14	60.5	S15	65	S16	62	S17	60.5		
S10	58.5	S11	61.5	S12	66	S13	50.5	S14	58.5	S15	60	S16	75	S17	68		
S10	52	S11	62.5	S12	59.5	S13	60	S14	61.5	S15	69	S16	61	S17	64		
S10	68	S11	61	S12	60.5	S13	57.5	S14	51	S15	65.5	S16	64	S17	60		
S10	53	S11	66	S12	72	S13	59	S14	60	S15	62	S16	65.5	S17	70		
S10	59.5	S11	67.5	S12	75	S13	59	S14	55.5	S15	58	S16	65	S17	59.5		
S10	51.5	S11	65	S12	68	S13	63.5	S14	64	S15	53	S16	70.5	S17	65		
S10	56.5	S11	65	S12	64	S13	57	S14	54.5	S15	63	S16	63	S17	61.5		
S10	57	S11	70.5	S12	55.5	S13	63.5	S14	62	S15	63	S16	66	S17	72		
S10	54	S11	69.5	S12	68.5	S13	60.5	S14	65	S15	76	S16	67	S17	71		
S10	57.5	S11	63.5	S12	75.5	S13	69.5	S14	55	S15	71	S16	62	S17	68.5		
S10	54	S11	66.5	S12	61	S13	62	S14	57	S15	58	S16	54	S17	66		
S10	58.5	S11	64.5	S12	69	S13	57	S14	56	S15	65	S16	62	S17	66		
S10	56.5	S11	57.5	S12	66.5	S13	64.5	S14	51	S15	65	S16	68	S17	60.5		
S10	56	S11	61.5	S12	65.5	S13	55.5	S14	52.5	S15	63	S16	55	S17	60.5		
S10	63.5	S11	59	S12	60	S13	72	S14	59	S15	55	S16	71	S17	68		
S10	60	S11	60.5	S12	59.5	S13	54	S14	57	S15	77	S16	62	S17	64		
S10	54.5	S11	56.5	S12	56	S13	63.5	S14	59.5	S15	57	S16	66	S17	60		
S10	69.5	S11	59	S12	54.5	S13	60	S14	54.5	S15	63	S16	67	S17	70		
S10	62	S11	64	S12	51	S13	63	S14	55	S15	60	S16	62	S17	59.5		
S10	54	S11	57	S12	73.5	S13	62.5	S14	57	S15	64	S16	56	S17	65		
S10	57.5	S11	68	S12	72.5	S13	65.5	S14	62.5	S15	72	S16	67	S17	61.5		
S10	60.5	S11	71.5	S12	62	S13	63.5	S14	62	S15	66	S16	68	S17	72		
S10	63	S11	60	S12	65.5	S13	63.5	S14	54.5	S15	60	S16	66	S17	71		
S10	51.5	S11	60.5	S12	61.5	S13	69.5	S14	51	S15	63	S16	61	S17	68.5		
S10	67	S11	69	S12	66	S13	57	S14	60	S15	63	S16	58	S17	66		
S10	62	S11	55	S12	61.5	S13	72	S14	64	S15	63	S16	62	S17	66		
		S11	54	S12	66	S13	60	S14	65	S15	59	S16	68	S17	60.5		
		S11	70.5	S12	65	S13	62	S14	51					S17	68.5		
		S11	55	S12	62.5	S13	57							S17	66		

		S11	61	S12	68	S13	64.5										
				S12	61												
				S12	59												
				S12	63.5												
				S12	59.5												
				S12	61.5												
				S12	68.5												
				S12	58												

