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Sex ratio distortion in bovine sperm correlates to recombination in the pseudoautosomal region

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Summary

A total of 2122 single sperm from 35 bulls belonging to six different paternal half-sib groups were analysed with respect to two markers in the bovine pseudoautosomal region (PAR) and sex-specific loci on the X and Y chromosomes, respectively. A segregation ratio significantly different from 1:1 was observed in a test over all families, with a higher proportion of X-bearing gametes (53·5%). The analysis of recombination conducted separately for X- and Y-bearing sperm showed that X-bearing sperm cells possess highly significant individual and between-family variability in recombination rate, whereas Y-bearing sperm show linkage homogeneity. To test whether the two phenomena are related, different logistic regression models were fitted to the data. The results show that sex ratio significantly correlates with changes in recombination rate among X-bearing but not among Y-bearing sperm. Different hypotheses to explain these observations are discussed.

1. Introduction

The chromosome sorting during male meiosis is expected to be random so that either member of each homologous pair, including X and Y chromosomes, should end up in the newly formed germ cells in a 1:1 ratio. The following spermatogenesis, however, is a complex developmental process where not all meiotic products necessarily become mature sperm. In a number of species, distortion of Mendelian segregation often referred to as meiotic drive, is found in at least some populations. Meiotic drive of the sex chromosomes is best studied in *Drosophila*, where the sex ratio is often distorted towards an excess of females by increasing the proportion of X-bearing gametes relative to the functional Y-bearing gametes (for review see Lyttle, 1991). If several loci acting on drive are polymorphic in a population, allelic combination inducing the greatest distortion will be selected for, unless fitness is affected (Cazemajor *et al.*, 1997). 'Driving' allele combinations may be created and split up by crossing over between pairs of homologous chromosomes. In humans and many other mammals studied, the most noted sex-specific chiasma is that between the X and Y chromosomes in the pseudoautosomal region (PAR). The gradient of sex linkage is due to the obligatory presence of at least one chiasma formed during each male meiosis, which holds the sex chromosomes together during metaphase I of meiosis, and thus ensures correct disjunction during the first division (Shapiro *et al.*, 1989).

Although the role of the sex chromosomes in mammalian primary sex determination is well known, their function in gametogenesis is less well understood. There is accumulating evidence that the mammalian Y chromosome, in addition to its testis-determining function, contains several genes that are essential for normal sperm development (Burgoyne *et al.*, 1992; Reijo *et al.*, 1995; Blendy *et al.*, 1996), but the biological role of many of these has not been determined. It has also been known for years that correct sex-chromosome pairing is essential for normal sperm development (Miklos, 1974; Burgoyne *et al.*, 1992). Another attribute in mammalian meiosis is the

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genomic imprinting of the sex chromosomes in males, also called meiotic sex chromosome inactivation (MSCI). As suggested by McKee & Handel (1993), MSCI is probably a meiotic adaptation to prevent the initiation of potentially damaging recombination events in non-homologous regions of the X and Y chromosomes. Since male meiosis in mammals is characterized by both sex chromosome inactivation and chromosome pairing in the human PAR, there may very well be a correlation between the two phenomena (Handel & Hunt, 1992).

While studies in yeast and *Drosophila* species have also yielded remarkable advances in understanding how meiotic recombination occurs in these organisms (Roeder, 1995), less information is available in mammals. As a large number of offspring is necessary to estimate individual recombination rates with sufficient accuracy, pedigree size is the main limiting factor in studies of humans and animals with low reproductive rate. However, due to an almost unlimited number of sperm (i.e. meioses) available from any male, single sperm typing (Li *et al*., 1988) offers a unique opportunity to overcome this problem, and a recent sperm typing experiment showed evidence for individual and between-family variability of the recombination rate in the bovine PAR (Simianer *et al*., 1997). In the current work, the same data set was analysed with respect to sex chromosome ratio among sperm and its possible correlation with recombination rates in the bovine PAR.

2. Material and methods

(i) *Data*

In the study by Simianer *et al*. (1997) a total of 2214 sperm cells (approximately 60 sperm per bull) were genotyped. The material was organized according to a design consisting of 37 bulls belonging to six different paternal half-sib families (35 sons and 2 sires), which allowed the detection of between-family variability of the recombination rate as shown for one marker interval in the PAR. For more details about the structure of the data, sperm isolation protocol and genotyping see Simianer *et al*. (1997). For the purpose of the current study only microsatellite markers *MAF45* and *TGLA325* in the bovine PAR (Barendse *et al*., 1994), and the sex-specific loci *ZFX* and *ZFY* (Kirkpatrick & Monson, 1993) from 35 sons were analysed. Since crossing over on the sex chromosomes occurs only in the PAR, the *ZFX*/*ZFY* loci marked the boundary of the PAR (PA boundary).

