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ORIGINAL ARTICLE

Prevalence of sex-chromosome aneuploidy estimated using SNP genotype intensity information in a large population of juvenile dairy and beef cattle

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Abstract

Aneuploidy is a genetic condition characterized by the loss or gain of one or more chromosomes. Aneuploidy affecting the sex chromosomes can lead to infertility in otherwise externally phenotypically normal cattle. Early identification of cattle with sex chromosomal aneuploidy is important to minimize the costs associated with rearing infertile cattle and futile breeding attempts. As most livestock breeding programs routinely genotype their breeding populations using single nucleotide polymorphism (SNP) arrays, this study aimed to assess the feasibility of integrating an aneuploidy screening tool into the existing pipelines that handle dense SNP genotype data. A further objective was to estimate the prevalence of sex chromosome aneuploidy in a population of 146,431 juvenile cattle using available genotype intensity data. Three genotype intensity statistics were used: the LogR Ratio (LRR), *R*-value (the sum of X and Y SNP probe intensities), and B-allele frequency (BAF) measurements. Within the female-verified population of 124,958 individuals, the estimated prevalence rate was 0.0048% for XO, 0.0350% for XXX, and 0.0004% for XXY. The prevalence of XXY in the male-verified population was 0.0870% (i.e., 18 out of 20,670 males). Cytogenetic testing was used to verify 2 of the XXX females who were still alive. The proposed approach can be readily integrated into existing genomic pipelines, serving as an efficient, large-scale screening tool for aneuploidy. Its implementation could enable the early identification of infertile animals with sex-chromosome aneuploidy.

KEYWORDS

Illumina, monosomy, probe intensity, single nucleotide polymorphism, trisomy

1 | INTRODUCTION

Aneuploidy is a genetic condition characterized by the deletion (i.e., monosomy) or duplication of a (i.e., trisomy) chromosome (Hassold & Hunt, 2001). Cattle with sex chromosome aneuploidy, which generally occurs on the X-chromosome, are more likely to survive than cattle with autosomal aneuploidy (Iannuzzi et al., 2021). However, cattle and other species including river buffalo sheep and humans with sex chromosomal aneuploidy are typically infertile (Iannuzzi et al., 2021; Raudsepp & Chowdhary, 2016).

Early identification of such cattle with sex chromosomal aneuploidy is important to minimize the monetary cost associated with rearing infertile cattle with the intention of breeding, as well as the futile attempts of serving the animal. The purchase of potential infertile bulls can also have serious long-term monetary repercussions. Compounding the issue of infertility is the fact that the majority of female cattle with X-chromosome aneuploidy remain undetected until breeding as their external characteristics are generally phenotypically normal (Gustavsson & Johansson, 1980; Iannuzzi et al., 2021; Szczerbal & Switonski, 2016). However, female cattle with X-chromosome aneuploidy generally have underdeveloped internal genitalia (Berry et al., 2017; Norberg et al., 1976) comparable to those of prepubertal heifers with normal XX karyotypes (Pinheiro et al., 1987).

Furthermore, cattle with the XXY karyotype also known as Klinefelter syndrome, often have issues with the development of their internal sex organs especially the seminiferous tubules in males as well as issues with their somatic development (Burgoyne et al., 2002). Testicular hypoplasia, oligospermia (i.e., low sperm count) and even azoospermia (i.e., absence of sperm in the ejaculate) are commonly observed in male cattle with the XXY karyotype (Iannuzzi et al., 2021; Slota et al., 2003; Szczerbal & Switonski, 2016) leading to sterility (Eldridge, 1985).

Cattle are tested for aneuploidy in many countries (Ducos et al., 2008) to ensure they have a normal karyotype and can reproduce successfully. The testing is undertaken using cytogenetic techniques like karyotyping, fluorescence in situ hybridization (FISH) and comparative genomic hybridization (CGH) (Martin & Warburton, 2015). However, these methods are laborious and expensive and typically not applied on a large scale, except in (some) high-value animals or when individuals show suboptimal fertility. Berry et al. (2017) proposed using routinely available SNP-array genotype intensity data to screen for monosomy in cattle at no additional cost to the breeder or producer. Such an approach also proved successful at detecting aneuploidy in cattle, chicken and fish embryos (Bouwman et al., 2023) as well as in sheep (Berry et al., 2018) and humans (Treff et al., 2010; Tuke et al., 2017; Xiong et al., 2014). Most livestock breeding programs routinely genotype (a proportion of) their breeding populations with SNP arrays at a young age. Therefore, SNP data could potentially be routinely used for automated screening for aneuploidy in large populations, enabling producers to make more informed decisions about the fate of individual animals at an early stage of their lives without incurring additional cytogenic costs. The objective of the present study was to explore the feasibility of integrating a sex-chromosomal aneuploidy screening tool into pipelines handling the now routinely generated dense SNP genotype data. A further objective was to quantify the prevalence of sex chromosomal aneuploidy in a large population of 146,431 juvenile cattle genotyped using SNP arrays.

2 | MATERIALS AND METHODS

2.1 | Genotype data

SNP genotype intensity data from the International Dairy and Beef Version 3 custom Illumina Beadchip (Illumina, 2011) were available from 146,431 dairy and beef cattle. Of these 27,804 animals were purebred and the remaining 118,626 were crossbred beef cattle; crossbred cattle predominate in Ireland. Of the purebreds, 27% were Holstein-Friesian while 69% were beef breeds. Of the purebred beef population, 95% were either Angus Charolais Hereford Limousin Salers Shorthorns or Simmental cattle. All animals were under 15 months at the time of genotyping, had a call rate $\geq$ 90% and had sex recorded by the producer. The custom genotype panel includes a total of 52,691 SNPs. To improve the accuracy of sex prediction from individual animal genotypes, SNP quality control edits were implemented prior to the sex prediction process. Only the 287 X-chromosome SNPs recorded to be located on the non-pseudoautosomal (nPAR) region, had a call rate $\geq$ 90% across all animals, and displayed <5% heterozygosity in recorded males were retained. Y-chromosome SNPs that exhibited a call rate < 90% in recorded males were omitted, leaving just 5 Y-chromosome SNPs.

