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The incidence of aneuploidy and mosaicism in 2,045 genotyped cattle blastocysts

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In brief

Embryo selection through preimplantation genetic testing for aneuploidy before transfer has been reported to improve pregnancy and live birth rates in cattle. This study demonstrates that the single nucleotide polymorphism-based preimplantation genetic testing for aneuploidy algorithm used in these studies can also detect mosaicism, reporting a mosaicism incidence of 25.6% among 311 aneuploid embryos from a cohort of 2,045 blastocysts.

Abstract

Chromosomal abnormalities are the most common cause of developmental arrest in mammalian embryos. They can be present consistently in all cells of the embryo or occur as admixtures of karyotypically distinct lineages (mosaics). The estimated incidence of mosaicism ranges from 14% to 82% in human embryo biopsies at the blastocyst stage. In cattle, mosaicism is not well described at a whole-genome level, with findings limited to sex chromosomes. Here, we conducted a retrospective analysis of published data spanning three studies from our laboratory to establish the incidence and nature of mosaicism in 2,045 bovine blastocysts genotyped using single nucleotide polymorphism-based approaches. We classified mosaic embryos as those where the inner cell mass and trophectoderm differed in ploidy and/or where embryos had a percentage of cells with aneuploidy ranging from 20% to 80%. We report an aneuploidy incidence of 15.2% (n = 311/2,045), with 25.6% of the aneuploid embryos (80/311) being mosaic. Mosaicism was particularly common (87.5%, *n* = 7/8) in embryos affected by multiple types of chromosomal errors and in embryos affected only by segmental aneuploidies (50.0%, *n* = 9/18). The chromosomal abnormalities with the highest incidence of mosaicism were segmental aneuploidies (48.1%, n = 13/27). Most errors leading to mosaicism had a paternal origin (44.9%, n = 22/49), followed by post-zygotic errors (37.3%, n = 19/51). Our results reveal an incidence of mosaicism in bovine embryos similar to that of human embryos. Additionally, we demonstrate that ploidy and mosaicism screening can be performed in embryos using the same single nucleotide polymorphism genotyping data obtained to calculate genomic estimated breeding values.

Keywords embryology, bovine, PGT-A, chromosomal abnormalities

Introduction

Chromosomal abnormalities [including aneuploidy and (hypo/hyper)polyploidy] are the most common cause of embryo developmental arrest, implantation failure, and spontaneous miscarriages in mammalian reproduction (Hassold & Hunt, 2001; Marquard et al., 2010). These chromosomal abnormalities can affect all cells of the embryo consistently or a proportion of cells, resulting in the presence of karyotypically distinct lineages within the same embryo (mosaicism) (Bavister & Brenner, 2006). Mosaicism can arise from post-zygotic chromosome segregation errors (mitotic non-disjunction, anaphase lag, unattached chromosome, tripolar spindle formation and endoduplication, or failed cytokinesis). Less commonly, mosaicism can arise from an embryo that was originally aneuploid or polyploid, followed by a "rescue" event (when some of the cells return the chromosome pair to a diploid state) (Levy et al., 2021). Recent studies in human in vitro fertilization (IVF) and preimplantation genetic testing for aneuploidy (PGT-A) have investigated mosaic embryos and their developmental outcomes. This has become one of the most debated topics in the field. Initial reluctance to transfer embryos where any form of aneuploidy (including mosaicism) was diagnosed gave way to the realization that many embryos in which mosaicism is detected via embryo biopsy can lead to live

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births. Euploid/aneuploid mosaic embryos have lower implantation success compared to fully euploid embryos, but a large proportion proceed to term (Capalbo et al., 2021; Victor et al., 2019; Viotti et al., 2021). Euploid/aneuploid mosaic embryos arising by a "rescue" event, however, usually result in a phenotype such as pregnancy loss or issues associated with uniparental disomy.

In human IVF, PGT-A is typically performed when a biopsy of 3–10 cells from the trophectoderm (TE) is taken from the blastocyst and diagnosis is facilitated by molecular cytogenetic and bioinformatic methodologies. Historically, studies involved fluorescence in situ hybridization, followed more recently by comparative genomic hybridization and then next generation sequencing (NGS), with PCR-based protocols and single nucleotide polymorphism (SNP) arrays used less frequently (Capalbo et al., 2021; Girardi et al., 2023; Lal et al., 2020). In cattle, recent studies have tended to biopsy between 3 to 20 TE cells (González-Rodríguez et al., 2022; Zeng et al., 2024). These biopsies are usually taken with the primary purpose of establishing the genomic estimated breeding value (gEBV) of the embryo before transfer. The combination of gEBV and chromosomal analyses in the same test is powerful, as it gives not only an indication of the likely genetic traits of the animal but also a reasonable estimate of the chances that embryo transfer will result in a live birth (Silvestri et al., 2021).

