Received: 12 October 2022 | Accepted: 22 November 2022

DOI: 10.1111/age.13280

RESEARCH ARTICLE

ANIMAL GENETICS WILEY

Characterisation of eight cattle with Swyer syndrome by whole-genome sequencing

Donagh P. Berry[1] | **Emily K. Herman**[2] | **Tara R. Carthy**[1] | **Rebecca Jennings**[3] | **Nahid Bandi-Kenari**[2] | **Rebecca E. O'Connor**[3] | **John F. Mee**[1] | **Jim O'Donovan**[4] | **Daragh Mathews**[5] | **Paul Stothard**[2]

[1]Teagasc, Animal & Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland

[2]Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada

[3]School of Biosciences, University of Kent, Canterbury, UK

[4]Department of Agriculture, Food and the Marine, Regional Veterinary Laboratory, Cork, Ireland

[5]Irish Cattle Breeding Federation, Ballincollig, Co. Cork, Ireland

Correspondence
Donagh P. Berry, Teagasc, Animal & Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland.
Email: donagh.berry@teagasc.ie

Funding information
Science Foundation Ireland; Government of Ireland, Grant/Award Number: 16/RC/3835

Abstract

Swyer syndrome is where an individual has the karyotype of a typical male yet is phenotypically a female. The lack of a (functional) *SRY* gene located on the Y-chromosome is implicated in some cases of the Swyer syndrome, although many Swyer individuals with an apparently fully functional *SRY* gene have also been documented. The present study undertook whole genome sequence analyses of eight cattle with suspected Swyer syndrome and compared their genome to that of both a control male and female. Sequence analyses coupled with female phenotypes confirmed that all eight individuals had the 60,XY sex reversal Swyer syndrome. Seven of the eight Swyer syndrome individuals had a deletion on the Y chromosome encompassing the *SRY* gene (i.e., *SRY*−). The eighth individual had no obvious mutation in the *SRY* gene (*SRY*+) or indeed in any reported gene associated with sex reversal in mammals; a necropsy was performed on this individual. No testicles were detected during the necropsy. Histological examination of the reproductive tract revealed an immature uterine body and horns with inactive glandular tissue of normal histological appearance; both gonads were elongated, a characteristic of most reported cases of Swyer in mammals. The flanking sequence of 11 single nucleotide polymorphisms within 10 kb of the *SRY* gene are provided to help diagnose some cases of Swyer syndrome. These single nucleotide polymorphisms will not, however, detect all cases of Swyer syndrome since, as evidenced from the present study (and other studies), some individuals with the Swyer condition still contain the *SRY* gene (i.e., *SRY*+).

KEYWORDS
cattle, disorder of sexual development, gonadal dysgenesis, intersexuality, karyotype, sex reversal

INTRODUCTION

Sex reversal can occur when a discrepancy exists between the karyotype-dictated sex of an individual and its external phenotype (Parma et al., 2016). Gonadal dysgenesis is used to describe any congenital developmental disorder of the reproductive system in either males or females. XY gonadal dysgenesis, which is more commonly known as Swyer syndrome, was first coined by Swyer in 1955 (Swyer, 1955) and is a type of hypogonadism. In bovines, an individual with Swyer syndrome has a 60,XY karyotype and generally, although not always (Sharma

et al., 1980), presents with normal female external genitalia. Swyer syndrome is a rare type of disorder (or difference) of sexual development (DSD) and can also be called testicular feminisation syndrome (Morris, 1953). Several cases of XY sex reversals have been reported in bovines (Chapman et al., 1978; Hare et al., 1994; Kondoh et al., 1992; Macmillan et al., 1984; Sharma et al., 1980) as far back as 1967 (Henricson & Åkesson, 1967). In most cases, the reproductive tract is underdeveloped and ovaries, if present, are usually elongated (Chapman et al., 1978; Henricson & Åkesson, 1967; Kondoh et al., 1992; Sharma et al., 1980). Swyer syndrome has also been reported in a multitude of other species including humans (Khare et al., 2017), horses (Raudsepp et al., 2010), dogs (Whyte et al., 2009), and sheep (Ferrer et al., 2009).

The cause of XY gonadal dysgenesis is not known in all cases. In humans, the *SRY* gene is deleted in approximately 10%–15% of Swyer individuals with a mutated *SRY* gene present in an additional 10%–15% (Da Silva et al., 2015; King & Conway, 2014). The *SRY* gene is located on the Y chromosome of mammals and this gene initiates both a genetic and hormonal pathway leading to the formation of male testes and the male phenotype in general (Albarella et al., 2020). Individuals without a functional *SRY* gene (i.e., often termed *SRY*− individuals as opposed to *SRY*+ individuals that are carrying the *SRY* gene) will activate a different pathway directing the development of ovaries (De Lorenzi et al., 2018) and an eventual female phenotype. In cattle and buffalo, both *SRY*− (Kawakura et al., 1996) and *SRY*+ (De Lorenzi et al., 2018; Iannuzzi et al., 2004) Swyer syndrome individuals have been reported. *SRY*+ and *SRY*− individuals with Swyer syndrome have also been reported in other species such as horses (Raudsepp et al., 2010; Switonski et al., 2005) and humans (Khare et al., 2017; Michala et al., 2008). Several other putative genes have also been linked with Swyer syndrome (Khare et al., 2017).

In Ireland, commercial producers and seedstock breeders submit ear biopsies from a selection of their female cattle for genotyping as part of a voluntary cattle breeding scheme. The objective of the scheme is to accelerate genetic gain in the national beef herd by financially incentivising producers to retain their genetically elite females. The goal was to genotype approximately 800 000 females annually. During the operation of the scheme, discrepancies arose between the producer-reported sex of a submitted sample (i.e., female) and the sex derived from its genotype. A total of eight individuals where such discrepancies persisted despite repeated animal sampling and analyses were investigated further. The objective of the present study was to examine the whole genome sequence of these eight individuals in an attempt to first confirm the presence of both X and Y chromosomes followed by identifying polymorphisms that could be incorporated into genotype panels to diagnose Swyer conditions. A necropsy of one of the individuals was also undertaken.

MATERIALS AND METHODS

Single nucleotide polymorphism genotyping

Eight suspected Swyer individuals were identified using 52 691 single nucleotide polymorphisms (SNPs) genotyped on a custom genotype platform developed for Ireland (i.e., International Dairy Beef, Version 2). All individuals were genotyped three times. The genotype panel used includes 386 SNPs reportedly on the non-pseudoautosomal part of the X chromosome and 19 SNPs on the Y chromosome. All but three SNPs in the non-pseudoautosomal part of the X chromosome were homozygous across all eight suspected Swyer individual whereas a genotype was called for all SNPs on the Y-chromosome of each suspected Swyer individual; these three SNPs are likely to be positional errors since they were also heterozygous in a large proportion of other females genotyped nationally. A genotype (and stored DNA) was also available for the dam of one of the suspected Swyer individuals. With the exception of a purebred Angus and a purebred Charolais, all six remaining suspected Swyer individuals were crossbred animals.

Necropsy

The complete reproductive tract and mammary gland of one of the individuals were collected immediately after slaughter in a commercial abattoir. Both organs were examined grossly and photographed. The entire reproductive tract and four representative samples of mammary tissue were fixed in 10% formalin within 1 h of death. The ovaries and cross sections of the tubular reproductive tract at the uterine body and horns and mammary tissue, were paraffin-embedded and processed as 5-μm sections and stained with haematoxylin and eosin. Heparinised blood samples were also taken and, along with samples of the reproductive tract and mammary gland, were all sent for separate cytogenetic analyses.