Sex was predicted based on decision rules that considered both the percentage of the called Y-chromosome SNPs, along with the percentage of nPAR X-chromosome SNPs reported to have a heterozygous (BAF); the BAF is the ratio of measured intensities from each of the two alleles, indicating the relative quantity of the B allele compared to the A allele (Attiyeh et al., 2009). In a diploid genome, the genotypes AA AB and BB correspond to mean BAF values of 0, 0.5 and 1, respectively (Illumina, 2017). For producer-recorded female heterozygous X-chromosome

AB genotypes, the mean BAF was 0.51 with a standard deviation of 0.049. As a result, the anticipated heterozygous X-chromosome BAF was expected to be in the range of 0.45–0.55. In contrast, a haploid genome has no heterozygous AB genotype, resulting in only two possible BAF values around 0 (A) or 1 (B) (Illumina, 2017). Consequently, XX females are expected to exhibit X-chromosome SNPs within the heterozygous BAF range of 0.45–0.55, consistent with a diploid genome, while XY males should not. The distribution of the percentage of X-chromosome SNPs in the BAF range of 0.45–0.55 in the producer-recorded male and female population was analysed, and 2% was the lowest point of the frequency distribution where a clear distinction between the two clusters of the data existed (Figure 1). Animal sex was therefore predicted to be female if the animal had >2% of X-chromosome SNPs in the heterozygous BAF range of 0.45–0.55, as well as having none of the 5 Y-chromosome SNPs called. Animals with ≤2% of X-chromosome SNPs in the BAF range of 0.45–0.55 and with ≥80% (i.e., at least 4 of the available 5) of the Y-chromosome SNPs called were classified as male. Of the 146,431 animals in the dataset, this approach defined 20,670 as male, with a further 124,809 defined as female, regardless of the producer-recorded sex. A total of 952 animals remained unclassified of which 706 had only 20–60% of the Y-chromosome SNPs called and were therefore deemed unclassifiable given the small number of SNPs on the Y chromosome; these animals were removed from the analysis. A further 246 ambiguous animals that had either 0% or ≥80% of the Y-chromosome SNPs (i.e., 0 or ≥4 SNPs of the available 5) called were retained for exploration.

2.2 | Genotype intensity data

Three genotype intensity metrics were considered in the present study. The first was the *R*-value, which is the sum of the raw signal intensity channels of fluorescent dyes used to call the A and B alleles of Illumina genotypes (Staaf et al., 2008). The second genotype intensity metric considered was the logarithm normalized R ratios (LRR), which is the logarithm to the base 2 of the ratio of the observed *R*-value to the expected *R*-value relative to a reference sample (Peiffer et al., 2006). An LRR value of zero indicates a neutral copy number, while positive values suggest copy number gain and negative values indicate copy number loss (Hashem et al., 2016). The expected mean LRR for a normal genome is 0 (Illumina, 2017). The BAF was the third genotype intensity metric considered.

In a normal genome, a BAF value of 0 or 1 indicates a homozygous genotype (AA and BB, respectively), whereas a value of 0.5 indicates a heterozygous genotype (AB). Where a (chromosomal) duplication exists, two heterozygous BAF bands generally appear around the values of 0.33 (AAB) and (or) 0.67 (ABB), along with the homozygous BAF bands at 0 (AAA) and 1 (BBB) (Illumina, 2017). The LRR and *R*-values for duplication will be higher than their respective values on a diploid chromosome. In the

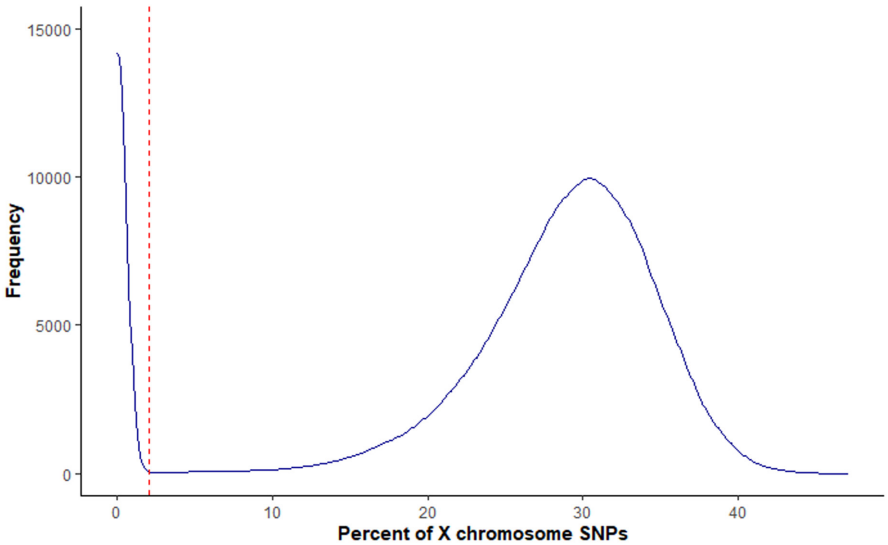

FIGURE 1 The percent of X-chromosome single nucleotide polymorphisms (SNPs) in the expected heterozygous B-allele frequency (BAF) range of 0.45–0.55 for a diploid genome in the male and female population. XX females are expected to have SNPs in this heterozygous BAF range, whereas XY, XO and XXX animals are not. Based on the observed distribution, animals with ≤2% of X-chromosome SNPs within the BAF range of 0.45–0.55, denoted by the vertical red line, were identified as having a missing heterozygous BAF band at 0.5. Consequently, these animals may potentially be classified as XO, XXY, XXX, or inbred XX, depending on their genotype intensity metrics and the number of Y-chromosome SNPs called.

case of a (chromosomal) deletion, the only two possible BAF bands occur at 0 (A) and 1 (B), along with lower LRR and *R*-values (Illumina, 2010). In the case of full chromosomal duplications and deletions, SNPs generally do not exhibit a BAF in the range of 0.45–0.55, making it a distinguishing characteristic for identifying aneuploidy.

2.3 | Detecting aneuploidy

Potential cases of monosomy and trisomy were detected in the present study based on the principles proposed by Berry et al. (2017) which used the LRR and *R*-values of SNP genotypes, but this approach was expanded to also take the BAF into consideration, as per Silvestri et al. (2021) and Tuke et al. (2017). There was no overlap between animals used in the study of Berry et al. (2017) and those used in the present study. For the 124,809 animals classified as female based on the previously described decision rules, the mean LRR and the mean *R*-value of the SNPs on the X chromosome for each animal were expressed in standard deviation units relative to the average and standard deviation of the LRR and *R*-values of the SNPs per chromosome for the respective autosomes for that individual. The terms 'standardized LRR' and 'standardized *R*-values' will be used henceforth to refer to these calculated statistics. The mean and standard deviation of standardized LRR and *R*-values on the X chromosome were calculated within the female verified population. For the 246 animals where their sex had not been classified as per the previously described decision rules, standardized LRR and *R*-values were calculated for each animal, along with the percentage of SNPs on the X-chromosome with a BAF of 0.45–0.55, and the percentage of genotypes called on the Y-chromosome. Animals with Turner syndrome (XO), X-chromosome trisomy (XXX), or Klinefelter (XXY) were classified based on these approaches as outlined in Table 1. The LRR and BAF for all animals diagnosed as having XO, XXX, or XXY karyotypes were visualized using Manhattan plots of the entire genome, as per Silvestri et al. (2021) and Tuke et al. (2017), and compared to the Manhattan plots of animals classified as male and female.