The issue of whether mosaicism should be a consideration in bovine PGT-A, however, remains under-explored. Given that mosaic cells are either located in the TE and/or inner cell mass (ICM), the biopsy taken may not fully represent the chromosomal ploidy status of the entire embryo. If mosaicism is detected in the TE, the embryo is considered mosaic. However, if mosaicism is not detected (i.e., the biopsy result indicates all euploid or aneuploid cells), it remains uncertain if there is mosaicism in other parts of the TE or the ICM. (Vera-Rodriguez & Rubio, 2017). Moreover, recent reports indicate a high concordance between the TE and ICM (Tutt et al., 2021) or between a TE biopsy and remaining blastocyst cells (Takahashi et al., 2021). Comprehensive chromosomal analyses have been conducted in human (Coll et al., 2021; Katz-Jaffe et al., 2017; Munné et al., 2019) and mouse embryos (Treff et al., 2010), but incidences of mosaicism in bovine embryos remain to some degree under-investigated. The reported incidence of mosaicism in human blastocysts is estimated to be between 14% and 59% (Capalbo et al., 2021; Chavli et al., 2022; Popovic et al., 2020). However, a recent study individually assessing multiple cells from the same blastocyst reported 82% of the embryos contained at least some aneuploid cells, with the majority of chromosomal abnormalities affecting <20% of all blastocyst cells (Chavli et al., 2024). In cattle, mosaicism has been mainly inferred by studying the sex chromosomes (Tšuiko et al., 2017; Szczerbal et al., 2021) or, more recently, using single-cell analysis by investigating all blastomeres of the embryo (De Coster et al., 2022; Masset et al., 2022).

Our previous studies in cattle demonstrated that TE biopsy provides a representative sample of the embryo (Tutt et al. 2021) and that the application of PGT-A before embryo transfer can improve pregnancy and live births by 7.5 and 5.8 percentage points, respectively, over non-tested embryos (Silvestri et al., 2021). Moreover, neither ovarian stimulation (Tutt et al., 2021) nor the composition of in vitro maturation media (Tutt et al., 2023) affect the ploidy status of pre-elongation embryos. Building on this work, the present study utilises the same SNP genotyping data to assess the nature and extent of mosaicism in cattle embryos by analysing log R ratio (LRR) values. We classified an embryo as mosaic if (i) its ploidy status differed between ICM and TE and/or (ii) it contained at least one chromosome with an LRR value between 20% and 80% of a full trisomy or monosomy, following previous human IVF guidelines (Cram et al., 2019). Values of LRR < 20% were considered normal/euploid, while values > 80% indicated full abnormality/aneuploidy. We report the overall incidence of mosaicism, its prevalence by chromosome, differences between ICM and TE, and associations with blastocyst stage and quality. As cattle IVP breeding programs increasingly rely on SNP genotyping for genetic merit evaluation (Fujii et al., 2019; Mullaart & Wells, 2018; Silvestri et al., 2021), the integration of PGT-A for copy-number variation and mosaicism assessment could provide a more comprehensive genetic profile before embryo transfer.

Materials and methods

SNPChip genotyping data

This study is a retrospective evaluation of SNP data from 2,256 blastocysts genotyped by the breeding company Boviteq (Saint-Hyacinthe, Canada) and the University of Nottingham through Neogen Europe Ltd (Ayr, Scotland, UK), as reported previously (Silvestri et al., 2021; Tutt et al., 2021, 2023) (Table 1). Briefly, embryos were produced as follows: cumulus–oocyte complexes were obtained via ovum pick-up using a standard protocol for oestrous synchronisation and ovarian stimulation/no stimulation. These cumulus–oocyte complexes were matured in vitro for 24 hr

Table 1 Overview of the data included in the study.

Type of biopsy	Data source	Illumina SNPChip platform	Number of SNPs	Embryos tested (*n*)	Progenitors tested (*n*)
TE biopsy of 15 cells	Silvestri et al., 2021	GGP Bovine HD 150k v01	138,892	379	–
		GGP Bovine HD 150k v03	139,376	1241	–
		GGP Bovine HD 150k v04	140,668	112	–
		GGP Bovine LD v4	30,105	5	–
		GGP BovineSNP50	45,187	–	241
TE + ICM separated	Tutt et al., 2021	GGP 50K SNP	50,452	199	14
TE isolated	Tutt et al., 2023	GGP 100K SNP	95,256	320	13
Total number of samples				2,256	268

Note. TE = trophectoderm; ICM = inner cell mass; SNP = single nucleotide polymorphism.

and then fertilised with frozen/thawed sperm. While Silvestri et al. (2021) used sperm from several sires, Tutt et al. (2021, 2023) used sperm from a single sire. Embryos were investigated for aneuploidy and mosaicism using three sampling methods: TE biopsy of ∼15 cells (n = 1,713 embryos), the entire TE and ICM analysed separately (n = 199 embryos), and whole TE only (n = 320). Previous reports indicate a high concordance between the TE and ICM (Tutt et al., 2021) or between a TE biopsy and remaining blastocyst cells (Takahashi et al., 2021), allowing us to pool the data from these three different studies. Moreover, data pooling permitted us to perform a more comprehensive analysis of mosaicism, since the average of aneuploidy incidence was 19.5% and mosaicism is only present in a fraction of these. Data from the genetic parents of the embryos was also available, enabling haplotyping and the determination of aneuploidy origin. Due to the presence of different SNPChips in these studies, only shared SNPs were used for the analysis. At the time of biopsy, blastocysts presented a minimum morphological quality of fair/good, with developmental stages ranging from 5 to 9 (i.e., early to hatched blastocysts), according to the International Embryo Technology Society (Stringfellow et al., 1990) and Bo and Mapletoft, (2013).

Chromosome error screening

Only samples with a minimum call rate ≥ 0.8 were included in the screening (n = 2,045). For all samples, three distinct aneuploidy screening algorithms were applied, as described previously (Silvestri et al., 2021; Tutt et al., 2021, 2023): Karyomapping (Handyside et al., 2010), signal intensity data as B-allele frequency (BAF) and LRR (Staaf et al., 2008), and Gabriel–Griffin plots (Gabriel et al., 2011). Copy number variation was detected through BAF and LRR plots, alongside Karyomapping to understand the parental origin of the error. The meiotic origin [meiosis I (MI), meiosis II (MII), or mitotic] of errors was identified in trisomies using Gabriel–Griffin plots. Due to the limited number of SNPs available and the nature of sex chromosomes, haploblock tracing using Karyomapping and Gabriel–Griffin plots was not possible for chromosome Y.