Cytogenetic analysis

Cytogenetic analyses of the animal that underwent necropsy were undertaken by the University of Kent in the UK. The heparinised blood samples were cultured for 72 h in PB MAX Karyotyping medium (Invitrogen) at 37°C, 5% CO_2. Cell division was arrested by adding colcemid at a concentration of 10.0 μg/ml (Gibco) for 35 min before hypotonic treatment with 75 mM potassium chloride and fixation to glass slides using 3:1 methanol:acetic acid. Metaphases for karyotyping were stained with 4′,6-diamidino-2-phenylindole in VECTASHIELD® antifade medium (Vector Laboratories). Image capturing was performed using an Olympus BX61 epifluorescence microscope with cooled charge coupled device camera

and SmartCapture (Digital Scientific UK) system for 10 samples. SMARTTYPE software (Digital Scientific UK) was used for karyotyping purposes and chromosomes were arranged according to the International System for Chromosome Nomenclature of Domestic Bovids (2000) (Cribiu et al., 2001).

Fluorescently labelled bovine DNA probes for the X chromosome were hybridised to metaphase chromosomes of the sample. The Y chromosome was detected in all hybridisations observed. To rule out Freemartins (Padula, 2005), or blood chimaeras, karyotypes were produced from metaphase chromosomes of both the reproductive tract and mammary gland. Fluorescence in situ hybridisation (FISH) probes were developed using bacterial artificial chromosomes (BACs) in the subtelomeric region of the p-arm (CH240-121E1) and q-arm (CH240-47J20) of the X chromosome. These were identified from the Btau 4.6.1 NCBI database and ordered from the CHORI-240 Bovine BAC library. BAC DNA was isolated using the Qiagen Miniprep Kit, the products of which were then amplified and directly labelled by nick translation with FITC-Fluorescein-12-UTP (Roche) for clone CH240-121E1 and Texas Red-12-dUTP (Invitrogen) for clone CH240-47J20 prior to purification.

Metaphase preparations were fixed to slides and dehydrated through an ethanol series (2 min each in 2×SSC, 70%, 85% and 100% ethanol at room temperature). Probes were diluted in a formamide buffer (Cytocell) with Bovine Hybloc (Insight Biotech) and applied to the metaphase preparations on a 37°C hotplate before sealing with rubber cement. Probe and target DNA were simultaneously denatured on a 75°C hotplate prior to hybridisation in a humidified chamber at 37°C overnight. Slides were washed post-hybridisation for 2 min in 0.4×SSC at 72°C followed by 30 s in 2×SSC/0.05% Tween 20 at room temperature, then counterstained using VECTASHIELD anti-fade medium with DAPI (Vector Labs). Images were captured using an Olympus BX61 epifluorescence.

Sequence data

Ear biopsy tissue samples from seven of the eight Swyer animals and one dam as well as a blood sample from the individual that underwent necropsy were sequenced at Edinburgh Genomics using the Illumina HiSeqX system and a HiSeqX Ten Reagent kit v2.5. Sequence reads were aligned to the ARS-UCD1.2_Btau5.0.1Y.fa reference genome sequence (autosomes from assembly ARS-UCD1.2 and the Y chromosome from assembly Btau5.0.1) using BURROWS-WHEELER ALIGNER v0.7.17 (Heng & Durbin, 2009). Potential variant sites were detected using GATK HaplotypeCaller, and the resulting GVCF files were jointly genotyped with GATK GenotypeGVCFs. SMOOVE v0.2.6 (Pedersen et al., 2020; Pedersen & Quinlan, 2018) was used to detect and genotype structural variants (SVs), excluding gapped regions and regions where coverage was more than four standard deviations higher than the mean coverage, calculated across 10 000 bp windows by MOSDEPTH v0.3.2 (Pedersen & Quinlan, 2018).

Sex chromosome assessment from sequence data

Several measurements were used to estimate the sex chromosome complementation of samples from the high-throughput sequencing results: X chromosome heterozygous SNP proportion, Y chromosome missing genotype proportion, X:Y coverage ratio, X:autosome coverage ratio, and Y:autosome coverage ratio. To calculate coverage ratios, the SAMTOOLS v1.12 idxstats command (Li et al., 2009) was used to count the number of reads in the BAM files mapping to the X and Y chromosomes and chromosome 10. The counts were divided by the respective chromosome length prior to calculating the final ratios. X chromosome heterozygous SNP proportion was calculated from the SNP VCF file using the VCFTOOLS v0.1.16 het option (Danecek et al., 2011). Only SNPs with a FILTER value of PASS were used in the calculation. Y chromosome missing genotype proportion was calculated using the BCFTOOLS v1.8 query command (Danecek et al., 2021). Fold coverage of each genome was calculated using GATK DepthOfCoverage with –minBaseQuality 15 and –minMappingQuality 30. These metrics were calculated using sequence data from an additional 20 control animals (9 female and 11 male) available from NCBI SRA (BioProject accession PRJNA783321) after it was processed using the same alignment and variant calling procedures.

BAM file visualisation

A list of 60 genes linked to DSDs in humans was compiled (Table S1) from two recent review papers (Audi et al., 2018; Baetens et al., 2019). The locations of the autosomal, X-linked, and chromosome-unassigned bovine orthologs of these genes were obtained for the ARS-UCD1.2 assembly using Ensembl BioMart (release 105) (Howe et al., 2021). The location of *SRY* on the Btau5.0.1 assembly Y chromosome was retrieved from NCBI (release 105) (Sayers et al., 2021). INTEGRATED GENOMICS VIEWER (IGV) v2.12.3 (Thorvaldsdóttir et al., 2013) was used to inspect the DSD gene regions (including 1 kb upstream and 1 kb downstream of the transcription start and end site, respectively) in each sample. Briefly, the BAM files from all samples were loaded into integrative genomics viewer (IGV) along with the DSD gene locations with added flanking sequence in BED format. An attributes file was supplied so that sample information was displayed in IGV. Each gene region was inspected for visual evidence of low or absent reads. Read pairing

and insert size information was examined for evidence of deletions, insertions, inversions, and translocations. A similar IGV analysis was conducted for the Y chromosome. For this analysis, all Y chromosome gene locations were obtained from NCBI and inspected to identify regions of missing coverage in the Swyer samples relative to the control samples. This visualisation was used in conjunction with the automated SV detection performed using Smoove.

Sliding-window read depth plots were created for the samples sequenced in this study and a control XY animal (NCBI BioSample ID SAMN23427834) using a custom Python script that first determines read counts at all positions using the SAMTOOLS v1.12 depth command (Li et al., 2009), then calculates mean depth for each window, and finally plots the results using the MATPLOTLIB v3.5.1 (Hunter, 2007). A window size of 10 000 bp was used for all plots.

SNPs and indels were annotated with functional impact predictions using SnpEff v5.0e (Cingolani, Platts, et al., 2012) and the ARS-UCD1.2.99 SnpEff annotation database. Structural variants annotation was performed using Ensembl VEP (release 105) (McLaren et al., 2016). Because these approaches did not assign impact predictions to Y-chromosome variants (due to the absence of Y chromosome information in the reference databases), the input VCF files were manually inspected for variants overlapping with the *SRY* gene.