Appendix A Table 2: MLH1 Counts in Icelandic Rams

Icelandic Ram	MLH1	Icelandic Ram	MLH1	Icelandic Ram	MLH1	Icelandic Ram	MLH1	Icelandic Ram	MLH1
I1	62.5	I2	53	I3	58.5	I4	56	I5	66.5
I1	56.5	I2	54	I3	58.5	I4	58	I5	69
I1	61	I2	65	I3	57	I4	59.5	I5	69.5
I1	53	I2	52	I3	64	I4	69	I5	65
I1	61	I2	52	I3	66	I4	55	I5	66.5
I1	62.5	I2	61.5	I3	58	I4	60.5	I5	66.5
I1	68	I2	54	I3	66	I4	62	I5	70
I1	63.5	I2	53	I3	65	I4	56	I5	62.5
I1	58.5	I2	51	I3	70	I4	53	I5	63.5
I1	64.5	I2	55	I3	62.5	I4	56	I5	60
I1	59.5	I2	62	I3	66.5	I4	60	I5	58.5
I1	56.5	I2	52	I3	66.5	I4	65	I5	69.5
I1	55.5	I2	58	I3	61	I4	67.5	I5	63.5
I1	55	I2	57	I3	53.5	I4	61.5	I5	66
I1	67.5	I2	53	I3	68	I4	63	I5	67.5
I1	58	I2	63	I3	64.5	I4	67.5	I5	66
I1	51	I2	55	I3	50.5	I4	59	I5	73
I1	55	I2	47.5	I3	54	I4	58	I5	74
I1	59	I2	49	I3	55.5	I4	54	I5	72
I1	72.5	I2	66	I3	63.5	I4	68	I5	61
I1	66	I2	67	I3	54	I4	66.5	I5	71
I1	61	I2	59	I3	57.5	I4	57	I5	75.5
I1	60	I2	62.5	I3	60.5	I4	64	I5	73
I1	57	I2	58.5	I3	69.5	I4	64	I5	72.5
I1	60	I2	57	I3	67	I4	60	I5	66
I1	62.5	I2	59	I3	66	I4	64.5	I5	66.5
I1	70.5	I2	59	I3	66.5	I4	67.5	I5	74
I1	68.5	I2	55.5	I3	69.5	I4	59	I5	72
I1	72.5	I2	55	I3	66	I4	65	I5	69.5
I1	64	I2	54.5	I3	67	I4	59	I5	68
I1	76	I2	58.5	I3	61.5	I4	68.5	I5	66.5
I1	65.5	I2	51	I3	67.5	I4	62	I5	61
I1	69	I2	58	I3	66	I4	60	I5	73.5
I1	60	I2	62.5	I3	61	I4	58	I5	69
I1	54.5	I2	61	I3	68.5	I4	65	I5	75.5
I1	61	I2	58	I3	70.5	I4	55.5	I5	69
I1	60.5	I2	47.5	I3	67.5	I4	63	I5	70.5
I1	70.5	I2	55.5	I3	68	I4	69	I5	72
I1	65	I2	54.5	I3	65	I4	57.5	I5	75.5
I1	63.5	I2	47.5	I3	60	I4	69.5	I5	65.5
I1	65	I2	54	I3	62.5	I4	64	I5	70
I1	66	I2	58	I3	68	I4	64	I5	76
I1	65	I2	58.5	I3	62	I4	63	I5	73
I1	65.5	I2	55.5	I3	67	I4	63	I5	70
I1	60	I2	52.5	I3	62	I4	69	I5	68.5
I1	62.5	I2	57	I3	62	I4	59	I5	74.5
I1	59	I2	55	I3	68	I4	59	I5	62
I1	63	I2	60	I3	63	I4	56	I5	69
I1	64	I2	52.5	I3	61	I4	53	I5	78