Mosaicism detection

Mosaicism was investigated in two ways: (i) per cell line of the blastocyst and (ii) per chromosome using LRR values. First, we studied whether TE and ICM presented different ploidy statuses. Then, we assessed mosaicism at the whole-chromosome level as well as segmental aneuploidy (i.e., involving parts of the chromosome). In the case of segmental aneuploidies, the mosaicism analysis was restricted to the affected chromosomal region. Mosaicism level, defined as an estimate of the proportion of cells in the whole embryo affected by a chromosomal error (given as a percentage), was calculated as the ratio between the LRR value measured for each chromosome (LRRa) and the expected LRR value (LRRe) for a non-mosaic aneuploidy. Because there is no consensus for LRRe values for both monosomy and trisomy (Glessner et al., 2021; Verdyck et al., 2025), we estimated LRRe empirically using our dataset by pooling all embryos classified as aneuploid. A standardised correction factor was applied to both LRRa and LRRe measurements, equivalent to the difference between the mean LRR of all euploid samples and zero (theoretical average LRR value for euploid samples). For each chromosome abnormality previously detected (by Karyomapping, LRR, and/or BAF analysis), the ratio LRRa/LRRe (as a percentage) was used to indicate the presence and percentage of mosaicism. We classified an embryo as mosaic if (i) its ploidy status differed between ICM and TE and/or (ii) it contained at least one chromosome with an LRR value between 20% and 80% of a full trisomy/monosomy. LRR values < 20% were considered normal/euploid, while values > 80% were considered fully abnormal/aneuploid. These thresholds follow previous human IVF guidelines (Cram et al., 2019), since human and cattle expanded blastocysts contain a similar number of cells (∼150–250 cells). Moreover, the number of cells biopsied was > 5 cells, aligning with the guidelines. All calculations were performed in R.

Statistical analysis

All statistical analyses were performed using RStudio 2013.12 (R Core Team, 2021). Confidence intervals for proportions were calculated by applying the Wilson interval. For statistical analysis, appropriate tests were selected depending on the specific experiment, following the guidelines presented in McDonald (2009), and the α value for statistical significance was set at 0.05. The statistical tests used in this study were: chi-square test, Fisher's test, t-test and Pearson's test.

Results

Overall aneuploidy and mosaicism incidence

The overall percentage of embryos diagnosed with chromosomal errors was 15.2% (n = 311/2,045), with 25.6% of these being mosaic (n = 80/311) (Supplementary Table S1, see online supplementary material). Ten of the mosaic embryos were diagnosed as mosaic because the ploidy status differed between the ICM and TE cell lines. In total, therefore, mosaicism was detected in 3.9% of all embryos (n = 80/2,045). Focusing on the distinct error types, whole chromosome errors (WCEs) were the most observed category, occurring in 12.0% of embryos (n = 245/2,045). Other types of chromosome error were identified at lower frequencies, including segmental aneuploidies (0.9%, n = 18/2,045) and (hypo/hyper)triploidy (1.9%, n = 39/2,045). Only one case of haploidy was found (0.05%), while complex chromosomal errors, defined as the presence of multiple error types (e.g., a combination of WCEs and segmental aneuploidies), were observed in 0.4% of embryos (n = 8/2,045) (Figure 1).

Mosaicism was also present in embryos with other types of errors, except for the haploid embryo. The highest prevalence of mosaicism was observed in embryos with complex errors (87.5%, n = 7/8), followed by those with segmental aneuploidies (50.0%, n = 9/18) and (hypo/hyper)triploidy (48.7%, n = 19/39). Although WCE was the most frequently detected chromosomal abnormality, only 18.4% of embryos with this type of error exhibited mosaicism (n = 45/245, P < 0.001, chi-square test).

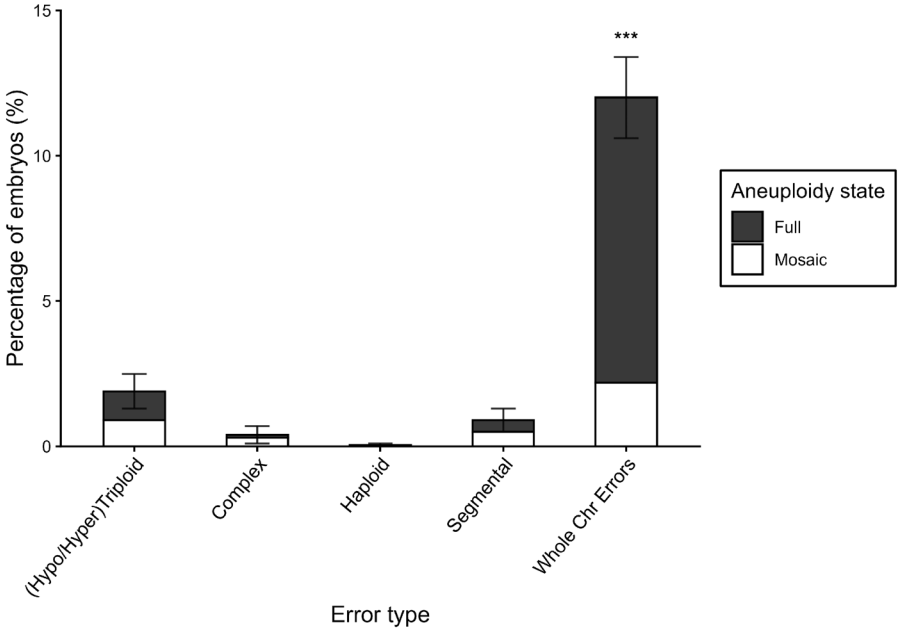

Figure 1 Incidence of chromosomal errors by type, classified as full (non-mosaic) or mosaic (n = 2,045 embryos) using preimplantation genetic testing for aneuploidy algorithms. Data presented as percentages (%) with 95% confidence intervals. ***p < .001.

