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Meiotic Recombination in Ruminant Livestock Species

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Summary

Homologous recombination is an important component of gametogenesis that contributes to genetic variation, and ensures proper chromosome segregation. Despite the importance of this process, we know very little about the factors that control and/or influence global meiotic recombination/crossover (CO) in livestock. Previous research recognizes that a least one CO per chromosome arm is required to ensure proper chromosome segregation. Even though cattle and sheep are different species and beefalo are a cattle hybrid, they have the same number of chromosome arms. This study uses a direct cytological approach to quantify, and characterize the number of COs in beefalo, cattle, and sheep spermatocytes. Here we report that beefalo exhibit on average 5% fewer COs per spermatocyte compared to cattle, and cattle exhibit 28% fewer COs compared to sheep. Further, we examined the number of COs for each homologous chromosome pair in a subset of spermatocytes for each species. We found a positive correlation between the numbers of COs and the length of a chromosome. Overall, sheep exhibited as many as 9 COs per chromosome; whereas the maximum number observed in cattle and beefalo was 5 and 4, respectively. Importantly, when compared to cattle, 11% of beefalo spermatocytes exhibited chromosomes with structural defects and 9% were lacking a CO, both of which lead to improper chromosome segregation, and ultimately apoptosis and reduced fertility. While hybrid species and subspecies crosses have the potential to provide valuable phenotypic traits, understanding chromosomal differences will help resolve breeding difficulties. This research contributes valuable information towards understanding meiotic recombination in livestock, for use in both genetic predictions and selection strategies.

Keywords: meiosis, cattle, sheep, beefalo, crossovers

Introduction

Homologous recombination is an important component of gametogenesis that contributes to genetic variation and ensures proper chromosome segregation. Despite the importance of this process, little is known about the factors that control or influence global meiotic recombination in livestock species. However, it is clear from previous studies in humans and model organisms, that at least one recombination event/CO per chromosome arm is necessary for proper chromosome segregation (Vogt *et al*., 2008, Handel & Schimenti, 2010). Furthermore, inadequate or misplaced COs can lead to improper segregation of homologous chromosomes during meiosis (Hassold *et al.,* 2007).

Meiotic recombination is initiated by the protein SPO11, which establishes double strand breaks (DSB) (Keeney *et al.,* 1997, Boateng, *et al.,* 2013). Subsequently, DSB are resected to yield 3’ overhangs, and strand invasion proteins RAD51 and DMC1 produce double-Holliday junctions (Hunter & Kleckner, 2001). Mismatch repair proteins, MLH1 and MLH3, then localize to the majority of CO events (Edelmann *et al.,* 1996, Lipkin *et al.,* 2002*)*. In order

for a CO to occur, homologous chromosomes must pair and synapse together by a protein complex called the synaptonemal complex (SC), composed of synaptonemal complex proteins 1 and 3 (SYCP1 and SYCP3) (Baudat *et al.,* 2013). The location of COs can exhibit preferences (hotspots), and the presence of one CO can "interfere" with a second CO in close proximity. Histone methyl transferase PRDM9 binds specific DNA motifs and different alleles of PRDM9 exhibit altered binding specificity for recombination hotspots (Baudat *et al.,* 2010, Grey *et al.,* 2011). Additionally, linkage studies in humans have identified an association between number of COs and genetic variation in *RNF212* (Kong *et al.,* 2008, Reynolds *et al.,* 2013).

While these analyses have provided valuable insight into meiotic recombination, only a few studies, to date, have been done in livestock species. Previous studies reported single nucleotide polymorphisms (SNP) in *REC8*, *RNF212* and *PRDM9* associated with COs in cattle (Sandor *et al.,* 2012). A different study utilizing SNP data in Holstein cattle, reported a decrease in male COs over time (Ma *et al.,* 2015). Furthermore, other studies have identified chromosomal regions associated with global CO numbers in mice (Murdoch *et al.,* 2010, Dumont & Payseur) and cattle (Weng *et al.,* 2014). The previous studies in cattle used genome based approaches, which rely on the quality of the reference genome assembly, can only detect COs in genomic regions with genetic variation, and require large pedigrees to provide meaningful statistical comparisons. Additionally, due to independent assortment of chromosomes, this approach only capture approximately half of the CO events that occurred during meiosis. Conversely, the use of cytogenetics, commonly used in humans and model organisms, does not suffer from these constraints. Cytogenetics can identify all meiotic recombination events and detect meiotic, synaptic, and chromosomal defects.

