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(54) **BOS TAURUS CELL TYPE 'HO840003210132823'**

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(57) **ABSTRACT**

The disclosure relates to Bovine germplasm of *Bos taurus* HO840003210132823. Included in the present disclosure are cells comprising the genome of Bovine HO840003210132823 characterized by the presence of homozygous loci and spermatozoa obtained from said cells. Also provided by the present disclosure are tissue cultures of cells, animals obtained from said cells, and parts thereof, including F1 spermatozoa. The disclosure further provides for methods of breeding, selecting, and using the germplasm to improve existing commercial cattle herds generated from in vitro fertilization methods and progeny cattle obtained from in vitro fertilization and implantation and artificial insemination methods.

Specification includes a Sequence Listing.

BOS TAURUS CELL TYPE 'HO840003210132823'

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of and priority to U.S. Provisional Application 63/031,761, filed on May 29, 2020. 63/031,761 is hereby incorporated by reference in its entirety.

INCORPORATION OF SEQUENCE LISTING

[0002] The Sequence Listing, including the file named DG-66-2020-US1-SEQLST.txt, which is 12,898,823 bytes in size, was created on May 18, 2021 and is hereby incorporated by reference in its entirety.

FIELD

[0003] The present disclosure relates to the field of *Bos taurus* breeding. In particular, the present disclosure related to *Bos taurus* HO840003210132823 having high multi-trait selection indices and high trait transmissibility.

BACKGROUND

[0004] There are numerous steps in the development of any new, desirable *Bos taurus* germplasm. *Bos taurus* breeding begins with the analysis and definition of problems and weaknesses of the current germplasm, the establishment of program goals, and the definition of specific breeding objectives. The next step is selection of germplasm that possesses the traits to meet the program goals. A goal is to combine in a single animal an improved combination of desirable traits from the parental germplasm. See Schefers et al., Animal Frontiers 2(1):1-9 (2012).

[0005] During breeding, cattle breeders have a variety of sources when making breeding decisions. In addition to genomic data, a number of agencies and organizations collect and release analysis of population data and indexes. Every three months, the Animal Improvement Programs Laboratory (AIPL) of the United States Department of Agriculture releases the newest USDA-DHIA (Dairy Herd Improvement Association) genetic evaluations for dairy bulls and cows. The AIPL calculates genetic evaluations for type for various breeds, and many breed associations provide their own indexes or other strategies for evaluating certain breed-relevant traits. U.S. dairy genetic evaluations are computed every four months by the Council on Dairy Cattle Breeding (CDCB) and Holstein Association USA (HAU). Both CDCB and HAU traits provide the breeder with important comparative data to evaluate the complex genetic and phenotypic traits to develop improved and desirable *Bos taurus* germplasm. For Holstein and Jersey sires, for example, evaluations are genomically enhanced and represent a blending of genomic data, pedigree information, and results from progeny. These genetic evaluations provide the breeder with important information for the selection of desirable germplasm and the development of new and valuable inseminates.

[0006] There is a continuous need to develop improved *Bos taurus* germplasm for use in improving production

SUMMARY

[0007] The present disclosure provides for and includes elite *Bos taurus* germplasm. In various embodiments, the present teachings can provide for a *Bos taurus* cell of type Animal, a representative sample of cells of type Animal comprising the Deposit. In some configurations, the present teachings can include a frozen vial, a cell culture, a tissue, a zygote, or an embryo comprising a plurality of the *Bos taurus* cell disclosed herein. In various configurations, the present teachings can include a bull comprising a plurality of the *Bos taurus* cell disclosed herein. In various configurations, the cell can be a sperm cell. In some configurations, semen can comprise a plurality of the sperm cell of the present teachings.

[0008] In various configurations, the present teachings can include an embryo produced by contacting the cell with a *Bos taurus* ovum.

[0009] In various configurations, the present teachings can include a composition of matter comprising the cell and a *Bos taurus* ovum.

[0010] In various embodiments, the present teachings can provide for a *Bos taurus* cell produced by somatic cell nuclear transfer of a *Bos taurus* cell of type Animal, a representative sample of cells of type Animal comprising the Deposit. In some configurations, the present teachings can include a frozen vial, a cell culture, a tissue, a zygote, or an embryo comprising a plurality of the *Bos taurus* cell. In various configurations, the present teachings can include a bull comprising a plurality of the *Bos taurus* cell. In various configurations, the cell can be a sperm cell. In some configurations, semen can comprise the sperm cell of the present teachings.

[0011] In various embodiments, the present teachings can provide for a *Bos taurus* cell from an F1 offspring of an animal comprising a cell of type Animal, a representative sample of cells of type Animal comprising the Deposit. In some configurations, the present teachings can include a frozen vial, a cell culture, a tissue, a zygote, or an embryo comprising a plurality of the *Bos taurus* cell from an F1 offspring. In various configurations an animal can comprise a plurality of the *Bos taurus* cell from the F1 offspring. In some configurations, the present teachings can include a container of semen produced by the animal wherein the animal is a bull. In some configurations, the animal can be a bull, a cow, or a heifer. In various configurations, the animal can be a bull. In various configurations, the animal can be a cow or heifer. In various configurations, the F1 offspring can be a gene edited animal.