Table 2 Number of chromosomal errors by class and origin (paternal, maternal, or embryo) in full (non-mosaic) or mosaic state.

Aneuploidy classes	Paternal		Maternal		Mitotic		Overall	
	Full	Mosaic	Full	Mosaic	Full	Mosaic	Full	Mosaic
Haploid	[a]	[a]	1	[a]	[a]	[a]	1	[a]
(Hypo/hyper)triploid	9[b]	12	13[b]	7	[a]	[a]	22	19
Parthenogenetic	[a]	[a]	[a]	1	[a]	[a]		1
Segmental	5	3	[a]	1	9	9	14	13
Deletion	4	3	[a]	1	7	7	11	11
Duplication	1	[a]	[a]	[a]	2	2	3	2
Androgenetic	1	[a]	[a]	[a]	[a]	[a]	1	[a]
Whole chromosome error	12	7	222	32	23	10	257	49
Monosomy	11	6	123[c]	14	4	1	138	21
Trisomy	1	[a]	98	18	18	7	117	25
Meiosis I	1	[a]	89[c]	18	[a]	[a]	90	18
Meiosis II	[a]	[a]	9	[a]	[a]	[a]	9	[a]
Mitotic	[a]	[a]	[a]	[a]	18[c]	7	18	7
Uniparental disomy	[a]	1	1	[a]	1	2	2	3
Total	27	22	236	41	32	19	295	82
	49		277		51		377	

[a]No error was found with those specifications.
[b]Case of hypertriploidy with both parental origin.
[c]One case per error type involving chromosome BTAX.

Origin of chromosomal errors

Chromosomal errors in embryos can be inherited from the oocyte (maternal origin), the sperm (paternal origin), or post-zygotically during mitotic and segregation events. Our analysis identified 377 distinct chromosome errors across 311 embryos (Table 2). Errors with a maternal origin were the most common (73.5%, n = 277/377, P < 0.001, chi-square test), while errors with a paternal origin and those arising post-fertilisation presented a lower, but similar incidence (13.0%, n = 49/377 and 13.5%, n = 51/377, respectively). Notably, chromosomal errors originating from sperm exhibited a higher incidence of mosaicism (44.9%, n = 22/49) compared to errors arising from the oocyte (14.8%, n = 41/277) (P < 0.001, chi-square test).

(Hypo/yyper)triploid cases had a similar incidence regarding parental origin, with paternal and maternal origins accounting for 51.2% (n = 21/41) and 48.8% (n = 20/41) of cases, respectively. Segmental aneuploidies were more prone to arise during mitotic events (66.7%, n = 18/27), followed by errors occurring during spermatogenesis (29.6%, n = 8/27). Interestingly, nearly half of the segmental aneuploidies were mosaics (48.1%, n = 13/27).

As previously described, WCEs were the most prevalent chromosomal error in the dataset. Among the 306 single WCEs identified, 83.0% (n = 254/306) were of maternal origin, with a mosa-

icism incidence of 12.6% (n = 32/254). Paternal WCEs accounted for 6.2% of cases (n = 19/306), among which 36.8% (n = 7/19) were mosaic. Finally, 10.8% of WCEs (n = 33/306) originated de novo during mitotic events, displaying a mosaicism incidence of 30.3% (n = 10/33).

Monosomies were the most frequently observed WCE of either maternal or paternal origin, encompassing 53.9% (n = 137/254) and 89.5% (n = 17/19) of cases, respectively. In contrast, trisomies were more prevalent among mitotic WCEs (75.0%, n = 25/33). The incidence of mosaicism differed between monosomies of distinct origins, with the lowest incidence observed in maternal errors (10.2%, n = 14/137) and the highest in paternal monosomies (35.3%, n = 6/17). Conversely, mosaic trisomies were more prevalent among the mitotic errors (38.9%, n = 7/18), followed by maternal meiotic errors (10.2%, n = 14/137). No mosaic paternal trisomy was found.

GG plot analysis was used to determine the origin of trisomies, distinguishing between those arising during gametogenesis (MI or MII) and those occurring post-fertilization due to mitotic errors. Among the 142 trisomies identified, 76.1% (n = 108) originated during MI, with only one case attributed to spermatogenesis. MII errors accounted for 6.3% (n = 9), while 17.6% (n = 25) of trisomies resulted from post-fertilization mitotic errors. Interestingly, all MII errors were non-mosaic trisomies at the time of biopsy, whereas 16.8% (n = 18/107) of MI errors and 28.0% (n = 7/25) of mitotic errors were mosaics.

Additionally, only one case of haploidy, parthenogenesis, and androgenesis were found, with the parthenogenetic case being in a mosaic state. Uniparental disomy (UPD) was diagnosed in a small subset of embryos, i.e., one mosaic case of paternal origin was found, plus a full UPD of maternal origin, and three UPD cases in which the parental and phase of origin were not determined.