Preliminary analyses revealed that the *SRY* gene was present in only one of the Swyer individuals, the individual that underwent the necropsy. For this case, variant filtering was used to identify other candidate causal variants. Three filters, termed 'recessive DSD', 'dominant DSD', and 'de novo', were applied. The 'recessive DSD' filter was used to identify high- or moderate-impact DSD gene variants with a homozygous non-reference-allele genotype in the *SRY*+ sample. The 'dominant DSD' filter was implemented in a similar manner but sought high- or moderate-impact variants with a heterozygous genotype in the *SRY*+ sample. These two filters were applied to SNPs, indels, and SVs. SNPs and indels passing these filters were then assessed in the 1000 Bull Genomes Project Run 9 data set, consisting of whole-genome SNP and indel genotypes from 5116 animals, to rule out common variants. The 'de novo' filter was applied to SNPs and indels and was used to identify high- or moderate-impact variants with a heterozygous genotype in the *SRY*+ individual that were not observed in any animal in the 1000 Bull Genomes Project Run 9 (Hayes & Daetwyler, 2019). Furthermore, only variants with a FILTER value of PASS were kept. These filters were implemented using the BCFTOOLS v1.8 isec command (Danecek et al., 2021), SnpSift v5.0e (Cingolani, Patel, et al., 2012) and standard command-line text-processing utilities.

RESULTS

Sequence analyses

Sequence coverage and genotype analysis supported the presence of an X and Y chromosome in all eight suspect Swyer individuals (Table S2, Figure 1). The external phenotypes of all eight suspect Swyer individuals suggested they were all female. Together, these two pieces of information confirmed that all eight individuals were indeed suffering from Swyer syndrome. IGV inspection of sequence read alignments (BAM files; Figure 2) and

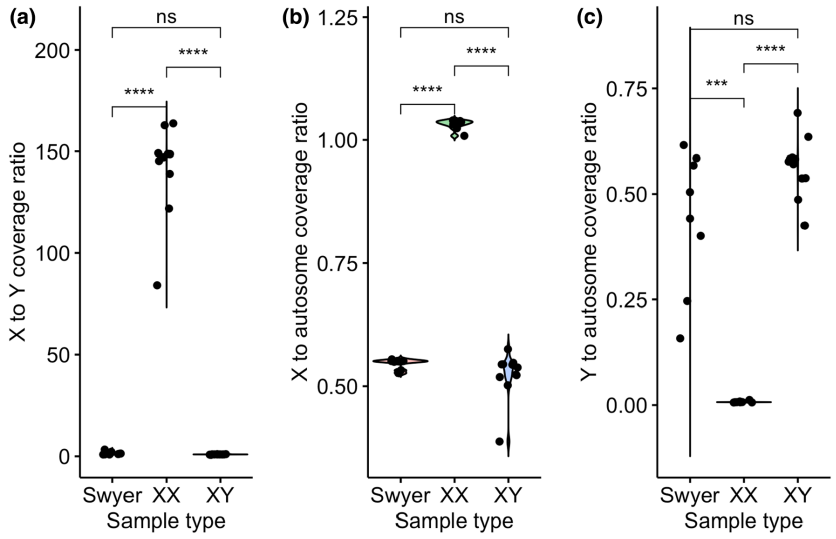

FIGURE 1 Sex chromosome assessment using sequence data of eight Swyer samples and 20 control animals (10 female, labelled as XX; 11 male, labelled as XY). Violin plots show the distribution in Swyer, XX, and XY groups of X to Y coverage ratio (the average is 1.5 in the Swyer group and 0.9 in the XY group; (a), X to autosome coverage ratio (b), and Y to autosome coverage ratio (c). Means were compared between groups using Welch's *t*-test and the results are displayed as $*p<0.05$, $**p<0.01$, $***p<0.001$, $****p<0.0001$, or ns, $p>0.05$.

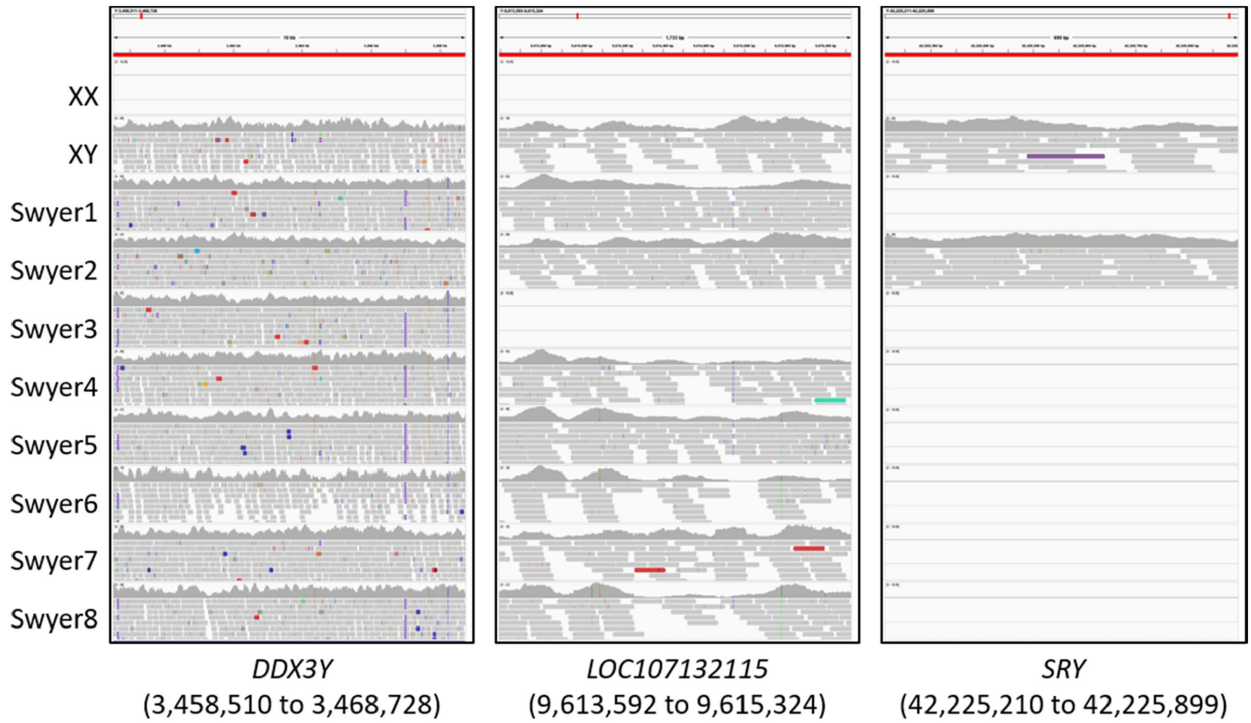

FIGURE 2 Integrative genomics viewer (IGV) visualisation of three loci on the Y chromosome in control (XX and XY) and Swyer individuals

sliding-window read depth plots (Figure 3) also indicated that all Swyer individuals and the XY individual had coverage of *DDX3Y*, indicating the presence of Y-chromosome sequence. One Swyer individual (Swyer 3 in Figure 2) had no coverage on *LOC107132115* and all but one Swyer individual (Swyer 2 in Figure 2) was missing coverage for the *SRY* gene. In six of these seven *SRY*− individuals, the deletion spanned from 42.1 Mb to the end of the chromosome (43.3 Mb); the deletion was much larger in seventh sample (from 10 to 43.3 Mb).

The coverage on the Y chromosome in the *SRY*− individuals was more uneven across the chromosome compared to the control where even coverage of the Y chromosome was evident (Figure 3). Sliding-window plots revealed peculiar coverage pattern on the Y chromosome of the Swyer individuals, where long segments of normal, intermediate, and missing coverage were observed.