Where possible, all animals classified as having X-chromosome aneuploidy were parentage verified. Where both parental genotypes were available, X-chromosome SNPs where the parents shared no common alleles were investigated in order to determine the parent of origin of the extra X-chromosome in the case of XXX and XXY animals; the approach was also used to deduce the inheritance of the single X chromosome in the case of XO animals. Subsequently, for each X-chromosome SNP without shared alleles between the sire and dam, we compared the genotype of the progeny to that of the sire and dam,

TABLE 1 Diagnostic criteria used for cattle with XO, XXX or XXY karyotypes based on X-chromosome (X-chr) log R ratio (LRR), *R*-value, percentage of single nucleotide polymorphisms (SNPs) with X-chromosomal B-allele frequency (BAF) between 0.45 and 0.55, and percentage of called SNPs on the Y chromosome.

Aneuploidy type	Standardised LRR from female mean standardised LRR	Standardised *R*-value from female mean standardised *R*-value	% X-chr SNPs BAF 0.45–0.55	% Y-SNPs called
XO	<3 SD	<3 SD	≤2%	0%
XXX	>3 SD	>3 SD	≤2%	0%
XXY	NA	NA	>2%	≥80%

Abbreviation: SD, standard deviation.

indicating which parent contributed the extra (or only) allele at that specific position. If the same parent contributed the additional (or sole) copy allele to the entire chromosome, it was established that the extra or single X-chromosome originated from that specific parent.

2.4 | Cytogenetic analysis

Cytogenetic analysis was conducted following the methodology of Berry et al. (2017) at the University of Kent on two animals that were identified as having XXX trisomy and were still alive. All XO and XXY animals identified were dead at the time of analysis. Blood samples were collected from the coccygeal vessels into 10 mL lithium heparin evacuated tubes (BD Vacutainer, LH 102 I.U.; BD, Plymouth, UK). To prepare the blood samples for karyotype analysis, heparinised blood was cultured in PB MAX Karyotyping medium (Gibco, Grand Island, NY) at 37°C and 5% CO_2 for 72 h. Cell division was stopped by adding colcemid (Gibco) at a concentration of 10.0 μg/mL for 30 min, followed by hypotonic treatment using 75 Mm potassium chloride and fixation on glass slides using a mixture of methanol and acetic acid in a 3:1 ratio. Metaphases for karyotyping were stained with 4′,6-diamidino-2-phenylindole in VECTASHIELD antifade medium (Vector Laboratories, Burlingame, CA). Image capturing was carried out using an Olympus BX61 epifluorescence microscope equipped with a cooled charge-coupled device camera and the SmartCapture software (Digital Scientific, Cambridge, UK) for a total of 20 metaphases per sample. Karyotyping was performed in at least 10 of 20 metaphases captured per sample with the help of SmartType software (Digital Scientific) and the chromosomes were organized following the International System for Chromosome Nomenclature of Domestic Bovids (2001).

3 | RESULTS AND DISCUSSION

The BAF, along with genotype intensity metrics of the LRR or *R*-values, are commonly used to detect aneuploidy or copy number variations from SNP genotype data (Hou et al., 2012). In the population of 145,725 cattle used in the present study, the BAF, LRR, and *R*-value statistics were used to detect 6 XO, 42 XXX, and 19 XXY karyotypes (Table 2). The whole-genome BAF and LRR Manhattan plots of one exemplar animal from each possible karyotype group (i.e., XX, XY, XO, XXX, and XXY) are shown in Figure 2. For XX and XXY animals, there are three clear clusters of BAF on the X chromosome at 0, 0.5 and 1; these correspond to the X chromosome genotypes AA, AB and BB. XO and XY animals, on the contrary, had all SNPs called homozygous on their X chromosome, meaning they only had two BAF clusters on their X chromosomes, one around 0 (A genotype) and the other around 1 (B genotype). The BAF plot for XXX animals revealed four clusters around 0 (AAA), 0.33 (AAB genotype), 0.66 (ABB genotype) and 1 (BBB genotype) on the X chromosome. On the Y chromosome, BAF values around 0 or 1 were present for both the XY and XXY animals. The LRR values per SNP across the genome are in Figure 2. For the XX and XXY animals, the LRR of the X chromosome was similar to that of the autosomes. The XY and XO animals had a lower X-chromosome LRR compared to the autosomes, indicating a chromosomal deletion. Conversely for the XXX female the X chromosome LRR was higher than her autosomal LRR, consistent with an extra X chromosome.

For XO and XXX animals, the missing heterozygous BAF cluster on the X chromosome was identified prior to plotting if animals had ≤2% of X-chromosome SNPs in the expected heterozygous BAF range of 0.45–0.55 for a diploid genome. The 2% threshold of X-chromosome SNPs in the BAF range of 0.45–0.55 was based on the distribution of the percentage of SNPs in this BAF range for the producer-recorded male and female population (Figure 1). For XO and XXX animals, one would expect no SNPs in the BAF range of 0.45–0.55.

While a missing heterozygous BAF band at 0.5 indicates a change in copy number (Liu et al., 2013) the BAF alone does not provide sufficient information to diagnose aneuploidy as inbreeding can be associated with an increase in X-chromosome homozygosity (Falconer & Mackay, 1996). If the extent of homozygosity reaches a point where the entire X-chromosome is homozygous, it can potentially result in misdiagnosing XX females as XO given that the characteristic X-chromosome heterozygous

TABLE 2 The number and prevalence of each type of aneuploidy and the parental origin of the single or extra X-chromosome.

Aneuploidy type	Number of animals diagnosed	Prevalence in the female population (%)	Prevalence in the male population (%)	Parental origin of aneuploidy
XO	6	0.0048	NA	80% maternal
XXX	42	0.0350	NA	77% maternal
XXY	19	0.0004	0.0870	100% maternal

Abbreviation: SD, standard deviation.