(ii) *Imputation of missing data*

For a certain number of isolated sperm it was impossible to identify whether they bore the X or Y homologue. The total number of X- and Y-bearing sperm and their recombination status accounting for the incomplete data fraction were imputed based on the information from *TGLA325*, following the approach proposed by Little & Rubin (1987):

$$\hat{\pi}_{ijk} = \hat{p}_{ik}\hat{p}_{ijk},$$

where

$\hat{\pi}_{ijk}$ is the proportion of haplotype *ij* estimated for the *k*th bull,

$\hat{p}_{ik} = \dfrac{n^{\mathrm{C}}_{ik} + n^{\mathrm{I}}_{ik}}{\sum_{i=1}^{2}(n^{\mathrm{C}}_{ik} + n^{\mathrm{I}}_{ik})}$ is the frequency of allele *i* at *TGLA325* estimated for the *k*th bull,

$\hat{p}_{ijk} = \dfrac{n^{\mathrm{C}}_{ijk}}{\sum_{j=1}^{2} n^{\mathrm{C}}_{ijk}}$ is the proportion of a *TGLA325*-*ZFX*/*ZFY* haplotype *ij* estimated for the *k*th bull from the complete data fraction.

n are appropriate allele or haplotype counts within complete (superscript 'C') and incomplete (superscript 'I') data fractions.

(iii) *Testing sex ratio distortion*

To test whether the counts of X- and Y-bearing sperm observed in the data follow the expected 1:1 segregation, Fisher's exact test was performed. The null hypothesis was:

$$H_0: \pi_{\mathrm{X}} = \pi_{\mathrm{Y}} = 0{\cdot}5,$$

where

$\pi_{\mathrm{X}} = \dfrac{n_{\mathrm{X}}}{n_{\mathrm{X}} + n_{\mathrm{Y}}}$, $\pi_{\mathrm{Y}} = \dfrac{n_{\mathrm{Y}}}{n_{\mathrm{X}} + n_{\mathrm{Y}}}$ and $n_{\mathrm{X}}, n_{\mathrm{Y}}$ are counts of X- and Y-bearing sperm respectively.

Using the likelihood ratio test:

$$\lambda = 2\ln\frac{L(\pi_{\mathrm{X}} = 0{\cdot}5)}{L[\hat{\pi}_{\mathrm{X}} = MLE(\pi_{\mathrm{X}})]}$$

as a test statistic and assuming that the binomial distribution describes the probability of observing a given constellation of n_{X} and n_{Y}:

$$P(n_{\mathrm{X}} \mid n_{\mathrm{X}} + n_{\mathrm{Y}}, \pi_{\mathrm{X}}) = \binom{n_{\mathrm{X}} + n_{\mathrm{Y}}}{n_{\mathrm{X}}} \pi_{\mathrm{X}}^{n_{\mathrm{X}}} (1 - \pi_{\mathrm{X}})^{n_{\mathrm{Y}}}$$

it is possible to obtain an exact probability of type I error (α) calculated by cumulating probabilities for all outcomes of the data set of size $n_{\mathrm{X}} + n_{\mathrm{Y}}$ under the null hypothesis which fulfil the condition:

$$\lambda_i \geqslant \lambda_D,$$

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where

λ_i is the value of the test statistic for the ith constellation of n_X and n_Y.

λ_D is the value of the test statistic for the observed data.

(iv) *Testing the X- and Y-sperm specific variability of recombination*

Individual and between-family variability of recombination was tested separately for X- and Y-bearing sperm. For testing individual variability, available gametes were partitioned between individual counts of informative meioses (N_{xi}, N_{yi}, respectively, for X- and Y-bearing sperm of the ith bull) and of recombinant meioses (K_{xi}, K_{yi}). To test between-family differences, all 35 sons were assigned to one of six half-sib families, so that subscript i by the numbers of recombinants and informative meioses denotes the ith half-sib family.

To test the variability of recombination the Morton test (Morton, 1956) was used. This statistic has already been described by Simianer *et al.* (1997). Additionally, other tests formulated in a Bayesian framework (Potthoff & Whittinghill, 1966; Risch, 1988) were also applied to this data. As their outcome did not differ markedly from the Morton test these results are not shown here but are available from the authors on request. More reliable empirical threshold values for the Morton test were derived from 10000 permutations of the original data (Churchill & Doerge, 1994).