No candidate causal SNP, indel, or SV in known DSD genes was identified in the *SRY*+ individual (using the 'recessive DSD' and 'dominant DSD' filters and the 1000 Bull Genomes Project Run 9 genotypes). The recessive DSD filter identified no SVs and 13 SNP/indels, but all were observed at moderate to high frequency in the reference set of genotypes (allele frequency ≥ 0.21). The dominant DSD filter identified no SVs and 25 SNP/indels but these too were not considered compelling candidate mutations due to their frequency in the reference data set (allele frequency ≥ 0.003). The 'de novo' filter produced 125 variants (Table S3), but due to the lack of known roles in sex determination/development of the affected genes, it is difficult to refine this list further to produce a specific candidate causal mutation.

All eight Swyer syndrome individuals in the present study were born as singletons. All but one had no recorded assistance at calving with one individual (not the *SRY*+ individual) having had experienced 'slight assistance' at birth. Of the dams that gave birth to all eight Swyer individuals, three were parity one, three were parity three, one was parity four (*SRY*+ individual) and one was parity nine. All Swyer individuals had half-sib females and, for six of the eight Swyer individuals, at least one female half-sib had at least one progeny; the *SRY*+ individual had three heifer half-sibs none of which had a recorded progeny. Moreover, at least one female half-sib of all eight Swyer individuals was genotyped with no noted discrepancy between the sex determined from the genotype vs. that reported by the producer. All Swyer individuals were slaughtered between age 530 and 888 days with the exception of two of the individuals being slaughtered at age 969 and 1043 days. None of the Swyer individuals had a recorded progeny and, in fact, none had a recorded service, either artificial or natural.

Cytogenetic and necropsy observations of *SRY*+ individual

All karyotype analysis of the blood, mammary, and reproductive tract of the *SRY*+ individual revealed 60,XY indicating no mosaicism with 60,XX; it also revealed the presence of the *SRY* gene.

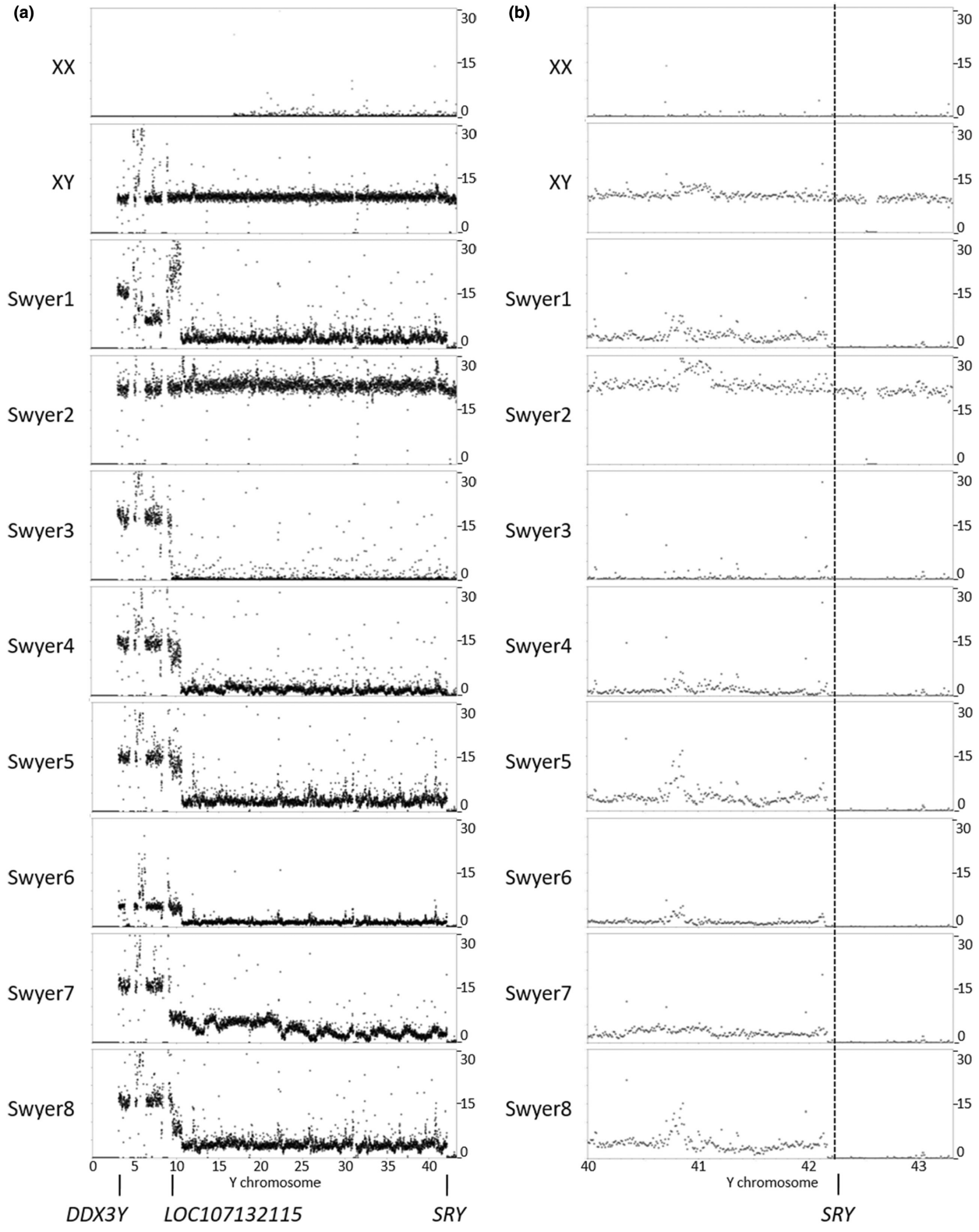

FIGURE 3 Y-chromosome sliding-window read depth plots for control (XX and XY) and Swyer animals. (a) Animals XY and Swyer2 have even coverage across the Y-chromosome whereas the other Swyer animals displayed reduced coverage starting near 10 Mb. (b) Coverage near the end of the Y chromosome falls to zero before the *SRY* gene (dashed line) except in the case of Swyer3, which appears to have a much larger deletion starting near 10 Mb.

Macroscopic examination of the reproductive tract of the single (*SRY*+) individual that underwent necropsy revealed the presence of a vulva, single cervix, single uterine body with two horns and two ovaries; no testicles were detected. The reproductive tract was underdeveloped but had normal gross morphology. The vulva, vagina, and cervix were unremarkable. The uterine body was 8 cm long from the external cervical os to the corneal

bifurcation and each horn was 6 cm long. Each ovary appeared flattened (3 × 1 × 0.5 cm) and vestigial with longitudinal surface striations; neither contained a corpus luteum nor evidence of ovulation. The mammary gland had four teats and a pad of fatty tissue approximately 10 cm thick.

Histological examination of the reproductive tract revealed an immature uterine body and horns with inactive glandular tissue of normal histological appearance. The ovarian cortices contained small numbers of recognisable oocytes (primordial follicles) and primary follicles and frequent atypical follicles with two or more layers of granulosa cells but no development of recognisable theca layers and no oocyte. The mammary gland contained adipose tissue traversed by several small and medium calibre arteries, veins and nerves but with no glandular elements (acini, ducts). The mammary skin had no recognisable adnexa in the dermis and very mild superficial eosinophilic perivascular dermatitis.