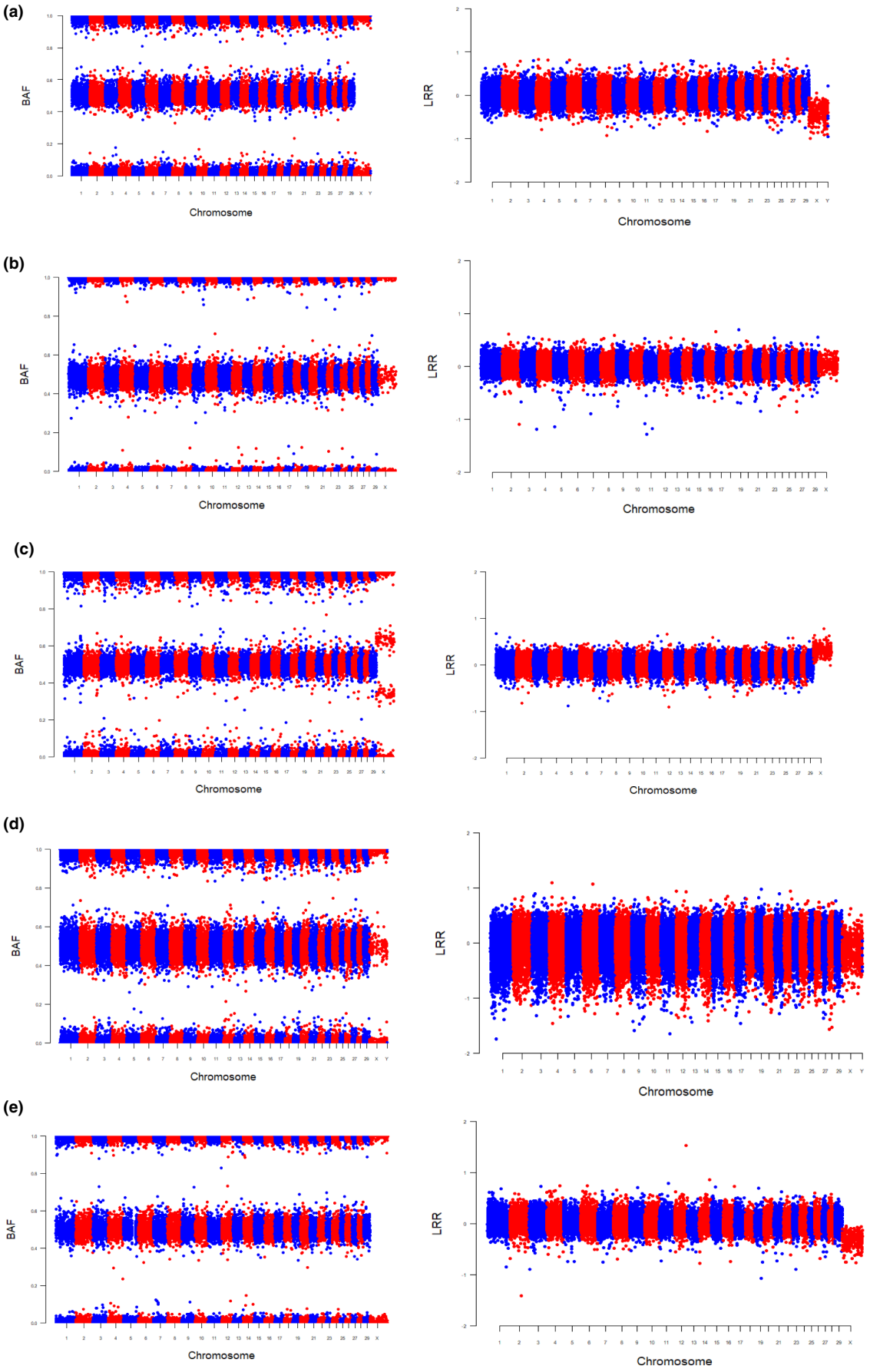

FIGURE 2 Real-life examples of Log R Ratio (LRR) and (BAF) plots for each detected X-chromosome karyotype. (a) BAF and LRR plot for an XY male. (b) BAF and LRR plot for an XX female. (c) BAF and LRR plot for XXX trisomy. (d) BAF and LRR plot for XXY. (e) BAF and LRR plot for XO monosomy.

BAF of 0.5 would be absent. Therefore, supplementary genotype intensity information is required to differentiate monosomy from inbreeding.

The concordance rate between the producer-recorded sex and the genomically predicted sex of the male and female verified population was 99.8%. Of the 246 animals whose sex could not be classified as per the developed decision rules, 149 females had standardized X-chromosome LRR and *R*-values within 3 SD of the respective female mean standardized X-chromosome LRR and *R*-values and had no Y SNPs called; however, the 149 females were homozygous for the entire X chromosome. Given that their X-chromosome genotype intensity metric was normal, these 149 females were likely to be inbred rather than being XO. The mean genomic inbreeding coefficient (*F*) of these females was calculated in Plink (Purcell et al., 2007) following the methodology proposed by Li and Horvitz (1953), which represents the proportion of observed autosomal homozygous SNPs relative to the expected number of autosomal homozygous SNPs assuming the Hardy–Weinberg equilibrium. The mean genomic inbreeding coefficient (*F*) calculated for the 149 females was 0.23 ranging from 0.022 to 0.336 while, on average 72% of their autosomal SNPs were homozygous, ranging from 63% to 78%. In comparison, the verified 124,809 female population had a lower mean genomic inbreeding coefficient (*F*) of 0.01, ranging from −0.173 to 0.312 and a lower mean autosomal homozygosity percentage of 65.4%.

As an example, the sire of the most inbred female ($F=0.336$) was also her grandsire and great-grandsire (Figure 3); the whole-genome BAF and LRR plots of the pedigree of this inbred female are presented in Figure 4. The X-chromosome BAF values for both the inbred female and her dam formed only two clusters each at 0 and 1 (Figure 4), suggesting chromosome-wide homozygosity or the presence of only one X chromosome. In contrast, the X-chromosome LRR metric for both females was similar to that of the autosomes, indicating the presence of two X chromosomes. Furthermore, the BAF Manhattan plot for both the inbred female and her dam highlighted the autosomal loss of heterozygosity, consistent with patterns of inbreeding. Of the 149 females, the female with the lowest inbreeding coefficient of 0.022 was investigated further and her whole-genome BAF Manhattan plot revealed that she did indeed have two X chromosomes and was not inbred. However, the heterozygous BAF clustered around 0.4 for the entire genome, deviating from the anticipated heterozygous BAF value of 0.5 (Figure 5). Therefore, these 149 females were subsequently considered as females for the purpose of calculating prevalence rates.