I1	58	I2	55	I3	53.5	I4	56	I5	64
I1	60	I2	53	I3	66	I4	63	I5	69.5
I1	63	I2	55.5	I3	57	I4	61	I5	74
I1	67	I2	54.5	I3	75	I4	56	I5	70
I1	66	I2	59	I3	73	I4	57	I5	69.5
I1	62	I2	55.5	I3	66.5	I4	64	I5	70.5
I1	65	I2	58.5	I3	61	I4	61	I5	64
I1	59.5	I2	58.5	I3	56	I4	63	I5	68.5
I1	62.5	I2	57	I3	64.5	I4	66	I5	71
I1	60.5	I2	64	I3	62	I4	66	I5	67
I1	63.5	I2	61	I3	60	I4	58	I5	69
I1	67	I2	53.5	I3	65.5	I4	61	I5	60.5
I1	64.5	I2	64.5	I3	64	I4	61	I5	76
I1	62	I2	50.5	I3	61	I4	66	I5	71
I1	63	I2	54	I3	68	I4	57	I5	73.5
I1	59	I2	55.5	I3	60.5	I4	58	I5	68
I1	62	I2	63.5	I3	71	I4	62	I5	63
I1	60	I2	54	I3	66.5	I4	61	I5	67.5
I1	70	I2	57.5	I3	67.5	I4	58.5	I5	74
I1	60	I2	60.5	I3	66	I4	57	I5	63
I1	62	I2	64	I3	61	I4	65.5	I5	68
I1	53	I2	64	I3	68.5	I4	70	I5	74
I1	58.5	I2	63	I3	57	I4	61.5	I5	64
I1	64	I2	63	I3	75	I4	50	I5	76
I1	65.5	I2	69	I3	73	I4	59.5	I5	68
I1	58	I2	59	I3	66.5	I4	66	I5	69
I1	60	I2	59	I3	61	I4	67	I5	64
I1	60	I2	56	I3	56	I4	56	I5	63
I1	72	I2	53	I3	58	I4	70	I5	66
I1	51.5	I2	56	I3	66	I4	72	I5	69
I1	69	I2	63	I3	65	I4	60	I5	76
I1	71	I2	61	I3	70	I4	56.5	I5	73
I1	60	I2	56	I3	62.5	I4	64	I5	71
I1	55	I2	57	I3	66.5	I4	66	I5	78
I1	67	I2	64	I3	68	I4	64	I5	76
I1	56.5	I2	61	I3	65	I4	57.5	I5	73
I1	57	I2	63	I3	60	I4	60.5	I5	62
I1	62.5	I2	59	I3	62.5	I4	60	I5	73
I1	67	I2	60.5	I3	68.5	I4	65	I5	69
I1	69	I2	69	I3	57	I4	60	I5	64
I1	69.5	I2	66	I3	75	I4	54	I5	66
I1	69.5	I2	66	I3	73	I4	56.5	I5	75.5
I1	68	I2	63	I3	58	I4	60.5	I5	78
I1	72	I2	58.5	I3	66	I4	56	I5	64.5
I1	64	I2	58.5	I3	65	I4	61	I5	73
I1	64	I2	69	I3	79.5	I4	61.5	I5	69.5
I1	62	I2	75	I3	65	I4	61	I5	66
I1	55.5	I2	59	I3	66	I4	60	I5	70.5
I1	59	I2	67	I3	65	I4	57	I5	71
I1	68	I2	65	I3	62.5	I4	66	I5	64
I1	58	I2	70.5	I3	64.5	I4	63.5	I5	75
I1	64.5	I2	69	I3	73	I4	61	I5	62
I1	71.5	I2	75.5	I3	66	I4	56.5	I5	66.5