DISCUSSION

The extent that (breeding) animals are being genotyped for circa 50 000 SNPs is increasing globally. Many such genotype panels have SNPs on both the X and Y chromosome and individuals that are homozygous for the non-pseudoautosomal section of the X chromosome, as well as having called genotypes for SNPs on the Y chromosome, are interpreted as being male (McClure et al., 2018). Inconsistency between the predicted sex from the resulting genotype vs. that observed by the producer can lead to considerable resources being expended trying to reconcile the discrepancy. Moreover, whereas cases of discordance between phenotypic and genotypic sex are rare, they can fuel producer cynicisms of genotyping results and DNA-based breeding technologies. One objective therefore of the present study was to ideally identify genomic polymorphisms that could be incorporated into routine genotyping plans to expedite the reconciliation process by informing the producer that the individual may suffer from Swyer syndrome; this should all be achieved without the need for additional effort of cytogenic analyses.

All eight suspected Sywer individuals in the present study were confirmed to have the Swyer condition with seven of the eight not having the *SRY* gene (*SRY*− individuals) and the eighth individual having the *SRY* gene (*SRY*+) with no obvious mutation within. While there has been numerous reports on Swyer syndrome in cattle (Chapman et al., 1978; Hare et al., 1994; Henricson & Åkesson, 1967; Kondoh et al., 1992; Macmillan et al., 1984; Sharma et al., 1980), many of those were before the *SRY* gene was discovered in 1990 (Sinclair et al., 1990) meaning that the *SRY* status of the individuals in those studies could not be determined. Kawakura et al. (1996) reported on three *SRY*− cattle from different breeds while De Lorenzi et al. (2018) reported on what they described as the first reported case of *SRY*+ in Holstein cattle. Neither study had access to whole genome sequence data as was the case in the present study.

None of the Swyer individuals had a recorded progeny and, in fact, none had a recorded service, either artificial or natural. While it cannot be ruled out that all females had been reared for meat production rather than breeding, the lack of any observable expression of oestrus is a common feature of both cattle (Chapman et al., 1978; Henricson & Åkesson, 1967; Sharma et al., 1980) and humans (Ferrer et al., 2009) with Swyer syndrome.

Sequence data

The presence of variability in the length of deletions in the *SRY*− individuals in the present study has also been previously documented in other species like horses (Raudsepp et al., 2010). Lange et al. (2009) speculated that deletions on the Y chromosome could be due to interchromatid recombination events between repeated sequences contributing to a deletion in a given sperm but a duplication in another. The fact that at least one female half-sib of the eight Swyer individuals had been genotyped with no reported discrepancy between the genotype-derived sex and the producer-reported sex, along with the fact that no mutation in the *SRY* gene was detected in the one sequenced dam of one of the Swyer individuals, suggests that the deletions on the Y chromosome are de novo deletions in the *SRY*− individuals. Nevertheless, familial cases of Swyer syndrome have been documented in humans (Michala et al., 2008). The significance of the pattern of uneven coverage of the Y chromosome in the *SRY*− individuals in the present study is not known. Perhaps further Y chromosome deletions occur at high frequency in these individuals, leading to variable coverage when DNA is extracted from a population of cells.

The cause of DSDs in *SRY*+ individuals is unknown, with Raudsepp and Chowdhary (2016) speculating that it could involve both sex-linked and autosomal factors but that the effects are likely to be heterogeneous. The influence of non-genetic effects can also not be ruled out. Raudsepp and Chowdhary (2016) concluded that only a small proportion of DSDs in domesticated animals (both XX and XY) can be explained by aberrations in the sex chromosome with the cause of the outstanding cases remaining unknown, including that of the *SRY*+ individual in the present study. De Lorenzi et al. (2018) also reported on an *SRY*+ Holstein animal but other than information on 18 microsatellites which are used for parentage testing in cattle, genomic information from no other part of the genome was available. The present study failed to resolve this dilemma any further, given that no strong candidate causal SNP, indel, or SV in known DSD genes were identified in the *SRY*+ individual.

Cytogenetic and necropsy observations of *SRY*+ individual

FISH analyses detected the Y chromosome in all hybridisations observed for the *SRY*+ individual (Figure 4). Given that 70 interphase nuclei FISH images and 17 metaphase FISH images along with 11 karyotypes were examined, mosaicism of ≥4.5% can be excluded with 99% confidence or mosaicism of ≥3% with 95% confidence (Hook, 1977).

The underdeveloped reproductive tract observed in the present study is in line with clinical examinations reported elsewhere of Swyer cattle (Chapman et al., 1978; De Lorenzi et al., 2018) albeit others like Henricson and Åkesson (1967) reported a normal sized uterus in their two Swyer Swedish Red and White dairy individuals. Smaller uterine size has also been reported in Swyer humans relative to controls (Michala et al., 2008). Consistent with the present study, a flattened ovary has also been documented in an almost 3-year-old Swyer Charolais individual (Chapman et al., 1978) albeit only one gonad was detected in that heifer. Elongated, streaked or 'almond-shaped' (Henricson & Åkesson, 1967) gonads is a common feature of cattle with Sywer syndrome (Kondoh et al., 1992; Sharma et al., 1980). Similar to the present study, primordial follicles have been detected on the ovaries of Swyer cattle (Chapman et al., 1978).

Whether explicitly mentioned (De Lorenzi et al., 2018; Henricson & Åkesson, 1967; Kondoh et al., 1992), or implied from the lack of any report of such from internal clinical examinations of cattle with Swyer syndrome (Chapman et al., 1978), in line with the present study, no testicles appear to exist in cattle with the Swyer condition. In their analysis of 18 XY sex reversals in horses, Raudsepp et al. (2010) suggested that the lack of testes is a feature of *SRY*− individuals while three of their *SRY*+ horses displayed male pseudohermaphrodite characteristics. Of the two known cases of *SRY*+ in cattle (i.e., the present study, De Lorenzi et al., 2018), no evidence of testes were observed.

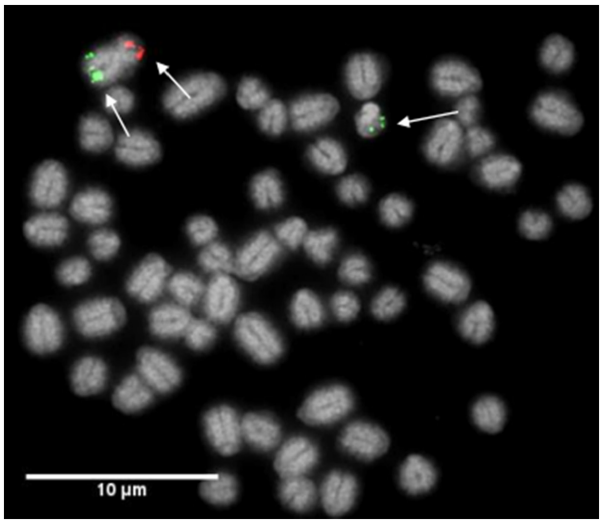

FIGURE 4 Fluorescence in situ hybridisation image showing X chromosome BACs on individual that underwent necropsy. Labelled bovine X chromosome BAC probes hybridised to reproductive tract metaphase chromosomes. Fluorescence in situ hybridisation result indicates the presence of Y chromosome with BAC CH240-121E1 (FITC) hybridising to the pseudoautosomic region of the Y chromosome. Co-localisation observed on the X chromosome (magnification ×1000)

Implications

Karyotype abnormalities in cattle have been reviewed in detail elsewhere (Iannuzzi et al., 2021; Popescu, 1990; Raudsepp & Chowdhary, 2016). In general, however, both structural and numerical abnormalities in the sex chromosomes of mammals is more tolerated than abnormalities in the autosomes, probably due to the effect of gene dosage compensation (Lyon, 1961). Nonetheless, abnormalities in the sex chromosomes tend to be associated with impaired reproductive performance including infertility (Benchikh et al., 2021; Iannuzzi et al., 2004; Kondoh et al., 1992). The incidence of Swyer syndrome in cattle is unknown. The incidence in humans is, however, thought to be 1 in every 80 000 (Khare et al., 2017) meaning that it is less frequent than aneuploidy of sex chromosomes in humans (Le Gall et al., 2017). Given that 648 639 nulliparous females aged <36 months had been genotyped in Ireland when the last of the Swyer cases in the present study was detected, the incidence of Swyer syndrome in Irish cattle is, at the very least, 1 in every 81 080 which is very similar to the 1 in 80 000 incidence reported for humans. Swyer syndrome is thought to be the second most frequent sex chromosome condition in horses after Turner syndrome (Lear & Bailey, 2008; Villagomez et al., 2009). Moreover, XY sex reversal in cattle, and other species, seems to be far more common than XX sex reversal (Favetta et al., 2012).