In a study of 139,675 female cattle, Berry et al. (2017) detected 17 females exhibiting X-chromosome homozygosity, which was also speculated to be due to inbreeding. These 17 females displayed a greater prevalence of homozygous autosomal SNPs when compared to females who did not have full X-chromosome homozygosity. Moreover, the X-chromosome *R*-values of these 17 females were not outliers when plotted against the *R*-values of typical XX females. Zhang et al. (2016) also identified two female cattles with homozygous X-chromosomes, which they attributed to inbreeding from a bull present on both sides of the pedigree. This highlights that the BAF alone cannot discriminate between monosomy

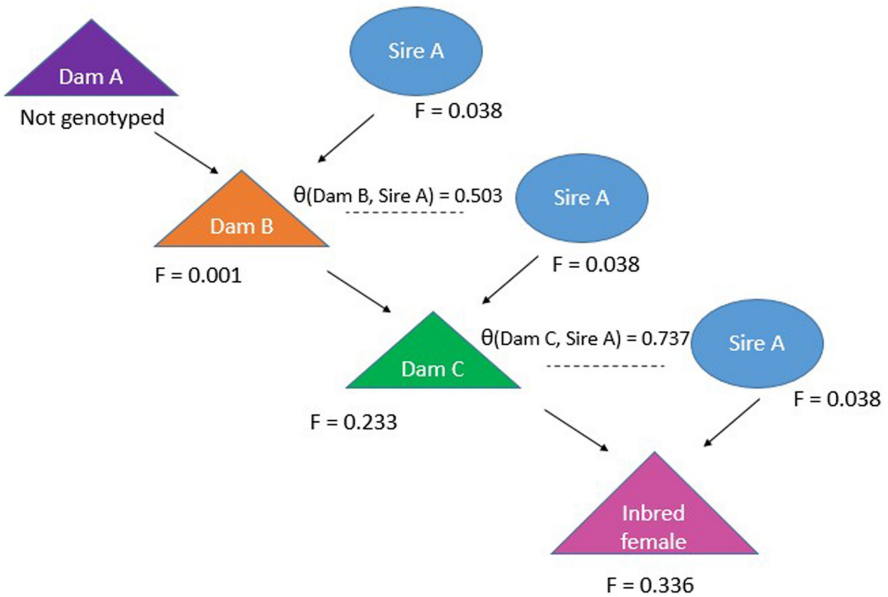

FIGURE 3 The pedigree of the most inbred female identified in the current study with the genomic inbreeding coefficient (*F*) of each animal is also shown. The co-ancestry coefficient between the dam and sire (θ(dam, sire)) in the mating's is also shown which was calculated using a genomic relationship matrix.

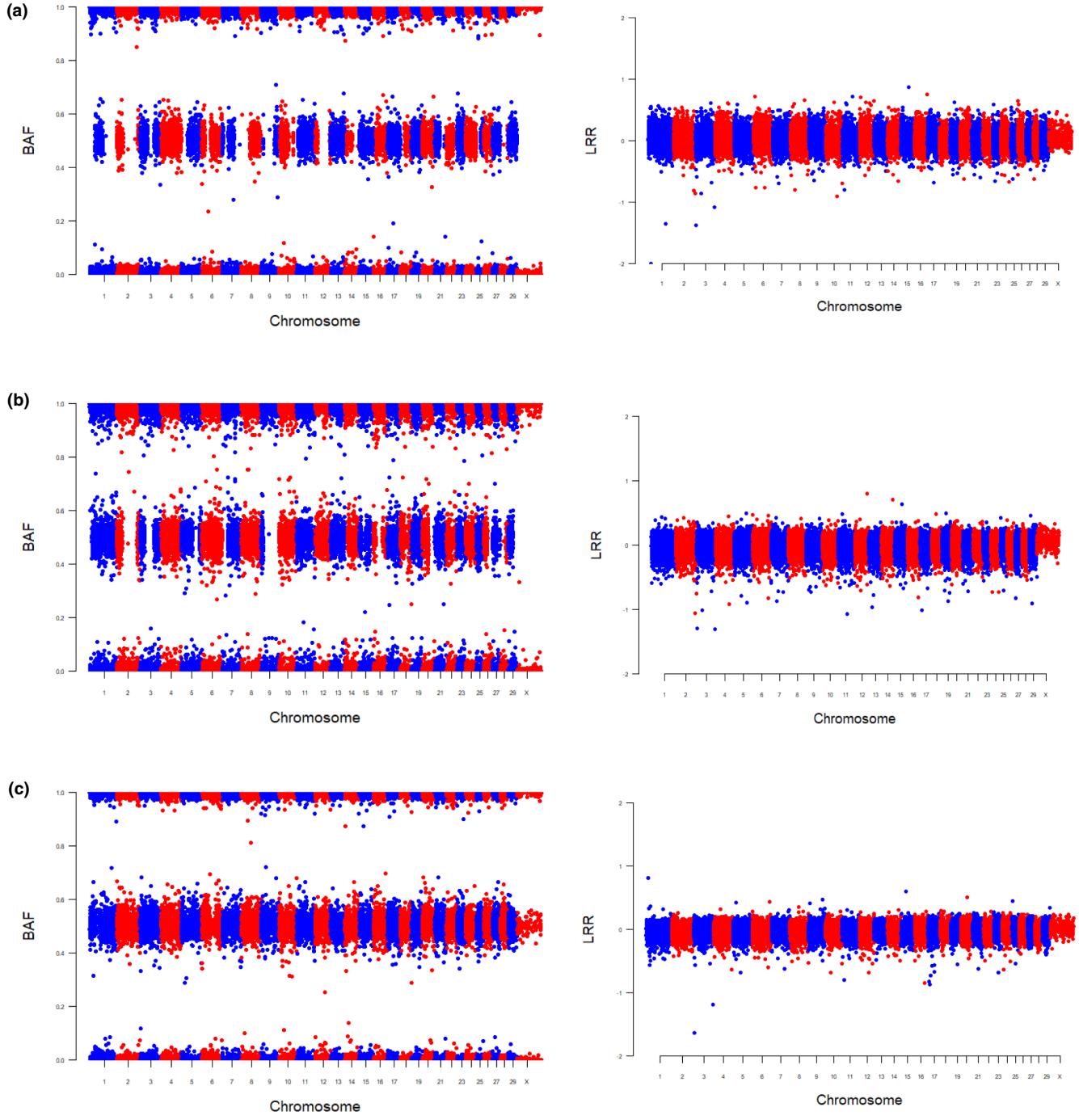

FIGURE 4 The B-allele frequency (BAF) and Log R Ratio (LRR) whole-genome Manhattan plots for (a) the most inbred female identified in the study, (b) her dam and (c) her grand-dam, where all three females had the same sire.