I1	54	I2	60.5	I3	75	I4	61.5	I5	64
I1	60	I2	69	I3	70	I4	68.5	I5	63.5
		I2	62	I3	69	I4	65	I5	77.5
		I2	74	I3	60.5	I4	57	I5	61
		I2	61	I3	72	I4	62.5	I5	69.5
		I2	72	I3	70	I4	67	I5	73
		I2	72	I3	63	I4	59.5	I5	76
		I2	69	I3	66	I4	58.5	I5	62.5
		I2	71	I3	65	I4	61	I5	67.5
		I2	71	I3	65	I4	63.5	I5	73
		I2	63.5			I4	64.5	I5	65.5
		I2	60			I4	65.5	I5	63.5
						I4	66	I5	72
						I4	63	I5	68.5
						I4	65	I5	69.5
						I4	58.5	I5	74
						I4	55.5	I5	73
						I4	63.5	I5	70
						I4	61	I5	73.5
						I4	66	I5	68.5
						I4	58.5	I5	75.5
						I4	64.5	I5	64
						I4	65.5	I5	71
						I4	58.5	I5	76.5
						I4	64	I5	73.5
						I4	58	I5	70.5
						I4	56.5	I5	70
						I4	62	I5	64
						I4	66	I5	72.5
						I4	59.5	I5	71.5
						I4	73	I5	65
						I4	59.5	I5	68
						I4	58	I5	73.5
						I4	59.5	I5	70.5
						I4	61.5	I5	63
						I4	58	I5	66.5
								I5	59.5
								I5	64.5
								I5	68
								I5	61
								I5	68
								I5	64.5
								I5	68
								I5	80
								I5	65
								I5	70
								I5	59.5
								I5	70
								I5	65.5
								I5	69
								I5	69
								I5	69.5
								I5	70.5

								I5	60.5
								I5	72
								I5	69.5
								I5	74

Appendix A Table 3: MLH1 Counts in Targhee Rams

Targhee Ram	MLH1	Targhee Ram	MLH1	Targhee Ram	MLH1	Targhee Ram	MLH1	Targhee Ram	MLH1
T1	68	T2	71	T3	69	T4	65	T5	56.5
T1	71.5	T2	68	T3	70.5	T4	59.5	T5	58
T1	63.5	T2	68.5	T3	65	T4	71.5	T5	50.5
T1	62.5	T2	61.5	T3	55	T4	79	T5	59.5
T1	74	T2	70	T3	73	T4	73	T5	63.5
T1	68	T2	66	T3	77	T4	67	T5	63
T1	64	T2	71	T3	71	T4	65	T5	55.5
T1	69.5	T2	76	T3	62	T4	75	T5	58
T1	66.5	T2	63.5	T3	76.5	T4	66	T5	56
T1	62.5	T2	73	T3	74	T4	60	T5	52.5
T1	68	T2	75	T3	63	T4	67.5	T5	52.5
T1	68	T2	65	T3	56.5	T4	66.5	T5	63
T1	60.5	T2	75.5	T3	72	T4	69	T5	61.5
T1	56	T2	64.5	T3	56	T4	71	T5	55.5
T1	71	T2	64	T3	63	T4	62.5	T5	53
T1	58	T2	75.5	T3	73	T4	71	T5	53.5
T1	68	T2	66	T3	60	T4	56.5	T5	59.5
T1	67	T2	74	T3	71	T4	69.5	T5	60.5
T1	75	T2	70	T3	59	T4	64	T5	61.5
T1	55	T2	68	T3	68	T4	65.5	T5	58
T1	64	T2	59	T3	63	T4	68	T5	60
T1	70	T2	72	T3	64.5	T4	74	T5	57.5
T1	67.5	T2	69	T3	60	T4	69	T5	61
T1	65	T2	69	T3	60	T4	67	T5	66.5
T1	61.5	T2	74.5	T3	72	T4	68	T5	59.5
T1	74.5	T2	74	T3	58.5	T4	77.5	T5	67
T1	72	T2	59	T3	66	T4	72	T5	63
T1	63	T2	57.5	T3	71	T4	70	T5	66.5
T1	71.5	T2	78	T3	54	T4	74	T5	65.5
T1	70	T2	70.5	T3	58.5	T4	71.5	T5	55
T1	74	T2	73	T3	56	T4	81	T5	54
T1	57	T2	71	T3	70	T4	68	T5	54
T1	72	T2	73	T3	59	T4	66.5	T5	57.5
T1	63	T2	73	T3	61	T4	66	T5	56.5
T1	55	T2	74	T3	56	T4	66.5	T5	62.5
T1	66	T2	67.5	T3	70.5	T4	74	T5	56.5
T1	70.5	T2	72.5	T3	67	T4	66.5	T5	60
T1	59.5	T2	74	T3	72.5	T4	66.5	T5	69
T1	62	T2	65	T3	68	T4	78	T5	66
T1	57	T2	58.5	T3	58.5	T4	72.5	T5	65
T1	63	T2	70.5	T3	68.5	T4	72	T5	56
T1	74	T2	72.5	T3	73	T4	67	T5	58.5
T1	67.5	T2	52.5	T3	64	T4	72.5	T5	57.5
T1	63	T2	65.5	T3	65	T4	72	T5	56