(v) *Haplotype determination*

Differences in haplotypes at the *MAF45–TGLA325–ZFY* loci are one of the possible sources affecting the frequency of recombination and the sex chromosome ratio. The most likely haplotypes for each of 35 bulls were determined separately at both available intervals using:

$$P(Ph1) = \frac{P(\theta \mid K, N)}{P(\theta \mid K, N) + P(\theta \mid N-K, N)},$$

$$P(Ph2) = \frac{P(\theta \mid N-K, N)}{P(\theta \mid K, N) + P(\theta \mid N-K, N)}$$

and

$\theta \in [0, 0{\cdot}5]$,

where

$P(Ph)$ is the probability of a given haplotype phase,

$$P(\theta \mid N1, N1+N2) = \binom{N1+N2}{N1} \theta^{N1}(1-\theta)^{N2}, \quad \text{for}$$

$N1, N2 \in \{K, N-K\}$,

N is the total count of sperm haplotypes available for a bull in a given interval,

$K, N-K$ are the numbers of both possible kinds of sperm haplotypes in this interval.

(vi) *Logistic regression modelling*

In order to identify how the numbers of X- and Y-bearing gametes produced depend on recombination rate, models based on the logistic regression transformation of the individual proportions of X-bearing sperm among the total number of sperm were fitted to the data. Considered effects are:

Y-sperm haplotype at *MAF45–TGLA325–ZFY* loci,
sire,
recombination rate calculated in Y-bearing sperm, X-bearing sperm, or in X and Y sperm pooled.

Models were fitted to the data using the SAS package (SAS, 1989–96). For comparison between nested models the difference in their deviance was used as a test statistic. This is equivalent to the likelihood ratio test for these models:

$$\lambda = D_1 - D_2 = -2\ln\left(\frac{ML(m_1)}{ML(m_2)}\right) \sim \chi^2_{df(m_1)-df(m_2)},$$

where

$D_i = -2\ln\left(\frac{ML(m_i)}{ML(m_f)}\right)$ is the deviance for model i,

$ML(m_i)$ is the maximum value of the likelihood function under model i,

subscripts: 1, 2, f denote, respectively, more parsimonious, less parsimonious, and the full model.

The Akaike information criterion, AIC (Akaike, 1970) can be used in addition to compare non-nested models: $\mathrm{AIC} = -2\ln[ML(m_i)] + 2p$, where p is the number of parameters in model i. This statistic provides no formal test and has no asymptotic distribution, but is a quantity which summarizes the models' fit and parsimony.

3. Results

Significant distortion in the ratio of X- to Y-bearing sperm was observed in a test over all families, with higher proportion of X-bearing (53·5%) than Y-

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Table 1. *Proportion of X- and Y-bearing sperm*

Family	*N*	*ZFX*	X-sperm	*ZFY*	Y-sperm	% X	*P*
1	363	192	210	122	146	59·0	0·0008
2	368	142	158	122	143	52·5	0·420
3	314	150	161	113	140	53·5	0·249
4	362	85	94	74	86	52·2	0·602
5	355	132	139	133	143	49·3	0·858
6	360	87	90	73	82	52·3	0·594
Total	2122	788	852	637	740	53·5	0·005

Number of isolated sperm samples (*N*), observed number of X-bearing sperm (*ZFX*) and Y-bearing sperm (*ZFY*), estimated total number of X-bearing sperm (X-sperm) and Y-bearing sperm (Y-sperm), percentage of X-bearing sperm (% X), and type I error probabilities for testing for equal numbers of X- and Y-bearing sperm (*P*), analysed separately in six paternal families and over all individuals.

Table 2. *Variability of recombination rate in X- and Y-bearing sperm*

	X-bearing sperm					Y-bearing sperm				
Interval	N_X	θ_X	Z_X	P_{XI}	P_{XF}	N_Y	θ_Y	Z_Y	P_{YI}	P_{YF}
MAF45–TGLA325	757	0·028	186·2	0·037	0·226	637	0·047	139·2	0·402	0·642
TGLA325–PA boundary	903	0·076	166·0	0·014	0·0004	822	0·043	184·6	0·818	0·415

Number of informative X-bearing (N_X) and Y-bearing (N_Y) sperm, maximum likelihood estimates for the recombination rate in X-bearing (θ_X) and Y-bearing (θ_Y) sperm, LOD scores (Z_X) and (Z_Y), error probabilities for the test on individual (P_{XI}) and between-family (P_{XF}) variability of the recombination rate in X-bearing sperm, and error probabilities for the test on individual (P_{YI}) and between-family (P_{YF}) variability of the recombination rate in Y-bearing sperm for the two marker intervals studied.

bearing (46·5 %) gametes. Estimated proportions are given in Table 1. When testing one family at a time, only family 1 showed a significantly higher ratio of X-bearing sperm (59·0 %). The proportion of X-bearing sperm in the six families ranged between 49·3 % and 59·0 %. In total, 19 of 35 individuals analysed had an excess of X-bearing sperm. The most significant departure from a 1 : 1 ratio was found on an individual level, with the three most extreme bulls exhibiting proportions of X-bearing sperm of 73·3 %, 71·9 % and 70·7 %. Two of these three bulls are half-brothers belonging to family 1 and one comes from family 3.