Mosaicism incidence by chromosome

To study differences in the incidence of mosaicism in cattle chromosomes, only abnormalities affecting a single chromosome were considered. A total of 306 WCEs and 27 segmental aneuploidies were identified (Figure 2). Cattle [Bos taurus (BTA)] chromosome 14 presented the highest incidence of WCEs (n = 34/306), followed by chromosomes BTA26 (n = 23/306), BTA1 (n = 22/306), BTA15 (n = 22/306), and BTA4 (n = 20/306). Trisomies were the predominant abnormality observed in BTA14 (n = 24/34), with the majority as non-mosaic (full aneuploidy) (n = 21/24). Conversely, chromosomes BTA7 and BTA18 showed only one WCE each. BTA7 was affected with a non-mosaic monosomy, whereas BTA18 presented a mosaic monosomy. Segmental aneuploidies were slightly more prevalent in BTA1 and BTA6 (n = 4/27 each), with one duplication and three deletions. Interestingly, all segmental errors were non-mosaic in BTA6, whereas in BTA1, only the duplication was non-mosaic.

Given the notable differences between WCE and segmental aneuploidies in cattle chromosomes, we investigated whether the size of the chromosome correlated with aneuploidy incidence. As illustrated in Figure 3A, chromosome length does not appear to be associated with the frequency of WCEs. The correlation between chromosome size and WCE frequency was close to zero (r = -0.03, P = 0.87, Pearson's test), indicating no significant relationship. However, a trend was observed in which larger chromosomes exhibited a higher incidence of segmental aneuploidies, although this association did not reach statistical significance (r = 0.24, P = 0.20, Pearson's test) (Figure 3B). When analysing only mosaic errors, the correlation between chromosome length and aneuploidy frequency remained weak for mosaic WCEs (r = 0.07, P = 0.70, Pearson's test) but was slightly stronger for mosaic segmental aneuploidies (r = 0.27, P = 0.12, Pearson's test), though still not statistically significant.

Prevalence of mosaicism in blastocysts of different stages and quality

According to the International Embryo Technology Society (Stringfellow et al., 1990) and Bo and Mapletoft (2013), blastocysts are classified into the following developmental stages: early blastocysts (stage 5), blastocysts (stage 6), expanded blastocysts (stage 7), hatching blastocysts (stage 8), and hatched blastocysts (stage 9). In this study, blastocysts were categorised into three groups: (i) stages 4 to 5 (n = 309), (ii) stage 6 (n = 668), and (iii) stages 7 to 9 (n = 950).

Our data revealed that the incidence of abnormal embryos and mosaicism decreases with advanced blastocyst developmental stage (Figure 4A). Early-stage embryos (stages 4 to 5) presented a higher prevalence of aneuploidy (18.4%, n = 57/309) and mosaicism (5.5%, n = 17/309). In contrast, embryos in stage 6 and stages 7 to 9 demonstrated similar incidences of aneuploidy (11.3%, n = 89/786 and 9.1%, n = 86/950, respectively; P < 0.001, general linear model) and mosaicism (3.7%, n = 29/786 and 3.5%, n = 33/950, respectively; P = 0.11, general linear model).

Cattle embryos are also classified into four quality categories: (i) excellent or good quality, (ii) fair quality, (iii) poor quality, and (iv) dead or degenerating embryos (Bo & Mapletoft, 2013). In the present study, all embryos analysed had a minimum quality code of fair; however, specific quality scores were available for 1,713 embryos (Figure 4B). Embryos classified as excellent or good quality had a significantly lower incidence of aneuploidy (10.7%, n = 115/1,073, P < 0.01, general linear model) and mosaicism (1.7%, n = 18/1,073, P < 0.001, general linear model) compared to fair-quality embryos, which had an aneuploidy incidence of 13.1% (n = 84/640) and a mosaicism incidence of 6.6% (n = 42/640).

Mosaicism incidence by embryo sex

Male (XY) embryos showed a significantly higher incidence of chromosomal abnormalities compared to female (XX) embryos (17.1% vs 12.6%, P < 0.001, chi-square test). However, the incidence of mosaicism was similar between sexes (25.9% in female embryos vs 24.5% in male embryos, P = 0.89, chi-square test). This dataset contained a significantly higher proportion of male embryos (54.9%, n = 1,121/2,042, P < 0.001, chi-square test) (Table 3). Embryos diagnosed with parthenogenesis, androgenesis, or haploidy (n = 3) were excluded from this analysis due to their genetic material originating from only one parent.