T1	61.5	T2	62	T3	61.5	T4	70	T5	61
T1	53	T2	62	T3	58	T4	74	T5	63.5
T1	61	T2	64	T3	51	T4	60	T5	63.5
T1	68	T2	62	T3	73	T4	73	T5	63
T1	66.5	T2	63	T3	69.5	T4	63	T5	63
T1	59	T2	65	T3	74	T4	63	T5	61.5
T1	64.5	T2	60	T3	66	T4	69.5	T5	61.5
T1	60.5	T2	71	T3	54	T4	69	T5	61
T1	60	T2	67	T3	72	T4	68.5	T5	66.5
T1	59.5	T2	67.5	T3	67	T4	82.5	T5	67
T1	70	T2	62	T3	60	T4	65	T5	63
T1	64.5	T2	62	T3	65	T4	65	T5	66.5
T1	71	T2	70.5	T3	69	T4	69	T5	65.5
T1	64.5	T2	65	T3	71	T4	73.5	T5	57.5
T1	73	T2	61	T3	65	T4	74	T5	60
T1	60	T2	65.5	T3	67	T4	71.5	T5	66
T1	71	T2	66	T3	67	T4	84.5	T5	65
T1	64.5	T2	62	T3	66	T4	75	T5	56
T1	59	T2	53.5	T3	56	T4	75	T5	58.5
T1	56	T2	54.5	T3	72	T4	75.5	T5	57.5
T1	62	T2	62.5	T3	74	T4	83	T5	56
T1	68	T2	62.5	T3	70.5	T4	72	T5	61
T1	67	T2	55.5	T3	68	T4	65	T5	59.5
T1	67	T2	58.5	T3	65	T4	76	T5	60.5
T1	61	T2	61.5	T3	67	T4	70	T5	61.5
T1	61	T2	67	T3	59	T4	70.5	T5	58
T1	71	T2	64	T3	70	T4	80	T5	60
T1	71	T2	61	T3	65	T4	73	T5	57.5
T1	70	T2	66.5	T3	69.5	T4	69.5	T5	61
T1	72	T2	53.5	T3	62	T4	65	T5	66.5
T1	62	T2	68	T3	71	T4	72	T5	59.5
T1	56	T2	71.5	T3	62	T4	77.5	T5	61.5
T1	75.5	T2	65	T3	71	T4	76	T5	61.5
T1	66	T2	67.5	T3	72	T4	85	T5	61
T1	69	T2	63	T3	77.5	T4	74	T5	66.5
T1	66	T2	70.5	T3	59.5	T4	78	T5	67
T1	64.5	T2	57	T3	58.5	T4	71	T5	63
T1	68	T2	57	T3	67	T4	62	T5	66.5
T1	61	T2	59.5	T3	65	T4	59.5	T5	60
T1	68	T2	57	T3	66	T4	68	T5	66
T1	59	T2	57	T3	63	T4	71.5	T5	65
T1	65	T2	56	T3	62	T4	71	T5	56
T1	67.5	T2	59	T3	60	T4	66	T5	58.5
T1	67	T2	60.5	T3	74	T4	75.5	T5	57.5
T1	62.5	T2	69	T3	73	T4	74		
T1	65	T2	66	T3	79.5	T4	75		
T1	58	T2	66	T3	57.5	T4	72.5		
T1	57	T2	63	T3	63	T4	72.5		