The recombination rates for marker intervals *MAF45–TGLA325* and *TGLA325*–PA boundary were estimated separately for X- and Y-bearing sperm (Table 2). The data were fully sufficient to assume the gametic phase known for each individual. Significant individual variability was detected in the interval *MAF45–TGLA325* and the *TGLA325*–PA boundary, and highly significant between-family variability in recombination rate was detected in the interval *TGLA325*–PA boundary among X-bearing sperm (Table 2). In contrast, no significant variability was found among Y-bearing sperm. As can be seen in Fig. 1, family 1 shows a much higher recombination rate in X-bearing ($0{\cdot}116 \pm 0{\cdot}041$) than Y-bearing ($0{\cdot}028 \pm 0{\cdot}025$) sperm for the interval *TGLA325*–PA boundary. The same tendency was also observed for family 3.

Thanks to the large number of gametes available for each bull it was possible to determine their haplotypes at *MAF45–TGLA325–ZFY* with a very high degree of certainty ($P < 0{\cdot}0001$). Five different haplotypes were identified, but their distribution among bulls was not uniform, with one predominant haplotype occurring in 19 of 27 available cases (70·4 %). This fact makes it impossible to differentiate half-sib families on the basis of their paternal haplotypes.

The results on fitting different logistic models show that changes in the proportion of X-bearing sperm follow the changes in recombination rate, but not in the Y-specific one. This correlation seems to be much more profound for X- than for Y-bearing sperm as well as for the interval *TGLA325*–PA boundary than for *MAF45–TGLA325*. Analysis of the influence of additional effects of the *MAF45–TGLA325–ZFY* haplotype and sire showed no significant evidence for their influence. The best of all fitted models was the one containing the effect of recombination rate at the

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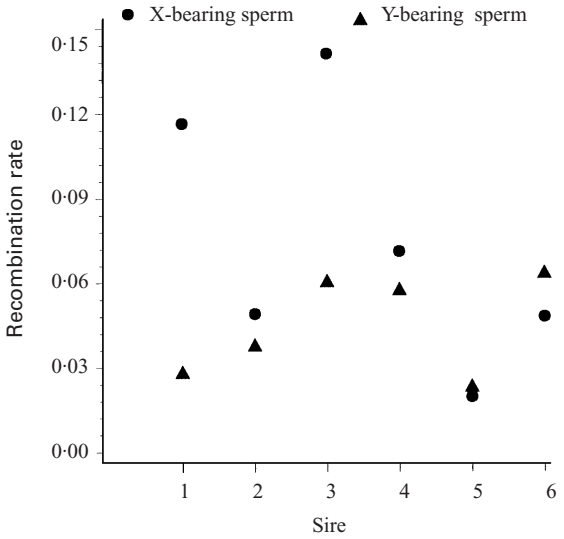

Fig. 1. Estimated mean recombination rates in X- and Y-bearing sperm for the marker interval *TGLA325*–PA boundary for each sire family.

interval *TGLA325*–PA boundary averaged over X- and Y-bearing sperm. This model had the lowest AIC and exhibited significantly better fit than the most parsimonious model assuming a uniform proportion of X-bearing sperm ($P = 0{\cdot}005$). Detailed results on model comparison are given in the Appendix (Table A1).

4. Discussion

Both the variability of recombination rate and distortions of the sex chromosome ratio have been well documented, particularly in populations of experimental species (see, for example, Mercot *et al.*, 1995; True *et al.*, 1996). Recently the availability of the single sperm typing technique (Li *et al.*, 1988) enabled the detection of variability of male recombination rates in humans (Yu *et al.*, 1996) and cattle (Park *et al.*, 1995). Moreover, results from several large studies in humans, analysing hundreds of thousands of sperm by a three-colour fluorescence *in situ* hybridization (FISH) technique, also show a higher proportion of X-bearing sperm (Chevret *et al.*, 1995; Martin *et al.*, 1995; Spriggs *et al.*, 1996), which, for cattle, is confirmed by the results presented in our study (Table 1). Two key observations specify the sex-chromosome distortion detected among bovine sperm: (1) the excess of X-bearing sperm was found in the majority of bulls, and (2) (of three) individuals with especially high distortion belong to the same family.