Externally, Swyer individuals often appear normal with unremarkable external genitalia (the present study; De Lorenzi et al., 2018); Sharma et al. (1980) did, however, report ill-defined external genitalia in their 6-year-old Swyer Haryana individual. Nonetheless, despite generally appearing normal, it is likely that (most) individuals suffering from Swyer syndrome are incapable of naturally establishing pregnancy (Kondoh et al., 1992). None of the Swyer syndrome individuals in the present study had any progeny, although none were recorded to have been served. Dumic et al. (2008) did report on a fertile XY human although different cell lines with different karyotypes were present in her ovaries. Similarly, pregnancy has been documented to be possible in Swyer humans following ova donations (King & Conway, 2014). All in all, unless of very high value, cattle with the Swyer condition are best drafted for meat production rather than for breeding. Because a genotype can be generated very early in life, this recommendation can be made well

before a decision is made on farm as to the eventual fate of the individual.

The flanking sequence and details of 11 SNPs within ±10 kb of the bovine *SRY* gene are included in the Appendix; these SNPs were extracted from Run 9 of the 1000 bull's genome project with a high allele count in that population. This information is useful for considering such SNPs on genotyping platforms along with the repertoire of other polymorphisms associated with infertility (VanRaden et al., 2011); approaches to identifying aneuploidy from available SNP data have also been documented for cattle (Berry et al., 2017). If genotypes are called for SNPs on the Y chromosome, but no SNP flanking the *SRY* gene is called (assuming a good overall call rate and homozygous genotypes in the non-pseudosomal region of the X chromosome), then it is a likely case of Swyer syndrome. If genotypes are, however, also called for the SNPs flanking the *SRY* gene and the individual has a female external phenotype, then biological samples could be bio-banked for further investigation into other likely causes of the sex reversal phenotype.

CONCLUSIONS

Sequence data indicated that all eight of the presumptive Swyer individuals had X and Y chromosomes confirming the condition; for one of the individuals, this was further confirmed by cytogenetic analysis. Deletions encompassing the *SRY* gene were detected in seven of the eight Swyer individuals. No strong candidate causal mutation in any of the reported DSD genes were detected that could possibly contribute to the Swyer phenomenon in the individual with the *SRY* gene (i.e., *SRY*+ individual). The addition of SNPs flanking the *SRY* gene on genotyping panels could help detect *SRY* deletions in XY individuals, in cases where discordance is detected between phenotypic sex and genotypic sex.

ACKNOWLEDGMENTS

Funding was provided by Science Foundation Ireland and the Department of Agriculture, Food and Marine on behalf of the Government of Ireland under the grant 16/RC/3835 (VistaMilk). This research was also enabled in part by support provided by the Prairie DRI Group and the Digital Research Alliance of Canada (alliance can-.ca). Open access funding provided by IReL. WOA Institution: Irish Agriculture and Food Development Authority Consortia Name: IReL hybrid OA 2022.

CONFLICT OF INTEREST

The authors have declared no potential conflicts.

DATA AVAILABILITY STATEMENT

Sequence data are available with the accession code NCBI BioProject Accession PRJNA850005.

ORCID

Donagh P. Berry https://orcid.org/0000-0003-4349-1447

REFERENCES

Albarella, S., Lorenzi, L.D., Rossi, E., Prisco, F., Riccardi, M.G., Restucci, B. et al. (2020) Analysis of XX SRY-negative sex reversal dogs. *Animals*, 10, 1667.

Audi, L., Ahmed, S.F., Krone, N., Cools, M., McElreavey, K., Holterhus, P.M. et al. (2018) The EU COST action. GENETICS IN ENDOCRINOLOGY: approaches to molecular genetic diagnosis in the management of differences/disorders of sex development (DSD): position paper of EU COST action BM 1303 'DSDnet'. *European Journal of Endocrinology*, 179, R197–R206.

Baetens, D., Verdin, H., De Baere, E. & Cools, M. (2019) Update on the genetics of differences of sex development (DSD). *Best Practice & Research Clinical Endocrinology & Metabolism*, 33, 101271.

Benchikh, S., Bousfiha, A., Razoki, L., Aboulfaraj, J., Zarouf, L., Elbakay, C. et al. (2021) Chromosome abnormalities related to reproductive and sexual development disorders: a 5-year retrospective study. *Biomedical Research International*, 2021, 8893467.

Berry, D.P., Wolfe, A., O'Donovan, J., Byrne, N., Sayers, R.G., Dodds, K.G. et al. (2017) Characterization of an X-chromosomal non-mosaic monosomy (59, X0) dairy heifer detected using routinely available single nucleotide polymorphism genotype data. *Journal of Animal Science*, 95, 1042–1049.

Chapman, H., Bruère, A. & Jaine, P. (1978) XY gonadal dysgenesis in a Charolais heifer. *Animal Reproductive Science*, 1, 9–18.

Cingolani, P., Patel, V.M., Coon, M., Nguyen, T., Land, S.J., Ruden, D.M. et al. (2012) Using *Drosophila melanogaster* as a model for genotoxic chemical mutational studies with a new program, SnpSift. *Frontiers in Genetics*, 3, 35.

Cingolani, P., Platts, A., Wang, L.L., Coon, M., Nguyen, T., Wang, L. et al. (2012) A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of *Drosophila melanogaster* strain w1118; iso-2; iso-3. *Fly*, 6, 80–92.

Cribiu, E.P., Di Berardino, D., Di Meo, G.P., Gallagher, D.S., Hayes, H., Iannuzzi, L. et al. (2021) International System for Chromosome Nomenclature of Domestic Bovids (ISCNDB 2000). *Cytogenetic and Genome Research*, 95, 283–299.

Da Silva, R.S., Monteiro, I.C.M. & Braz dos Santos, L.G. (2015) A case of Swyer syndrome associated with advanced gonadal dysgerminoma involving long survival. *Case Reports Oncology*, 8, 179–184.

Danecek, P., Auton, A., Abecasis, G., Albers, C.A., Banks, E., DePristo, M.A. et al. (2011) The variant call format and VCFtools. *Bioinformatics*, 27, 2156–2158.

Danecek, P., Bonfield, J.K., Liddle, J., Marshall, J., Ohan, V., Pollard, M.O. et al. (2021) Twelve years of SAMtools and BCFtools. *GigaScience*, 10, giab008.

De Lorenzi, L., Arrighi, S., Rossi, E., Grignani, P., Previderè, C., Bonacina, S. et al. (2018) XY (SRY-positive) ovarian disorder of sex development in cattle. *Sexual Development*, 12, 196–203.