T1	58	T2	58.5	T3	68	T4	69.5		
T1	59	T2	58.5	T3	63.5	T4	74		
T1	63	T2	69	T3	79.5	T4	73		
T1	63	T2	75	T3	65	T4	73		
		T2	59	T3	66	T4	62		
		T2	67	T3	65	T4	77.5		
		T2	65	T3	62.5	T4	70		
		T2	70.5	T3	64.5	T4	72		
		T2	69	T3	73	T4	80		
		T2	75.5	T3	66	T4	76		
		T2	60.5	T3	75	T4	70		
		T2	69	T3	70	T4	60.5		
		T2	62	T3	69	T4	71		
		T2	74	T3	60.5	T4	68		
		T2	61	T3	72	T4	79.5		
		T2	72	T3	70	T4	77		
		T2	72	T3	63	T4	78		
		T2	69	T3	66	T4	81		
		T2	71	T3	65	T4	80		
		T2	71	T3	65				
		T2	63.5						
		T2	60						

Appendix B: Abstracts Submitted and Accepted for Presentation

American Society of Animal Science 2016
Accepted for poster presentation

Meiotic recombination differences in ruminant livestock species

Homologous recombination or cross-overs (CO) ensures proper chromosome segregation while contributing to genetic variation. It is clear from previous studies that at least one CO per chromosome arm is necessary to avoid mis-segregation. Furthermore, it has been well documented that the locations of CO are not random, with some genomic regions exhibiting preferences, called hotspots. Global meiotic recombination rates determined from offspring studies underestimates the total number of meiotic recombination events due to independent assortment. Despite the importance of meiotic recombination in the production of viable gametes and towards predicting or estimating genetic breeding values, we know very little about meiotic recombination rates in livestock species. In this study we have used a cytological approach to quantify the number of recombination events in male sheep and cattle. Characterizing recombination events using a cytological approach allows us to accurately identify all recombination events during meiosis without the need for a large number of offspring and independent of an accurate reference genome that does not exist. Testicular tissue samples were taken from mature rams and bulls, and spermatocytes were spread and fixed on slides. Immunofluorescent staining was used to identify the synaptonemal complexes (SYCP3) and CO events (MLH1) of pachytene stage prophase cells. The total number of CO per meiocyte was quantified for different livestock species. Interestingly, the average number of CO per meiocyte in sheep is approximately 20% higher than in cattle despite having a similar number of chromosome arms and genome size. More specifically, sheep have on average a greater number of recombination events per chromosome arm (~ 2.8 CO per arm) in comparison to cattle (~ 1.7 CO per arm). This research provides important information regarding differences in recombination rates in sheep and cattle spermatocytes, and has a direct impact on the genetic predictions in these species. Moreover, this research contributes valuable information towards a greater understanding of the factors that control meiotic recombination in different species to enhance reproduction, improve accuracy of genetic prediction, and advance selection strategies that support the sustainability of the livestock industry.

International Society for Animal Genetics 2016
Accepted for poster presentation

The ramification of meiotic recombination differences in sheep

The production of viable gametes is an integral part of reproduction and therefore a critical aspect for the sustainability of the livestock industry. Homologous recombination or cross-overs (CO) contribute to genetic variation and ensures proper chromosome segregation. In

virtually all organisms studied thus far, it is clear that at least one CO per chromosome arm is necessary to avoid mis-segregation. Furthermore, the location of CO are not random, exhibiting some preferences (called hotspots) and with the presence of one CO "interfering" with the proximity of a second. Importantly, failure or improper placements of recombination represent a significant contribution to fetal loss and infertility. Despite the importance of these issues, we know very little about meiotic recombination rates in livestock species. Although global recombination rates are known to differ between strains of mice, this has not been evaluated in livestock breeds. In this study we characterized and quantified the number of recombination events in males from different breeds of sheep. Characterizing recombination differences between breeds of sheep will greatly enhance breed specific genetic predictions. Testicular tissue samples were taken from mature rams of different breeds and spermatocytes were spread and fixed on slides. Immunofluorescent staining was used to identify the synaptonemal complexes (SYCP3) and CO events (MLH1) of pachytene stage prophase cells. The total number of CO per meiocyte and their locations on the chromosomes were quantified. Our data suggests that global recombination rates are 10% higher in Targhee than in Suffolk rams. Despite having a similar number of chromosome arms and genome size, the number of recombination events in sheep spermatocytes are approximately 20% higher than in cattle. This research provides important information regarding recombination rates in sheep spermatocytes and has a direct impact on genetic breed predictions. Moreover, this research contributes valuable information towards a greater understanding of the factors that control meiotic recombination to enhance reproduction, improve genetic predictions, and advance selection strategies towards the sustainability of the livestock industry.