Despite beefalo being a bison /cattle hybrid, and cattle and sheep being different species, they have similar sized genomes, and the same number of chromosome arms. Cattle and beefalo have 29 telocentric (one arm) autosomes and sheep have 23 telocentric and 3 metacentric (two arms) autosomes. In this study, we quantified the number of global CO events and examined the relationship between CO numbers and chromosome length in spermatocytes of beefalo, cattle, and sheep. It is important to note that the mating of two different species can lead to reduced fertility from hybrid crosses. Therefore, we quantified the number of chromosomal defects observed in spermatocytes of beefalo and cattle. Improper chromosome pairing and/or misplaced CO placement during gametogenesis can result in hybrid sterility in males. This research will allow us to better understand the chromosomal properties of speciation, to move towards improved and successful breeding strategies of hybrid livestock. Additionally, these data contribute valuable information towards a greater understanding of meiotic recombination in livestock for use in both genetic predictions and selection strategies.

Materials and Methods

Animals

Testicular tissue samples were collected from sexually mature beefalo (n=7, unknown buffalo and cattle cross), cattle (n=10; 7 Angus, 1 Charolais, 1 Gelbvieh, and 1 Jersey), and sheep (n=27; 5 Icelandic, 17 Suffolk, and 5 Targhee). Beefalo are the hybrid (3/8 bison) resulting from cattle (*Bos taurus*) and bison (*Bison bison)* matings. All of the beefalo, cattle, and sheep samples were acquired post mortem from local abattoirs.

Immunofluorescence staining of testicular samples

Testicles were collected immediately postmortem and transported on ice to the laboratory for surface spread preparation as described in (Murdoch *et al.,* 2010). Briefly, testicular tissue was dissected into approximately one-gram pieces and incubated in a hypotonic buffer (30mM Tris, 50mM sucrose, 17 mM sodium citrate, 5 mM EDTA, 2.5 mM DTT, and 0.5 mM PMSF). Small sections of seminiferous tubule were cut to remove the cells, then mixed with 100mM sucrose, and fixed on slides with 1% paraformaldehyde. Slides were placed in a humid chamber overnight and either stained immediately or stored at -20ºC.

Immunofluorescent staining was performed to identify MLH1 and SYCP3 proteins using a modified protocol outlined previously (Murdoch *et al*., 2010). Slides were blocked in 1% Normal Donkey Serum, 3 mg/mL BSA, 0.05% Triton X-100, PBS for 1 h at 23 ºC. The polyclonal MLH1 antibody (Calbiochem, PC56-100UG) was applied to each slide. Slides were cover slipped, incubated at 37ºC for 12-14 h and washed twice. AlexaFluor 488-conjugated donkey anti-rabbit secondary antibody (Jackson ImmunoResearch, 711-005-152) was placed on the slides and incubated at 37ºC for 4 h. Slides were washed before the polyclonal rabbit anti-human SYCP3 antibody (Abcam, ab15093) was added. The slides were cover slipped and incubated for 2 h at 37ºC then washed twice before Rhodamine donkey anti-rabbit secondary antibody (Santa Cruz Biotechnology, sc-516249) was added. Slides were cover slipped and incubated for 1 h at 37ºC, then washed three times in PBS. Lastly, ProLong® Gold Antifade (Fisher Scientific, P36930) was applied to slides and cover slipped.

Acquisition of data and Analysis

A Leica DM6 B fluorescence microscope with appropriate filters (405, GFP, and Y3 cubes) were used for imaging. Pachytene stage cell images were captured with the use of Leica LASX software version 3.0. Quantification of COs were determined by counting the number of MLH1 foci observed on the SC for each of 100 spermatocytes. MLH1 foci observed on the pseudoautosomal region of the sex chromosomes, were not included. Total defects were quantified using 50 randomly identified pachytene stage spermatocytes per animal. Each cell was evaluated for absence or presences of defect(s). The SC length was measured (µm) for all individual SCs in a subset of 10 spermatocyte per individual. The distance was measured starting at one end of the SC to the first MLH1 focus, then subsequent MLH1 foci, and to the distal end of the SC using ImageJ v1.51 software.