Dumic, M., Lin-Su, K., Leibel, N.I., Ciglar, S. & Vinci, G. (2008) Report of fertility in a woman with a predominantly 46,XY karyotype in a family with multiple disorders of sexual development. *Journal of Clinical Endocrinology and Metabolism*, 93, 182–189.

Favetta, L.A., Villagómez, D.A.F., Iannuzzi, L., Di Meo, G., Webb, A., Crain, S. et al. (2012) Disorders of sexual development and abnormal early development in domestic food-producing mammals: the role of chromosome abnormalities, environment and stress factors. *Sexual Development*, 6, 18–32.

Ferrer, L.M., Monteagudo, L.V., García de Jalón, J.A., Tejedor, M.T., Ramos, J.J. & Lacasta, D. (2009) A case of ovine female XY sex reversal syndrome not related to anomalies in the sex-determining region Y (*SRY*). *Cytogenetic and Genome Research*, 126, 329–332.

Hare, J.E., Baird, J.D., Duignan, P., Saunders, J., Floetenmeyer, R. & Basrur, P.K. (1994) XY gonadal dysgenesis and tetralogy of Fallot in an Angus calf. *Canadian Veterinary Journal*, 35, 510–512.
Hayes, B.J. & Daetwyler, H.D. (2019) 1000 Bull genomes project to map simple and complex genetic traits in cattle: applications and outcomes. *Annual Review of Animal Biosciences*, 7, 89–102.
Heng, L. & Durbin, R. (2009) Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics*, 25, 1754–1760.
Henricson, B. & Åkesson, A. (1967) Two heifers with gonadal dysgenesis and the sex chromosomal constitution XY. *Acta Veterinary Scandandavia*, 8, 262–272.
Hook, E.B. (1977) Exclusion of chromosomal mosaicism: tables of 90%, 95% and 99% confidence limits and comments on use. *American Journal of Human Genetics*, 29, 94–97.
Howe, K.L., Achuthan, P., Allen, J., Allen, J., Alvarez-Jarreta, J., Amode, M.R. et al. (2021) Ensembl 2021. *Nucleic Acids Research*, 49, D884–D891.
Hunter, J.D. (2007) Matplotlib: a 2D graphics environment. *Computing in Science & Engineering*, 9, 90–95.
Iannuzzi, A., Parma, P. & Iannuzzi, L. (2021) Chromosome abnormalities and fertility in domestic bovids: a review. *Animals (Basel)*, 12, 802.
Iannuzzi, L., Di Meo, G.P., Perucatti, A., Incarnato, D., Palo, R.D. & Zicarelli, L. (2004) Reproductive disturbances and sex chromosome abnormalities in two female river buffaloes. *Veterinary Record*, 154, 823–824.
Kawakura, K., Miyake, Y.-I., Murakami, R.-K., Kondoh, S., Hirata, T.-I. & Kaneda, Y. (1996) Deletion of the *SRY* region on the Y chromosome detected in bovine gonadal hypoplasia (XY female) by PCR. *Cytogenetics and Cell Genetics*, 72, 183–184.
Khare, J., Deb, P., Srivastava, P. & Reddy, B.H. (2017) Swyer syndrome: the gender swayer? *Alexandria Journal of Medicine*, 53, 197–200.
King, T.F.J. & Conway, G.S. (2014) Swyer syndrome. *Current Opinion in Endocrinology, Diabetes, and Obesity*, 21, 504–510.
Kondoh, S., Miyake, Y., Nakahori, Y., Nakagome, Y. & Kaneda, Y. (1992) Cytogenetical and molecular biological studies on a bovine XY female. *The Journal of Veterinary Medical Science*, 54, 1077–1080.
Lange, J., Skaletsky, H., van Daalen, S.K.M., Embry, S.L., Korver, C.M., Brown, L.G. et al. (2009) Isodicentric Y chromosomes and sex disorders as byproducts of homologous recombination that maintains palindromes. *Cell*, 138, 855–869.
Le Gall, J., Nizon, M., Pichon, O., Andrieux, J., Audebert-Bellanger, S., Baron, S. et al. (2017) Sex chromosome aneuploidies and copy-number variants: a further explanation for neurodevelopmental prognosis variability? *European Journal of Human Genetics*, 25, 930–934.
Lear, T.L. & Bailey, E. (2008) Equine clinical cytogenetics: the past and future. *Cytogenetic and Genome Research*, 120, 42–49.
Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N. et al. (2009) The Sequence Alignment/Map format and SAMtools. *Bioinformatics*, 25, 2078–2079.
Lyon, M.F. (1961) Gene action in the X-chromosome of the mouse (*Mus musculus* L.). *Nature Cell Biology*, 190, 372–373.
Macmillan, K.L., Fielden, E.D., McNatty, K.P. & Henderson, H.V. (1984) LH concentrations in two cattle with XY gonadal dysgenesis. *Journal of Reproduction and Fertility*, 71, 525–531.
McClure, M.C., McCarthy, J., Flynn, P., McClure, J.C., Dair, E., O'Connell, D.K. et al. (2018) SNP data quality control in a national beef and dairy cattle system and highly accurate SNP based parentage verification and identification. *Frontiers in Genetics*, 9, 84.
McLaren, W., Gil, L., Hunt, S.E., Riat, H.S., Ritchie, G.R.S., Thormann, A. et al. (2016) The Ensembl variant effect predictor. *Genome Biology*, 17, 122.
Michala, L., Goswami, D., Creighton, S.M. & Conway, G.S. (2008) Swyer syndrome: presentation and outcomes. *BJOG: An International Journal of Obstetrics and Gynaecology*, 115, 737–741.
Morris, J.M. (1953) The syndrome of testicular feminization in male pseudohermaphrodites. *American Journal of Obstetrics and Gynecology*, 65, 1192–1211.
Padula, A.M. (2005) The freemartinism syndrome: an update. *Animal Reproduction Science*, 87, 93–109.
Parma, P., Veyrunes, F. & Pailhoux, M.E. (2016) Sex reversal in non-human placental mammals. *Sexual Development*, 10, 326–344.
Pedersen, B.S., Layer, R. & Quinlan, A.R. (2020) *smoove: structural-variant calling and genotyping with existing tools (0.2.8)* [Go]. Available from: https://github.com/brentp/smoove/blob/c68ae05317dc5cedcc848b0389fd1a6b9d0fb92e/CITATION.cff (Original work published 2018) [Accessed 22nd February 2022].
Pedersen, B.S. & Quinlan, A.R. (2018) Mosdepth: quick coverage calculation for genomes and exomes. *Bioinformatics*, 34, 867–868.
Popescu, P.C. (1990) Chromosomes of the cow and bull. *Advances in Veterinary Science and Comparative Medicine*, 34, 41–71.
Raudsepp, T. & Chowdhary, B.P. (2016) Chromosome aberrations and fertility disorders in domestic animals. *Annual Review of Animal Biosciences*, 4, 15–43.
Raudsepp, T., Durkin, K., Lear, T.L., Das, P.J., Avila, F., Kachroo, P. et al. (2010) Molecular heterogeneity of XY sex reversal in horses. *Animal Genetics*, 41, 41–52.
Sayers, E.W., Beck, J., Bolton, E.E., Bourexis, D., Brister, J.R., Canese, K. et al. (2021) Database resources of the National Center for Biotechnology Information. *Nucleic Acids Research*, 49, D10–D17.
Sharma, A.K., Vijaykumar, N.K., Khar, S.K., Verma, S.K. & Nigam, J.M. (1980) XY gonadal dysgenesis in a heifer. *Veterinary Record*, 107, 328–330.
Sinclair, A.H., Berta, P., Palmer, M.S., Hawkins, J.R., Griffiths, B.L., Smith, M.J. et al. (1990) A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. *Nature*, 19, 240–244.
Switonski, M., Chmurzynska, A., Szczerbal, I., Lipczynski, A., Yang, F. & Nowicka-Posluszna, A. (2005) Sex reversal syndrome (64,XY; *SRY*-positive) in a mare demonstrating masculine behaviour. *Journal of Animal Breeding and Genetics*, 122, 60–63.
Swyer, G.I.M. (1955) Male pseudohermaphroditism: a hitherto undescribed form. *British Medical Journal*, 17, 709–712.
Thorvaldsdóttir, H., Robinson, J.T. & Mesirov, J.P. (2013) Integrative genomics viewer (IGV): high-performance genomics data visualization and exploration. *Briefings in Bioinformatics*, 14, 178–192.
VanRaden, P.M., Olson, K.M., Null, D.J. & Hutchison, J.L. (2011) Harmful recessive effects on fertility detected by absence of homozygous haplotypes. *Journal of Dairy Science*, 94, 6153–6161.
Villagomez, D.A., Parma, P., Radi, O., Di Meo, G., Pinton, A., Iannuzzi, L. et al. (2009) Classical and molecular cytogenetics of disorders of sex development in domestic animals. *Cytogenetic and Genome Research*, 126, 110–131.
Whyte, A., Monteagudo, L.V., Díaz-Otero, A., Lebrero, M.E., Tejedor, M.T., Falceto, M.V. et al. (2009) Malformations of the epididymis, incomplete regression of the mesonephric tubules and hyperplasia of Leydig cells in canine persistence of Müllerian duct syndrome. *Animal Reproductive Science*, 115, 328–333.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Berry, D.P., Herman, E.K., Carthy, T.R., Jennings, R., Bandi-Kenari, N., O'Connor, R.E. et al. (2022) Characterisation of eight cattle with Swyer syndrome by whole-genome sequencing. *Animal Genetics*, 00, 1–11. Available from: https://doi.org/10.1111/age.13280