and inbreeding, or even a run of homozygosity spanning across an entire chromosome (Bouwman et al., 2023) given that both inbreeding and monosomy would not display a heterozygous BAF of 0.5 on the effected chromosome(s). However, the BAF when used in conjunction with other genotype intensity metrics, can be used to differentiate inbreeding from monosomy. For example, X-chromosome homozygosity (i.e., no X-chromosome SNPs with a BAF of 0.5) standardized LRR and *R*-value metrics within 3 SD of the mean of the respective X-chromosome standardized LRR or *R*-value of the female population suggest an inbred XX female, given that the X-chromosome genotype intensity metrics are consistent with animals that have two X chromosomes. In contrast, X-chromosome homozygosity, standardized LRR and *R*-value genotype intensity metrics <3 SD from the mean of the respective X-chromosome standardized LRR or *R*-value of the female population are indicative of monosomy, given that the X-chromosome genotype intensity metrics are lower than animals that have two X

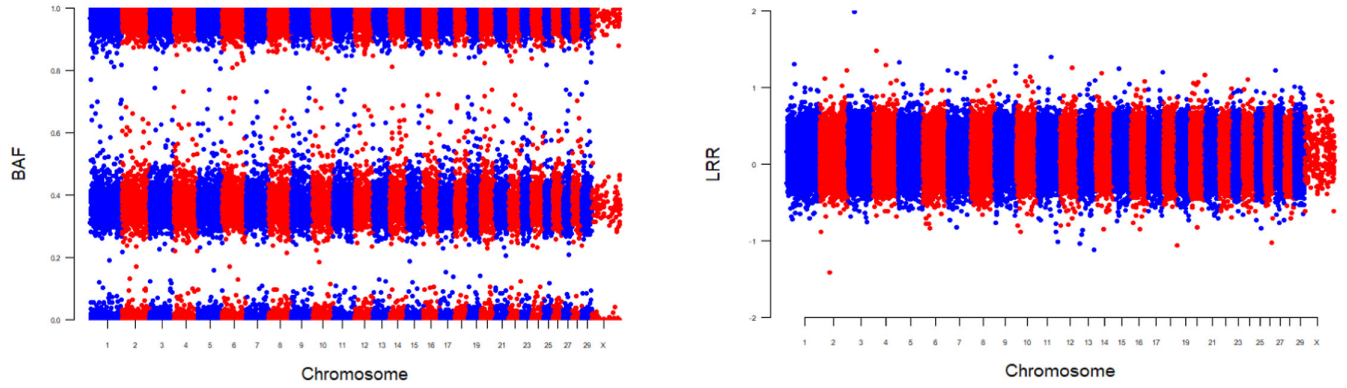

FIGURE 5 The B-allele frequency (BAF) and Log R Ratio (LRR) whole-genome Manhattan plots for a female displaying a heterozygous BAF around 0.4 across the genome instead of the expected 0.5.

chromosomes. Therefore, only genotype intensity values can differentiate between inbreeding involving the entire X-chromosome and monosomy.

Using only the LRR or *R*-value metrics independently for aneuploidy detection would have incorrectly labelled a total of 2399 and 2370 females, respectively, as XXX trisomy. These 4769 females all demonstrated a heterozygous BAF band at 0.5, indicating that they were XX females despite having either standardized X-chromosome LRR or *R*-values >3 SD from the mean of the respective X-chromosome standardized LRR or *R*-value of the female population. This underscores the significance of using the BAF along with both the LRR and *R*-values for proper large-scale aneuploidy screening. When one of the intensity values is susceptible to noise, the combined assessment of both LRR and *R*-values, along with the BAF, provides a more reliable and robust approach for detecting aneuploidy, preventing potential misclassifications of monosomies and trisomies.

3.1 | Prevalence and genetic mechanisms of Turner syndrome (XO)

The 6 XO individuals detected from the 146,431 genotyped animals in the present study equates to a prevalence of 0.0040% in the total population or 0.0048% in the female population (including the 149 inbred females; Table 2). Turner syndrome (XO) is more prevalent in humans and horses than in cattle, with previously recorded prevalence rates of 0.04% and 0.15% among live female human and filly births, respectively (Bondy & Cheng, 2009; Kakoi et al., 2005; Urbach & Benvenisty, 2009).

Of the 6 XO cattle, one was a Holstein-Friesian, whereas the remaining 5 were all crossbred beef cattle. All 6 XO females were also recorded as female by the producer. None of the 6 XO females identified in this study had offspring, suggesting that all six individuals were possibly infertile since they were all born ≥89 months before the date of data extraction. One of the six herds where a detected XO female resided was a dairy herd that used artificial insemination (AI) and that dairy XO female had no recorded inseminations, suggesting she may never even have displayed oestrus. Infertility has been a phenomenon reported for XO females of different species, including cattle (Berry et al., 2017) horses (Mäkinen et al., 2010) and humans (Folsom & Fuqua, 2015). In the current study, estimates of aneuploidy prevalence were derived from a population of animals that were genotyped at less than 15 months of age, approximately the age at first breeding. This approach was adopted to mitigate potential bias in prevalence estimates, as a substantial number of infertile animals with aneuploidy might be genotyped when they fail to produce offspring. However, SNP genotype intensity data were also available for an additional 121,126 animals that were genotyped at ≥15 months of age. Notably, one XO female was identified in this population of 121,126 animals and she did not have progeny.

Parental genotypes were available for 5 XO females, revealing that 4 inherited their single X chromosome from their dam while one inherited the X chromosome from its sire. The inheritance of the single X chromosome in XO humans is generally maternal (Hassold et al., 1988). In a pre-implantation study testing for aneuploidy in bovine embryos, Silvestri et al. (2021) reported that 120 XO embryos inherited the single X chromosome from the dam, whereas only 15 inherited the paternal X chromosome. Similarly, human studies have reported that 60–80% of XO females inherit the single X chromosome maternally (Sagi et al., 2007; Uematsu et al., 2002). The single X chromosome for the XO dairy female identified by Berry et al. (2017) was inherited paternally.