Recently, Simianer *et al.* (1997) detected individual and between-family variability of the recombination rate in the bovine PAR. When using the same material but taking the 'sex' of the sperm into account, almost all this variability can be attributed to the X-bearing sperm, while recombination in the Y-bearing sperm appears to be homogeneous (Table 2). When looking more precisely at the estimates of recombination rate for the interval *TGLA325*–PA boundary, we found that most of the between-family variability among X-bearing sperm can be attributed to families 1, 3 and 5. The comparison between X- and Y-bearing sperm reveals a considerable difference in the recombination rates for families 1 and 3. Strikingly different from these observations are the results for family 5, which displays the lowest recombination rate in both sperm types (Fig. 1).

A priori, the total number of X- and Y-bearing sperm together with the numbers of both types of recombined sperm are equal; thus our findings suggest that there may be a mechanism relating sperm survival to the recombination between X- and Y-bearing homologues during gametogenesis. This hypothesis is supported by two observations. Family 1, which has the highest proportion of X-bearing sperm (Table 1), also shows the largest difference in recombination rate between X- and Y-bearing sperm (Fig. 1). In contrast, family 5, which produces a lower proportion of X-bearing sperm, also has the lowest recombination rate for both sperm types.

Different hypotheses may be used to explain the findings in our study. The most straightforward one may be that recombination can produce a certain combination of alleles that are detrimental to Y-sperm viability or preferential to X-sperm viability. The overall linkage homogeneity among Y-sperm (Table 2), and the fact that Y is the only chromosome part consistently shared by paternal half-brothers, certainly suggest that it is the Y-recombinant products that are being lost at some stage during spermatogenesis.

An alternative explanation of our results relies on the fact that, although most of the sex chromosomes are thought to become transcriptionally inactive as they form the heterochromatic sex body during meiotic prophase (McKee & Handel, 1993), studies have revealed specific expression of genes from these chromosomes in spermatids (Shannon & Handel, 1993; Hendriksen *et al.*, 1995; Moss *et al.*, 1997). As a consequence, there may well be X-specific genes located close to the bovine PAR that are expressed in spermatids and affect the viability of the sperm. If their expression depends on the level of MSCI, this could also correlate with recombination rates in the PAR, as observed in our data. Suggestive support for this theory is the observation that the PAR region has a more open chromatin configuration than the sex-specific parts that do not recombine, and transcription from this region has been reported throughout spermatogenesis (Das & Raman, 1994).

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Appendix

Table A1. *Logistic regression models for the individual proportion of X-bearing sperm*

					Comparison of models		
	Model	d.f.	DEV	AIC	Models	Difference in DEV	*P*
1	logit (XPR) = $\mu+\theta_1+\theta_2$	24	35·84	2202	1 vs 4	9·38	0·009
2	logit (XPR) = $\mu+\theta_{X1}+\theta_{X2}$	24	37·79	2204	2 vs 4	7·43	0·024
3	logit (XPR) = $\mu+\theta_{Y1}+\theta_{Y2}$	24	44·64	2210	3 vs 4	0·58	0·750
1a	logit (XPR) = $\mu+\theta_1$	25	44·35	2208	1a vs 4	0·87	0·352
					1a vs 1	8·51	0·004
1b	logit (XPR) = $\mu+\theta_2$	25	37·29	2201	1b vs 4	7·93	0·005
					1b vs 1	1·45	0·229
2a	logit (XPR) = $\mu+\theta_{X1}$	25	43·69	2207	2a vs 4	1·53	0·217
					2a vs 2	5·90	0·015
2b	logit (XPR) = $\mu+\theta_{X2}$	25	38·57	2202	2b vs 4	6·65	0·010
					2b vs 2	0·78	0·377
4	logit (XPR) = μ	26	45·22	2207	—	—	—

Individual proportion of X-bearing sperm (XPR), individual recombination rates in X- and Y-bearing sperm pooled for intervals *MAF45–TGLA325* (θ_1) and *TGLA325*–PA boundary (θ_2), individual recombination rates in X-bearing sperm for intervals *MAF45–TGLA325* (θ_{X1}) and *TGLA325*–PA boundary (θ_{X2}), individual recombination rates in Y-bearing sperm for intervals *MAF45–TGLA325* (θ_{Y1}) and *TGLA325*–PA boundary (θ_{Y2}), number of degrees of freedom (df), deviance (DEV), Akaike information criterion (AIC), and type I error probabilities for testing differences in fit between models (*P*) (not adjusted for multiple testing).

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