APPENDIX

RS NUMBER, GENOMIC POSITION (BTAU 5.0.1 BUILD) AND FLANKING SEQUENCE OF SINGLE NUCLEOTIDE POLYMORPHISMS IN THE *SRY* GENE (BTAU5.0.1Y:42225210–42225899)

RS number	Position	Sequence
rs1114829560	42216850	AGCTTTAAGCCAACTTTTTCACTCTCCACTTTCACTTTCTTTAAGCGGCTTTTTA GTTCCTCTTCACTTTCTGCCATAAGGGTGGTGTCATCTGCATATC[A/T] GAGGTTATTGATATTTCTCCCTGCAATCTTGATTCCAGCTTGTGTTTCTTCCA GTCCAGCATTTCTCATGATGTACTCTGCATGTACTCTGCACGTAAAT
rs209105969	42218225	AAATAATCTTTGTCCGATGAATTGTACCCTATGGCTTCTTAGAATAGGGAGGGGTA TGCCTTTGCCAGTTCAAGAGACCCGTGGTAAAGAATGGCTGATA[G/A] AAAAGAACAGTGTGGCCAGGATGGATATGCCCATCTGGCCCCACCTGTGATAC TGCTTGGACCTCCCTAAAAGGTATTTGCAAAACCTCTCTCTCTTGAG
rs383837163	42218478	CTCTTCTAAACTGGGGAGGGTTGGGTATAATGTTGATGCAGTAGCTCCTGTCACAA TTTTTGGTACAATAGGAGGGAAAGAAACCTCCTCCACTGGGGGA[C/T] GCAGAGGAGGAATGTCCCTTCCTCTTCTTCTGGGGATCCCCTTCATGTTCTCCAG AATGGAGGGCATGGTGGGAGTTCCTACTCCTCCAGATCATATTTC
rs211246165	42219035	TACTTTCCTTCTATCACTTACAGCAAGTCCAACTTCTATTTCATCGCTCATTGCTAGG GTCTTGTACCCCAGCCCTGACTGAAGTCTCCACTCAAAGAAG[G/T] ACAAGGGCCACTGTACCTCAACTATGATCAAAGTGAAAAGGGTCTTACCTACCAA GTCTTTCCATGGTATTATCTCTCATGTTTTTCTGTTAGCAGTCAT
rs211064863	42220126	CTACAACATGCAGGTTCAGGCACTCTCATTAGTCTAACACAATCTGACCTTTACGTTC AGGCACTATCGTTAGTCTAACACAATCTGACCTTTACTTCCA[A/G] TCTTTGCCATCTGTGAGGATGGAGTTTCCTGACTTTAGTTTGACAATAGTTAACA GGGTTCAGGCCCAGTTTTCATCGTGCCTTAAGATAACTGACAGTC
rs452958779	42223235	CCAAAAGTGCAATTGTTACCGAGTCCAAGCTCACTCTGGTCACCTCATGACAGGCCA ATGAATCCAAGAGACAAGTTGTTGACACAATGAGAAGACTTTA[A/T] TCACAGAGCAGGCAGACTGAGAAGAGGCAGGCTAGTGCCTCAAAATAATTATCT TATTAGGGTCTGGATACCAGGTTCTGTTTAGATCAGAGAAAAAGAA
rs455433795	42225259	ACTGTAGTAAAATTGAGATAAAGAGCGCCTTTGTTAGCGAGAGTAAGGAAGTCAATA TTGAAAATAAGCACAAGAAAGTCCAGGCTCTAAGCTTTTGTTA[C/A] AGGGAAAGTCCGCCGAAATCCGTGTAGCCAATGTTACCTTATTGTGGCCCAGG CTTGTCCAGCTGCTGTGATGCTCCTTTTGCAGGAGTGAATTGGTTAT
rs380931777	42227449	TAAAACATGCCTCTTACTGTGGAAGCAAATGTTTAAATGACACTCATACACAAAAG CAGATTTTATAAACCTAGAAATTGTTTAATATATGAGAAAAATA[C/A] ATACCTTTTCAATGTTGAAATTAAAAGACAAAAAGTGATACATTCAGCAATGTGG AGGAACCTTGAAAACATCATACTGTGTTAAAAAAGGTACACAAGA
rs384536181	42227450	AAAACATGCCTCTTACTGTGGAAGCAAATGTTTAAATGACACTCATACACAAAAGCA GATTTTATAAACCTAGAAATTGTTTAATATATGAGAAAAATAC[A/T] TACCTTTTCAATGTTGAAATTAAAAGACAAAAAGTGATACATTCAGCAATGTGG AGGAACCTTGAAAACATCATACTGTGTTAAAAAAGGTACACAAGAG
rs384892968	42233003	CAGCTTTAGCACCAGTCCTTCCAAAGAACTCCCAGGGCTGATCTCCTTTAGAATGGA CTGGTTGGATCTCCTTGCAGTCCAAAGGACTCTCAAGACTCTT[C/T] TCCAACACTACAGTTCAAAAGCATTAATTCTTCAGCTGTCAGCCTTCTTC ACAGTCCAACTTTTACATCCATATGTGACCACTGGAAAAACCATAGGCTT
rs382514878	42234774	GGTGTTCAGGTTTGGGAACATGTGTACACCATGGCAGATTCATGTTGATGTATGGCA AAAGCAATACAATATTGTAAAGTAATTAACCTCCAATTAAAAT[A/T] AATAAATTTATATTAAAAATGCATCAATTCTTTGGCACTCAGCTTTCTTCACAT TCCAATACTCACATCCATACATGACTACTGGAAAATCATAGCCTTG