While only one X chromosome remains active regardless of karyotype (Iannuzzi et al., 2021; Migeon et al., 2008) genes on the PAR and several other regions on the X chromosome escape inactivation and are expressed on both the active and inactive X chromosome(s) (Bondy & Cheng, 2009; Brown et al., 1997). Consequently, XO

individuals exhibit haploinsufficiency of these escaping genes, contributing to the observed phenotypic effects (Brown et al., 1997; Urbach & Benvenisty, 2009; Zinn & Ross, 1998). Because genes in the PAR play critical roles in placental development and early embryonic growth (Urbach & Benvenisty, 2009) having only one X chromosome is expected to have consequences for embryo survival, the extent of which is likely to be influenced by the size and gene content of the PAR, which varies between species (Raudsepp et al., 2012). Cattle, for example, have a relatively large PAR region of over 9 Mb (Das et al., 2009) whereas horses and humans have a relatively smaller PAR region of 1.8 and 2.7 Mb, respectively (Raudsepp & Chowdhary, 2008). Raudsepp et al. (2012) proposed that the lower frequency of Turner syndrome in cattle relative to horses and humans may be due to their larger PARs, suggesting that the loss of an X chromosome results in haploinsufficiency for a more extensive genomic segment involving a greater number of genes than in species with smaller PARs, such as humans and horses. As a result, this could potentially contribute to more abortions, given the influence of PAR genes on embryonic survival and fewer live-born cases of Turner syndrome.

3.2 | Prevalence and genetic mechanisms in X-chromosome trisomy (XXX)

XXX trisomy was the most common form of aneuploidy detected in the females in the present study, comprising 0.035% of the female population (including the 149 females that were inbred or had a heterozygous BAF outside of the expected BAF range) or 0.030% of the total population (Table 2). Of the 42 XXX females, 5 were purebred Limousin ($n=2$), Charolais ($n=1$) or Holstein-Friesian ($n=2$) cattle. The remaining 37 XXX females were all crossbred beef cattle. All 42 XXX females were recorded as female by the producers. Two of the 42 XXX females identified were still alive at the time of analysis and both were confirmed by karyotype analysis to have a trisomy on chromosome X (61, XXX; Figure 6). Given the fact that at least 10 metaphases were examined by karyotyping, mosaicism of ≥3% can be excluded for both with 99% confidence, or mosaicism of ≥2% can be excluded with 95% confidence (Hook, 1977). XXX trisomy has been reported to occur in 0.1% of human female births (Tartaglia et al., 2010); this makes XXX trisomy the most frequent sex chromosome abnormality present at birth in human females (Powell, 1999). Only 12 of the 42 XXX females identified in the present study had progeny. The 42 females were all born ≥79 months before the date of data extraction. Of the 30 XXX females that had no progeny, 22 were from herds that had recorded AI information, but none of these females had any record of being served. Therefore, it is likely that these females may not have displayed oestrus.

The two XXX females that were karyotyped were fertile and had 5 and 6 progeny, each. For one of the two XXX females her dam was not genotyped, so the parental origin of the extra X chromosome could not be conclusively determined. However, two of her progeny were genotyped and neither the XX nor XY progeny inherited the X

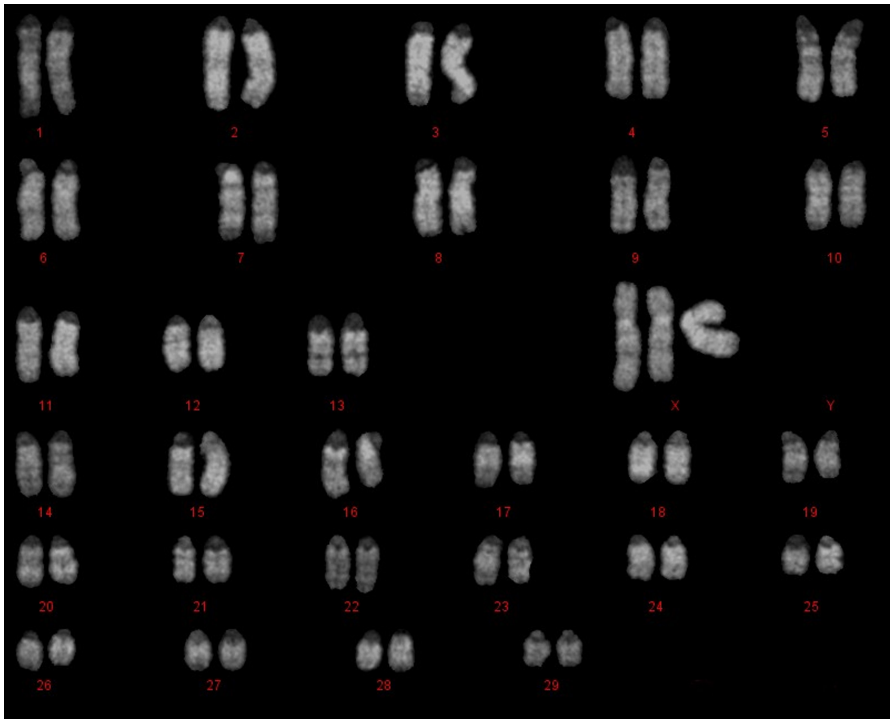

FIGURE 6 Karyotype of a cow carrying a trisomy on chromosome X (61, XXX). Chromosome staining was performed using DAPI (4′,6-diamidino-2-phenylindole).

chromosome from their maternal grand-sire (i.e., the sire of the XXX female) indicating that they inherited the X chromosome from their maternal grand-dam instead (i.e., the dam of the XXX female that was not genotyped). The other XXX female had one female progeny genotyped, also with a normal XX karyotype. Although this XXX female inherited two X chromosomes from her sire and one from her dam, her progeny inherited the maternal grand-dam's X chromosome. Both karyotype-verified XXX females were visually inspected by a veterinarian and neither was deemed to have any abnormal external features. To date, only one case of a fertile XXX cow has been reported and she gave birth to an XXY calf (Schmutz et al., 1994). Given that karyotype analysis is typically performed in cases of infertility, the less reported frequency of fertile XXX cows is not unexpected. The majority of XXX cattle previously reported have been infertile (Buoen et al., 1981; Herzog et al., 1977; Norberg et al., 1976) due to impairments to the internal reproductive organs, such as underdeveloped ovaries that lack follicular development and a smaller uterus body (Iannuzzi et al., 2021). These fertility issues observed in some cases of XXX are potentially a consequence of three active X chromosomes in early embryonic development before inactivation (Iannuzzi et al., 2021) or due to the overdose of PAR genes or other X-chromosome genes that escape inactivation (Tartaglia et al., 2010).