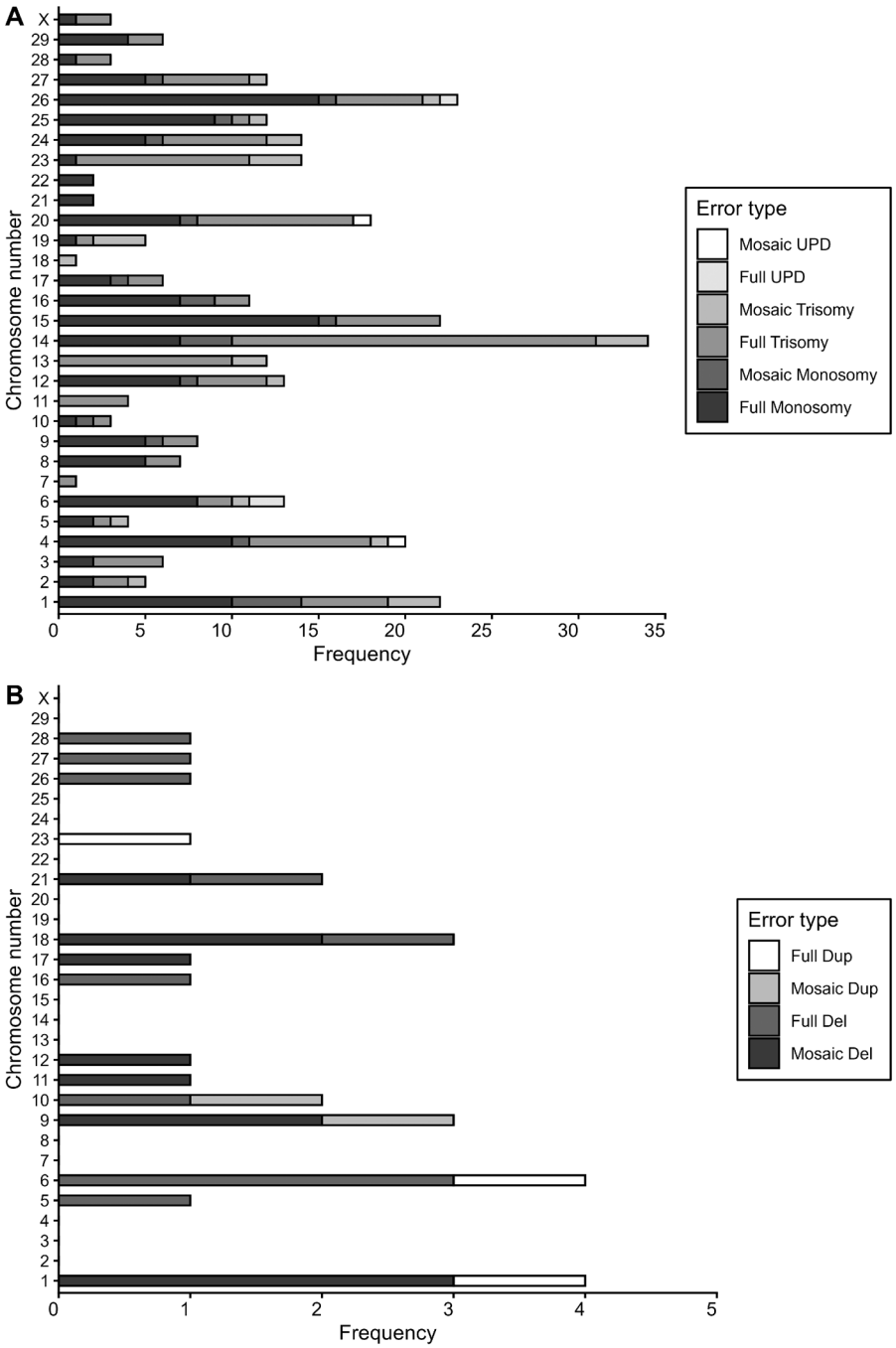

Figure 2 Frequency of mosaic and non-mosaic (full) in (A) WCE ($n = 306$) and (B) segmental errors ($n = 27$). Note: UPD = uniparental disomy.

Discussion

Many cattle breeding programmes rely on in vitro embryo production to perform genetic merit evaluation from SNPChip genotyping data (Fujii et al., 2019; Mullaart & Wells, 2018; Silvestri et al., 2021). In this and previous studies (Silvestri et al., 2021; Tutt et al., 2021, 2023), we have utilised SNPChip data to ask fundamental questions regarding the landscape of chromosome abnormality in early bovine development. In these previous studies, mosaicism was briefly mentioned, while here we expand on our findings and provide an in-depth analysis of this highly important phenomenon by integrating samples from two centres and three biopsy types. Despite variations in biopsy methods, previous human IVF research suggests high concordance between TE and ICM (Takahashi et al., 2021; Tutt et al., 2021; Victor et al., 2019), justifying the combined analysis of all biopsy types to evaluate chromosomal errors and mosaicism rates.

The current study, considered together with the findings of Tutt et al. (2021, 2023) and Silvestri et al. (2021), identified chromosomal errors in 15.2% of blastocysts and mosaicism in 25.6% of blastocysts with a chromosomal error, contributing significantly to the limited genome-wide understanding of chromosomal anomalies