Statistical analysis was performed using a non-parametric test to examine differences in the number of MLH1 foci between species and individuals within each breed (Hassold *et al.,* 2007). RStudio version 3.3.3 was used to run a Kruskal-Wallis with post-hoc Tukey-Kramer test for significant ($P < 0.05$) differences in number of MLH1 foci. To examine potential relationships between chromosome length and number of COs, Spearman's correlation was calculated between the number of MLH1 foci and SC length.

Results and Discussion

Number of crossovers in beefalo, cattle, and sheep spermatocytes

Between strains of mice, recombination numbers differ, however, this has not been evaluated extensively in livestock. We examined COs in meiotic cells from beefalo, cattle, and sheep. In total, 4,480 spermatocytes were examined and the number of MLH1 foci were quantified; 718 spermatocytes were from beefalo (n=7), 1,018 spermatocytes from cattle (n=10), and 2,749 sheep spermatocytes (n=27). Beefalo bulls exhibited significantly fewer COs ($\bar{X}$=42.6±0.70) in comparison to cattle ($\bar{X}$=44.7±0.79) and sheep, ($\bar{X}$=62.5±0.78) which

exhibited the greatest. The global number of COs for each spermatocyte in the three species is shown in *Figure 1.* Despite all three species having the same number of chromosome arms, they exhibit significantly different (P<0.01) global recombination.

To evaluate the variation in meiotic recombination within a species, individual cattle were compared. Of the cattle analyzed, the number of COs did not significantly differ in 6 of the Angus examined. However, bulls of a different breed exhibited a significantly (P<0.01) greater number of COs. The number of COs per spermatocyte was plotted for each individual cattle in *Figure 2.* Charolais, Gelbvieh and Jersey all had similar CO numbers, and 6 Angus were significantly lower (P<0.01). However, it is important to note, that only one bull from each of 3 breeds, Charolais, Gelbvieh, and Jersey and 7 Angus bulls are represented here. Interestingly, similar results were observed when examining breeds of sheep (Murdoch, unpublished). Overall, most Angus bulls, exhibited similar number of COs. Because different breeds may have a different number of COs, our approach may provide valuable insight into breed-specific genetic predictions in livestock.

Characterizing crossover numbers and synaptonemal complex length

To characterize meiotic recombination in livestock spermatocytes, we examined the relationship between SC length, and the number of COs present on that SC. In total 487 spermatocytes were evaluated, 61 spermatocytes from beefalo (n = 7), 92 from cattle (n=10) and 340 from Suffolk sheep (n=17). We examined the correlation between length of individual SC and the number of COs in beefalo (r=0.53), cattle (r=0.57), and sheep (r=0.70) in *Figure 3.* These data suggest that longer SCs have a greater number of COs on them. Confirming the correlation between SC length and the number of COs per SC provides valuable insight for understanding how many COs occur on chromosomes of differing sizes.

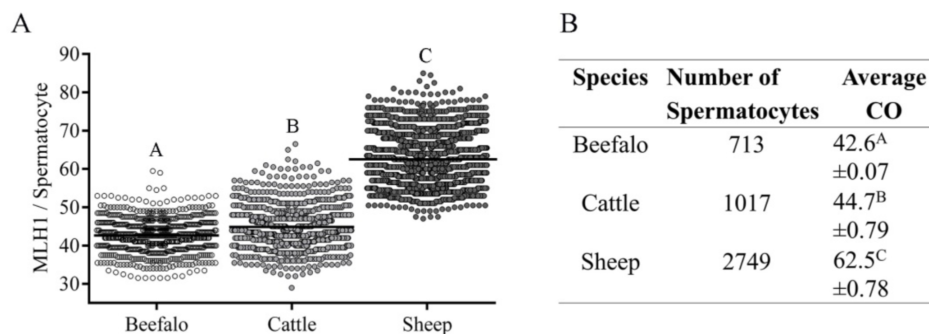
Chromosomal defects in spermatocytes of beefalo and cattle