Plant and Animal Genome Conference 2017
Accepted for poster presentation
Invited oral presentation in International Sheep Genomics Consortium

Understanding the Ramification of Recombination Variation in Sheep

Meiotic recombination is an important process during gamete formation that ensures proper chromosome segregation and controls genetic variation. It is clear from previous studies that at least one recombination event per chromosome arm is necessary to avoid mis-segregation. In addition, placement of recombination is not random, showing some preferences called "hotspots." According to previous studies using linkage maps, recombination rates can vary as much as 10% in domestic sheep breeds, and is reported to be lower in bighorn sheep. However, global recombination rates determined from linkage maps or haplotype phasing underestimate the number of recombination events per meiotic cell due to chromosomal independent assortment. To better understand and account for recombination differences between breeds of sheep, we used a cytological approach to quantify recombination events in spermatocytes. Testicular tissue samples were taken from mature Targhee, Suffolk, and Icelandic rams, and spermatocytes were spread and fixed on slides. In total, we examined over 2,200 spermatocytes and approximately 150,000 recombination events. Immunofluorescent staining was performed to identify the

synaptonemal complexes (SYCP3) and recombination events (MLH1) of pachytene stage prophase cells. The total number of recombination per meiotic cell was quantified and compared between the three breeds using an ANOVA with a subsequent Tukey-Kramer test. Interestingly, Targhee rams have significantly higher ($p < 0.01$) recombination than Suffolk and Icelandic rams. This research gives us the tools to better understand chromosomal inheritance that consequently improves the accuracy of genetic predictions and contributes valuable information towards the sustainability of the sheep industry.

International Society for Animal Genetics 2017
Accepted for poster presentation
Invited oral presentation in Applied Sheep and Goat Genomics Workshop

Investigating genetic associations with meiotic recombination in rams

Meiotic recombination is an important process during gametogenesis that contributes to genetic variation. Understanding the process of recombination will lead to enhanced genetic predictions that will promote the sustainability of the sheep industry. It is clear from previous studies that recombination is not random, and at least one recombination event or crossover (CO) per chromosome arm is necessary for proper chromosome segregation. In addition, CO experience location preferences termed "hotspots," as well as interference in that one CO cannot occur in too close proximity with another. Previous studies in sheep have identified loci associated primarily in females with recombination rate derived from linkage data. Our objective was to investigate genetic associations with CO counts in rams acquired cytogenetically. We quantified the number of CO in Suffolk, Icelandic, and Targhee rams using a cytogenetic approach because it allows us to accurately identify all recombination events during meiosis without a large number of offspring. In total, we examined over 165,000 CO events from approximately 2,600 spermatocytes. We identified significant differences in CO number between individual rams within Suffolk, Icelandic, and Targhee breeds ($p < 0.05$), as well as differences between breeds ($p < 0.01$). Using the mean CO counts obtained from the spermatocytes from individual rams as a quantitative phenotype, we performed a genetic association study. The OvineSNP50 BeadChip was used to genotype rams and an association study was performed with PLINK v1.09. The results of the association study identified genomic regions of interest on chromosomes 1, 4, 6, 9, 14, 22, 23, and 24 after a Bonferroni correction ($p < 1E-06$). This study identifies potentially important genomic regions of interest associated with the number of CO in these rams. These data contribute important information towards the understanding of individual and breed recombination differences. Furthermore, this research will advance breed specific selection strategies that support the sustainability of the sheep industry.