Of the 42 XXX females identified, parental genotypes were available for 26, revealing that 20 (77%) inherited two of their X chromosomes from their dam and the other from their sire, while 6 (23%) inherited one X chromosome from the dam with the remaining two X chromosomes having originated from the sire. Two copies of the same maternal or paternal X-chromosome were inherited in all 26 instances (i.e., no XXX females inherited two different X-chromosomes from the dam) and of the 20 that inherited two X chromosomes from the dam three had progeny while of the six that inherited the same X chromosome twice from the sire, two had progeny. However, the small sample sizes preclude definitive conclusions regarding the impact of parental origin of X chromosomes on fertility. In a preimplantation study testing for aneuploidy in bovine embryos, Silvestri et al. (2021) reported that two X chromosomes for XXX embryos originated from the dam in 90 instances and from the sire in just one instance. Errors during maternal meiosis 1 specifically, accounted for 73.5% of all trisomy cases documented by Silvestri et al. (2021).

3.3 | Prevalence and genetic basis of XXY Klinefelter syndrome in males

The 18 XXY males detected in the present study translate to a prevalence of 0.087% in the male population (Table 2). Of the 18 detected XXY males, 13 were either purebred Holstein-Friesian ($n=5$) Charolais ($n=1$) Limousin ($n=2$) Angus ($n=2$) Simmental ($n=2$) or Shorthorn ($n=1$) while the remaining 5 were crossbred beef cattle. All 18 XXY males were recorded by the producers as being male. The sex-determining region of the Y chromosome (SRY) and androgen receptor genes located on the Y and X chromosomes, respectively, are crucial for male development (Hu & Namekawa, 2015; Sinclair et al., 1990). The SRY gene is responsible for the differentiation of the bipotential gonads into testes, which then produce androgens including testosterone and anti-Müllerian hormone. The effects of these androgens are then mediated through the androgen receptor gene and play a crucial role in the development of male reproductive organs and the regression of female structures (Kashimada & Koopman, 2010). Given that one Y chromosome with the SRY gene is sufficient to induce testis development, XXY Klinefelters are almost exclusively phenotypically male yet infertile due to overdosage of X-chromosome genes that escape inactivation (Joerg et al., 2003; Schmutz et al., 1994; Slota et al., 2003). Of the 18 XXY males identified in the present study 10 were recorded as steers at the time of slaughter 6 were bulls that were ≤24 months of age at the time of slaughter and 2 were bulls that were >24 months of age at the time of slaughter. None of the 18 XXY males in the s-tudy had any recorded progeny. Furthermore, no XXY males were identified in the population of 121,126 animals genotyped at ≥15 months of age had progeny. Of the 18 XXY males identified, 4 individuals had both parents genotyped and analysis revealed that both X chromosomes were inherited from the dam for all 4 XXY males. Two of the four XXY males inherited one copy of each maternal X-chromosome (i.e., received two different X-chromosomes from the dam), whereas the other two XXY males inherited two copies of the same maternal X-chromosome (i.e., received two copies of the same X chromosome from the dam).

3.4 | Prevalence and genetic basis of XXY Klinefelter syndrome in females

Only one case of an XXY female was detected (0.0004% of the female population) and she was a crossbred beef female recorded as female both at the time of birth and at 23 months of age at the time of slaughter in an abattoir. XXY cattle that appear phenotypically as female have not been previously reported, although rare instances have been documented in humans (Müller et al., 1990; Röttger et al., 2000). Given the rarity of this XXY condition in females and the fact that only five SNPs on the Y chromosome were used, a further examination of the

X- and Y-chromosomes of the female in relation to those of her parents was undertaken to investigate the possibility of genotyping errors. Using the five Y-chromosome SNPs, there was a 100% concordance rate between the Y-chromosome genotype of the female and that of her sire and both X-chromosomes were inherited from the dam. A copy of each maternal X-chromosome was inherited. Therefore, it is likely that the female did indeed have the XXY karyotype. However, future genotype panels should include more Y-chromosome SNPs to provide greater confidence in the detection of the presence of a Y-chromosome. The XXY female did not have any progeny. In the case of the rare XXY female phenotype observed in humans, androgen insensitivity syndrome (AIS) has been identified as the main cause (Gerli et al., 1979; German & Vesell, 1966; Girardin et al., 2009; Müller et al., 1990; Uehara et al., 1999). AIS, also known as testicular feminization is a genetic condition characterized by the presence of a Y chromosome, yet individuals appear phenotypically female. This is due to mutations in the androgen receptor gene, which prevents the body from responding to androgens. Consequently, this leads to the absence of masculinization of external genitalia and the development of female secondary sexual characteristics (Galani et al., 2008; Oakes et al., 2008). These mutations can either arise spontaneously (de novo) or be inherited. In cases of inheritance, the mothers serve as carriers of the mutation and typically do not exhibit symptoms of the condition, which is transmitted as an X-linked disorder (Girardin et al., 2009). For example, Girardin et al. (2009) reported a case of a female XXY human who possessed two identical androgen receptor mutations, which she inherited from her asymptomatic mother.

Mutations or deletions in the SRY gene on the Y chromosome have also been linked to the XXY female phenotype in humans (Lin et al., 2014; Röttger et al., 2000). Röttger et al. (2000) reported a human XXY mother who had a son and two daughters and one daughter also had the XXY karyotype. In both the case of the mother and the daughter, the SRY gene was absent. However, the XXY female (human) with an SRY deletion described by Lin et al. (2014) was infertile. Berry et al. (2023) documented variability in the length of deletions in the SRY gene among XY females, which could potentially contribute to the fertility or infertility of XXY SRY-negative females, depending on the length of the deletion.

4 | CONCLUSION

A technique for identifying X-chromosome aneuploidy using the genotype intensity metrics of 146,431 animals is proposed that can help distinguish aneuploidy from inbreeding; cytogenetic testing was used to validate two XXX females. The proposed approach can be readily integrated into existing genomic pipelines serving as an efficient, large-scale screening tool for aneuploidy. Its implementation could enable the early identification of animals with X-chromosome aneuploidy and likely infertility without incurring additional cytogenic costs. Within the female verified population, the estimated prevalence rates were 0.0048% for XO 0.0350% for XXX and 0.0004% for XXY. The prevalence of XXY in the male verified population was 0.0870%. While only juveniles were used in the present study to get a more accurate estimate of the prevalence the reported prevalence estimates may potentially be underestimated considering the possibility that certain animals may have died in utero or were not genotyped due to undesirable characteristics such as a slow growth rate.

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CONFLICT OF INTEREST STATEMENT

'Berry, Donagh' is an Editorial Board member of the Journal of Animal Breeding and Genetics and a co-author of this article. To minimize bias, they were excluded from all editorial decision-making related to the acceptance of this article for publication.

DATA AVAILABILITY STATEMENT

The data used in the present study originated from a pre-existing database managed by the Irish Cattle Breeding Federation (ICBF). Data available on request from the authors.

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