We observed a number of chromosomal defects in the spermatocytes of beefalo in comparison to cattle. To quantify defects, 350 beefalo spermatocytes and 500 cattle spermatocytes were examined. The defects that were observed included ring chromosomes, missing COs on an SC, and gapping on the SC. On average, beefalo exhibit 10% more defects than cattle in spermatocytes (*Figure 4)*. Ring chromosomes occurred at significantly higher levels in beefalo (P<0.01) than cattle. Representative images of each type of defect can be seen in *Figure 4B, C.* Overall, beefalo exhibited greater numbers of defects in their spermatocytes when compared to cattle. This suggests, spermatocytes of hybrid species crosses can result in chromosomal defects which ultimately affect fertility; an important consideration with other hybrid animals, including *Bos taurus* x *Bos indicus*.

Conclusion

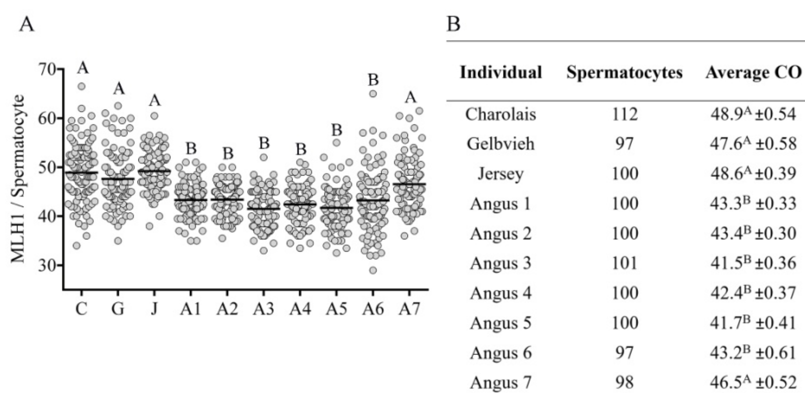
The use of cytogenetics to determine the number of COs has distinct advantages. This approach allows direct visualization of COs and can detect meiotic, synaptic, and chromosomal defects. Additionally, this method is independent of a reference genome, genetic variation and large pedigrees. Our data suggests there is variation between individuals, and the number of COs may differ in different breeds of the same species. Despite similar genome size and the same number of chromosome arms, the number of COs is greater in sheep than in cattle and beefalo. Furthermore, there is a positive correlation between the size of a chromosome and the number of COs observed. Beefalo, hybrid animals, have significantly greater numbers of chromosomal defects when compared to cattle

which may be reflected in other hybrid matings. Overall, this research provides valuable insight, such that we can predict how many COs are likely to occur on different size chromosomes, further enhancing our ability to forecast how genetic material will be recombined.



Species	Number of Spermatocytes	Average CO
Beefalo	713	42.6^{A} ±0.07
Cattle	1017	44.7^{B} ±0.79
Sheep	2749	62.5^{C} ±0.78

Figure 1. Meiotic crossovers in beefalo, cattle, and sheep spermatocytes. A) The number of COs (MLH1) for each spermatocyte from beefalo (n=7), cattle (n=10), and sheep (n=27). Each dot represents the number of COs from individual spermatocyte, black bars represent breed mean, and the letters above denotes significant differences (P<0.01). B) Table of the mean CO number (MLH1) plus and minus the standard error for beefalo, cattle, and sheep.



Individual	Spermatocytes	Average CO
Charolais	112	48.9^{A} ±0.54
Gelbvieh	97	47.6^{A} ±0.58
Jersey	100	48.6^{A} ±0.39
Angus 1	100	43.3^{B} ±0.33
Angus 2	100	43.4^{B} ±0.30
Angus 3	101	41.5^{B} ±0.36
Angus 4	100	42.4^{B} ±0.37
Angus 5	100	41.7^{B} ±0.41
Angus 6	97	43.2^{B} ±0.61
Angus 7	98	46.5^{A} ±0.52

Figure 2. Comparison of crossover per spermatocyte for individual cattle. A) The number of COs (MLH1) for each spermatocyte for individual bulls. Each dot represents the number of COs from individual spermatocyte, black bars represent individual bull mean, and the letters above denotes significant differences (P<0.01). B) The mean number of crossovers (MLH1) plus and minus the standard error for each bull. The Angus bulls are significantly different in comparison to the others.