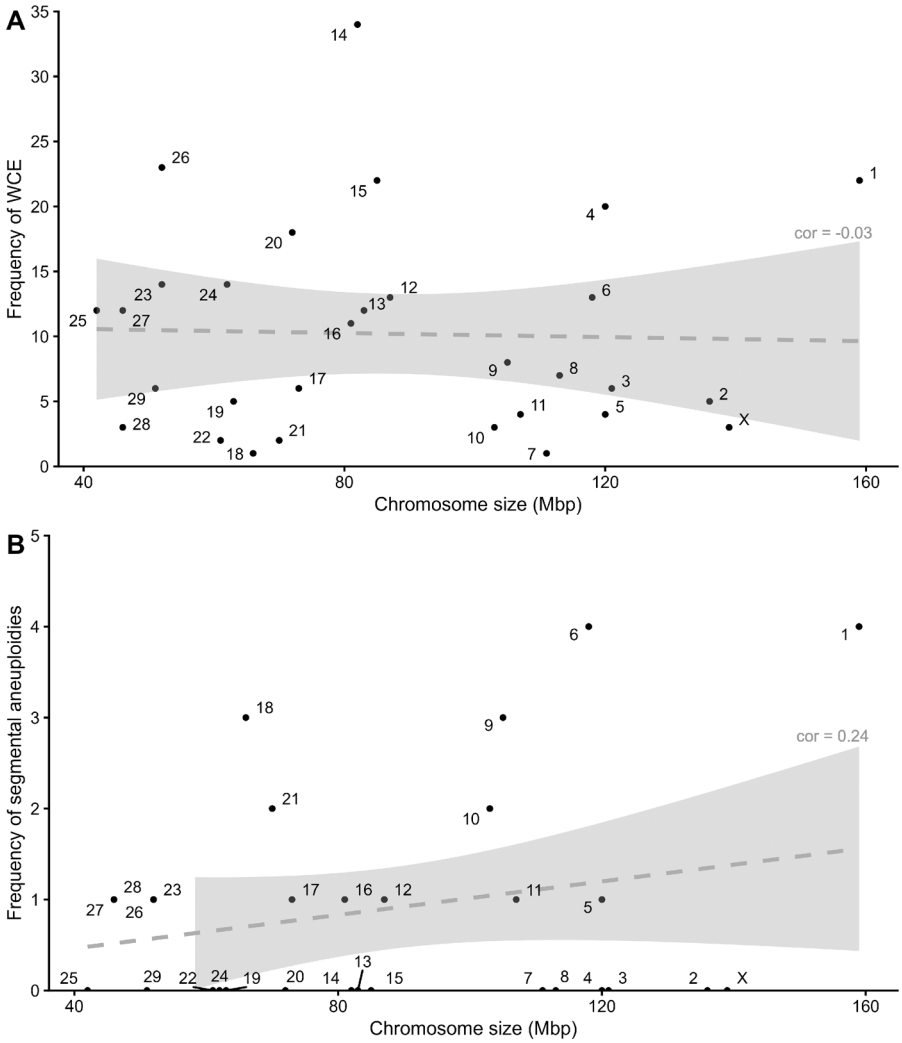

Figure 3 Frequency of (A) whole chromosome errors (WCE) ($n = 306$) and (B) segmental aneuploidies ($n = 27$) per chromosome and size. The grey area represents a linear fitting model with a 95% confidence interval. Note: cor = correlation.

in cattle embryos (Bouwman & Mullaart, 2023; Lonergan et al., 2004; Turner et al., 2019; Viuff et al., 1999). Reported bovine embryo aneuploidy rates in previous studies vary significantly due to differences in methodologies, with previous studies employing fluorescence in situ hybridization showing incidences as high as 100% (Lonergan et al., 2004; Viuff et al., 1999), while sequencing-based methods reported lower values, ranging from 8.9% (Bouwman & Mullaart, 2023) to 68.8% (Turner et al., 2019). Unlike previous studies that focused on a limited number of chromosomes, this study provides a more comprehensive genomic perspective.

Maternal-origin chromosomal errors were the most prevalent (73.5%), consistent with findings in humans, where oocytes contribute more errors than sperm (Greaney et al., 2018; Nagaoka et al., 2012; Wartosch et al., 2021). Whole chromosome anomalies, primarily maternal in origin, arose mainly during MI, aligning with other observations performed in human IVF (Hassold & Hunt, 2001; Rana et al., 2023) and cattle IVP studies (Bouwman & Mullaart, 2023; Turner et al., 2019). Mosaicism rates for WCEs were similar between monosomies (13.2%) and trisomies (17.6%), again a finding comparable to human studies (Coll et al., 2021; Mourad et al., 2021), highlighting the utility of the cattle model for genetic and embryological studies.

Segmental aneuploidies, predominantly mitotic in origin (66.7%), had a notable paternal contribution (29.6%), aligning with findings in human (McCarty et al., 2022; Rodrigo et al., 2019) and murine studies (Gao et al., 2023) describing how spermatogenesis is a key stage for segmental chromosomal defects. A recent study in mice suggested that spermatogenesis is the main stage during which segmental chromosomal errors arise, particularly during chromatin repackaging and the replacement of histones with protamines. The torsional forces during this stage can induce a high number of double-strand breaks, which likely contribute to the formation of segmental aneuploidies (Álvarez-González et al., 2022; Gao et al., 2023). The high mosaicism rate in segmental aneuploidies (48.1%) further supports findings from human IVF research (Gruhn et al., 2019; Girardi et al., 2020; Picchetta et al., 2023). Triploidy analysis, assessed via Karyomapping and SNP arrays, revealed a near-equal incidence of paternal (51.2%) and maternal (48.8%) origins, contrasting with previous bovine studies reporting maternal dominance (Bouwman & Mullaart, 2023; Turner et al., 2019).