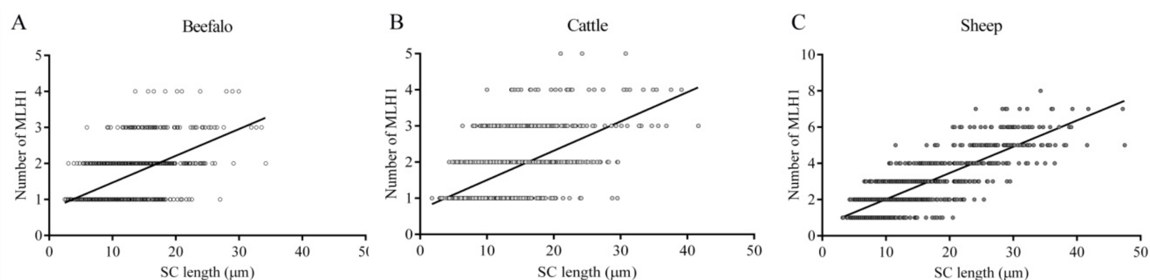
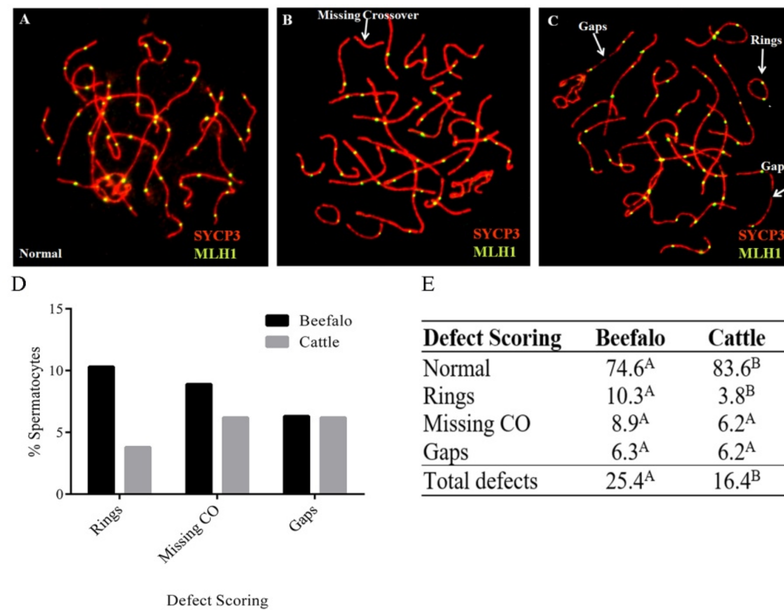


Figure 3. The number of crossovers for each synaptonemal complex from Beefalo, cattle and sheep spermatocytes. Each dot is plotted according to the number of COs (MLH1) and the length of the SC for individual spermatocytes. A) Beefalo (61 cells, r=0.53), B) Cattle (92 cells, r=0.57) and C) Sheep (340 cells, r=0.70) are statistically different (P<0.0001).



Defect Scoring	Beefalo	Cattle
Normal	74.6^{A}	83.6^{B}
Rings	10.3^{A}	3.8^{B}
Missing CO	8.9^{A}	6.2^{A}
Gaps	6.3^{A}	6.2^{A}
Total defects	25.4^{A}	16.4^{B}

Figure 4. Representative images of beefalo spermatocytes scored for defects. A) An image of a normal beefalo spermatocyte with no defects. B) A spermatocyte with a pair of homologous chromosomes, observable by the SC, with no MLH1 (CO). C) A spermatocyte with gaps in the SC, and a ring/fused chromosome. D) The percentage of chromosomal defects (ring/fused, missing MLH1 and gaps) in beefalo (n=7) and cattle (n=10) spermatocytes. E) A table representing the percent of defects observed in beefalo and cattle. The total percentage chromosomal defects in beefalo and cattle spermatocytes are 25.4% and 16.4%, respectively. Letters denote significant differences ($P<0.01$).