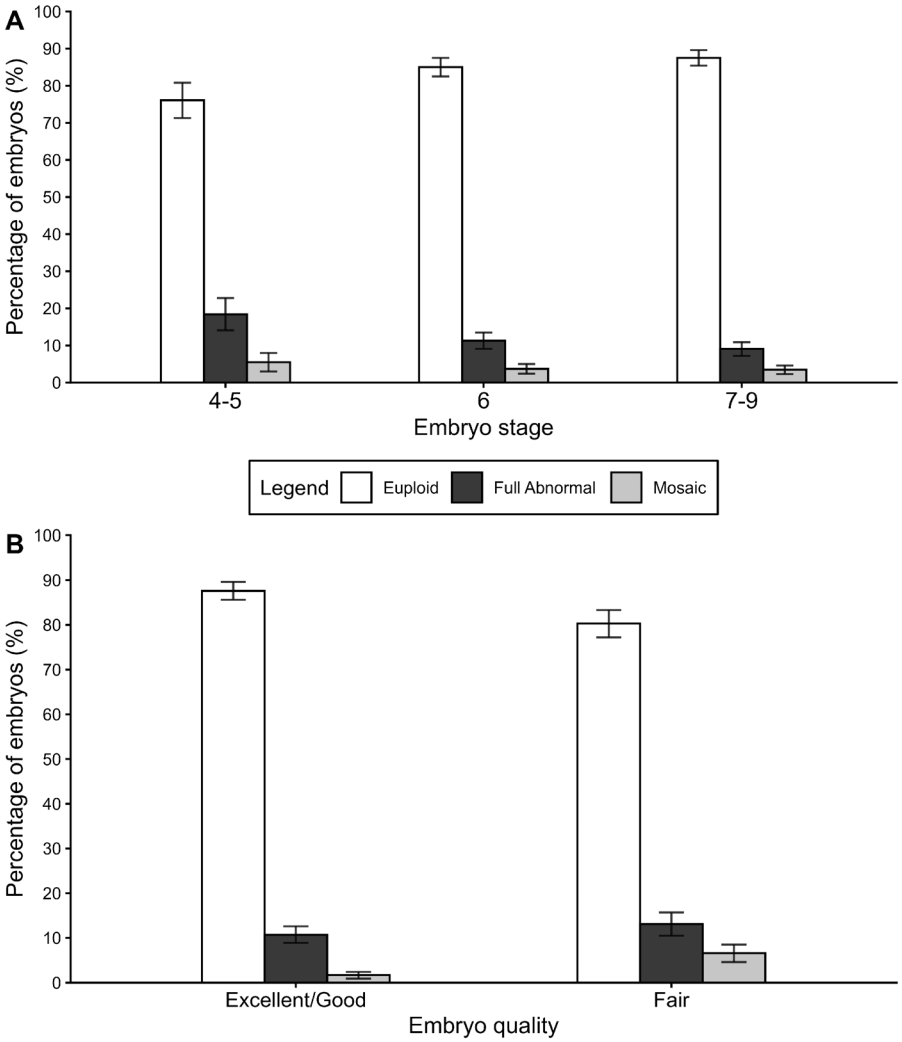

Figure 4 Incidence of ploidy status [euploidy, full abnormal (non-mosaic) and mosaic] per (A) embryo stage and (B) embryo quality (n = 1,713) after preimplantation genetic testing for aneuploidy analysis. Data presented as percentages with 95% confidence intervals.

Table 3 Sex ratio and incidence of chromosomal errors and mosaicism per embryo sex.

Sex	*n* (%)	Abnormal embryos (%)	Mosaic embryos (%)
Female	921 (45.1)	12.6 (116/921)	25.9 (30/116)
Male	1,121 (54.9)	17.1 (192/1,121)	24.5 (47/192)

The increased paternal contribution suggests alternative fertilization mechanisms, such as double-sperm fertilization or diploid sperm involvement (Rosenbusch, 2008). Mosaic triploidy, including mixoploidy, was also observed, reinforcing previous findings (De Coster et al., 2021, 2022).

WCEs were most frequent in chromosomes BTA1, BTA4, BTA14, BTA15, and BTA26. While previous studies identified BTA13 and BTA29 as having higher aneuploidy rates (Turner et al., 2019), the discrepancy likely stems from differences in SNPChip analysis methodologies and sample sizes. Unlike some human studies, no significant correlation between chromosome size and aneuploidy incidence was detected (Coll et al., 2021).

In this study, a 20% threshold for mosaicism detection was applied, consistent with prior human IVF guidelines (Cram et al., 2019). As such, any chromosome error that affects <20% of the embryonic cells will be discarded and the embryo labelled as euploid, indicating a limitation in detecting low-level mosaicism. In cases where mosaic errors originated in parents, an incomplete self-correction during embryo development might lead to this low proportion of cells affected by the chromosome error and potential misdiagnosis. These self-correction events in embryos have been reported in several species, including cattle (Nagai et al., 2021), humans (Barbash-Hazan et al., 2009; Orvieto et al., 2020), and mice (Bolton et al., 2016; Singla et al., 2020). We also found that the later the stage, the higher the incidence of euploidy (range 76.1% to 87.4%), while the prevalence of chromosome errors in their full or mosaic state decreased. These trends align with human (Yao et al., 2018; Barbash-Hazan et al., 2023) and bovine data, indicating that chromosomally abnormal embryos often exhibit reduced developmental potential. Similarly, excellent/good embryos presented fewer full (2.4%) and mosaic (4.9%) chromosome errors, indicating that abnormal embryos tend to be of lower morphological quality (as assessed visually), impacting

their development and leading to embryo fragmentation (Angel-Velez et al., 2023; McQueen et al., 2021; Pennetta et al., 2018; Ulloa et al., 2008; Yao et al., 2018).

Concluding remarks

This study advances our understanding of the incidence and nature of chromosomal mosaicism in bovine embryos. Our results reinforce previous findings obtained in human IVF studies, showing that these insights could possibly extend to other mammalian species. Nevertheless, investigation in other mammalian species would be important to confirm this. By identifying chromosomal abnormalities, breeders and researchers can make informed decisions about which embryos to transfer and in what order. For example, segmental aneuploidies show higher implantation potential than WCEs (Victor et al., 2019; Viotti et al., 2021). Through the gradual development of similar guidelines in cattle, these findings will contribute to refining embryo selection strategies and improving reproductive success in cattle breeding programs that utilise gEBVs.

Supplementary material

Supplementary material is available at *Reproduction* online.

Author contributions

C.C.-R. conceptualised the study, conducted the analysis, and drafted the manuscript. D.K.G. acquired funding and critically reviewed the manuscript. K.D.S. acquired funding, collected the data, and critically reviewed the manuscript. R.L. collected the data. M.F. supervised part of the study and drafted the manuscript. G.S. supervised the study and drafted the manuscript. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

R.L. was an employee of Boviteq at the time of the study.

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Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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