References

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

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

Boateng, K.A., Bellani, M.A., Gregoretti, I.V., Pratto, F., & Camerini-Otero, R.D., 2013. Homologous pairing preceding SPO11-mediated double-strand breaks in mice. Developmental cell [24]: 196-205.

Dumont, B.L., & Payseur, B.A., 2011. Genetic analysis of genome-scale recombination rate evolution in house mice. PLoS genetics [7]: e1002116.

Edelmann, W., Cohen, P.E., Kane, M., Lau, K., Morrow, B., Bennett, S., Umar, A., Kunkel, T., Cattoretti, G., Chaganti, R., et al. (1996). Meiotic pachytene arrest in MLH1-deficient mice. Cell 85: 1125-1134.

Grey, C., Barthes, P., Chauveau-Le Friec, G., Langa, F., Baudat, F., & de Massy, B., 2011. Mouse PRDM9 DNA-binding specificity determines sites of histone H3 lysine 4 trimethylation for initiation of meiotic recombination. PLoS biology [9]: e1001176.

Handel M.A., & Schimenti J.C., 2010. Genetics of mammalian meiosis: regulation, dynamics and impact on fertility. Nat. Rev. Genet. [2]: 124-36.

Hassold T, Hall H, & Hunt P., 2007. The origin of human aneuploidy: where we have been, where we are going. Hum. Mo.l Genet. [15]:16.

Hunter, N., &Kleckner, N., 2001. The single-end invasion: an asymmetric intermediate at the

double-strand break to double-holliday junction transition of meiotic recombination. Cell [106]: 59-70.

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

Kong, A., Thorleifsson, G., Stefansson, H., Masson, G., Helgason, A., Gudbjartsson, D.F., Jonsdottir, G.M., Gudjonsson, S.A., Sverrisson, S., Thorlacius, T., et al., 2008. Sequence variants in the RNF212 gene associate with genome-wide recombination rate. Science [319]: 1398-1401.

Lipkin, S.M., Moens, P.B., Wang, V., Lenzi, M., Shanmugarajah, D., Gilgeous, A., Thomas, J., Cheng, J., Touchman, J.W., Green, E.D., et al., 2002. Meiotic arrest and aneuploidy in MLH3-deficient mice. Nature genetics [31]: 385-390.

Ma, L., O'Connell, J.R., VanRaden, P.M., Shen, B., Padhi, A.,Sun, C., Bickhart, D.M., Cole, J.B., Null, D.J., Liu, G.E., Da, Y., & Wiggans, G.R., 2015. Cattle sex-specific recombination and genetic control from a large pedigree analysis. PLoS Genet. [11]:e1005387.

Murdoch, B., Owen, N., Shirley, S., Crumb, S., Broman, K.W., & Hassold, T., 2010. Multiple loci contribute to genome-wide recombination levels in male mice. Mammalian genome : official journal of the International Mammalian Genome Society [21]: 550-555.

Reynolds, A., Qiao, H., Yang, Y., Chen, J.K., Jackson, N., Biswas, K., Holloway, J.K., Baudat, F., de Massy, B., Wang, J., et al., 2013. RNF212 is a dosage-sensitive regulator of crossing-over during mammalian meiosis. Nature Genetics [45]: 269-278.

Sandor, C., Li, W., Coppieters, W., Druet, T., Charlier, C., & Georges, M. 2012. Genetic variants in REC8, RNF212, and PRDM9 influences male recombination in cattle.PLoS Genet. [7]:e1002.

Vogt, P. H., Falcao, C. L., Hanstein, R., & Zimmer, J., 2008. The AZF proteins. International Journal of Andrology, [31]: 383–394.

Weng, Z., Saatch, M.M., Schnabel, R.D., Taylor, J.F., & Garrick, D.J., 2014. Recombination locations and rates in beef cattle assessed from parent-offspring pairs. Genet. Sel. Evo. [29]:46:34.