[0012] In various configurations, the cell can be an ovum.

[0013] In various configurations, the cell can be a sperm cell. In some configurations, the present teachings can include semen comprising a plurality of the *Bos taurus* sperm cell.

[0014] In some various configurations, the present teachings can include an embryo produced using a gamete of the F1 animal of the present teachings. In various configurations, the present teachings can include an embryo produced by contacting the sperm cell of the F1 bull with a *Bos taurus* ovum. In various configurations, the present teachings can include an embryo produced by contacting an ovum of the F1 cow or heifer of the present teachings with *Bos taurus* sperm cell.

F1 animal of the present teachings and a gamete cell from a second *Bos taurus* parent. In various configurations, the present teachings can include a composition of matter comprising the F1 sperm cell of the present teachings and a *Bos taurus* ovum. In various configurations, the present teachings can include a composition of matter comprising the F1 ovum of the present teachings and a *Bos taurus* sperm cell. The present disclosure provides for and includes elite *Bos taurus* germplasm. In various embodiments of the present teachings can provide for a *Bos taurus* cell having a genome comprising at least 90% of loci comprising a Homozygous Genotype of Animal, a representative sample of which comprises the Deposit. In some configurations, the genome comprises 95% of loci comprising the genotype of Animal.

[0016] In various configurations, the *Bos taurus* cell can further comprise a plurality of the cell comprising a frozen vial of the plurality of the cell, a cell culture, a tissue, a zygote, an embryo, a calf, or a mature adult. In various configurations, the *Bos taurus* cell further comprises a plurality of the cell comprising a bull.

[0017] In various embodiments, the present disclosure provides for and includes a *Bos taurus* cell comprising a genome comprising at least 90% of loci comprising a Haploid Genotype of Animal, a representative sample of which comprises the Deposit. In some configurations, the genome comprises at least 95% of loci comprising a Haploid Genotype of Animal. In various configurations, the cell is a gamete. In various configurations, the cell is a sperm. In some configurations, the sperm further comprises a plurality of sperm comprising semen. In some configurations, the semen is contained in a straw. In some configurations, the straw of semen is cryopreserved.

[0018] In various embodiments, the present disclosure provides for and includes a *Bos taurus* cell from an F1 offspring of an animal having a genome comprising at least 90% of loci comprising the Homozygous Genotype of Animal, a representative sample of Animal comprises the Deposit. In some configurations, the F1 offspring cell comprises at least 70% of loci that comprise a Homozygous Genotype of Animal. In various configurations, the at least 70% of loci that comprise a Homozygous Genotype of Animal is at least 80% of loci that comprise a Homozygous Genotype of Animal. In various configurations, the at least 70% of loci that comprise a Homozygous Genotype of Animal is at least 90% of loci that comprise a Homozygous Genotype of Animal. In various configurations, the at least 70% of loci that comprise a Homozygous Genotype of Animal is at least 95% of loci that comprise a Homozygous Genotype of Animal. In various configurations, the at least 70% of loci that comprise a Homozygous Genotype of Animal is at least 98% of loci that comprise a Homozygous Genotype of Animal. In various configurations, the F1 offspring further comprises at least one gene edited sequence. In various configurations, the cell from an F1 offspring further comprises a plurality of the cell comprising meat. In various configurations, the cell from an F1 offspring further comprises a plurality of the cell comprising a frozen vial of the plurality of the cell, a cell culture, a tissue, a zygote, an embryo, a calf, or a mature adult. In various configurations, the cell from an F1 offspring further comprises a plurality of the cell comprising a bull.

[0019] In various embodiments, the present disclosure least 90% of loci comprising the Homozygous Genotype of Animal, a representative sample of Animal comprising the Deposit. In various configurations, the haploid cell comprises at least 50% of loci that comprise a Homozygous Genotype of Animal. In various configurations, the haploid cell comprises at least 66% of loci that comprise a Homozygous Genotype of Animal. In various configurations, the haploid cell comprises at least 75% of loci that comprise a Homozygous Genotype of Animal. In various configurations, the haploid cell comprises at least one gene edited sequence. In various configurations, the cell is a gamete. In various configurations, the cell is an ovum. In various configurations, the cell is a sperm. In some configurations, the sperm further comprises a plurality of sperm comprising semen. In various configurations, the semen is contained in a straw. In various configurations, the straw of semen is cryopreserved.

[0020] In various embodiments the present disclosure provides for and includes a method of producing an embryo comprising crossing Animal to a cow or heifer. In some configurations the embryo can be a cow embryo. In various configurations, the embryo can be a calf embryo. In various configurations, the present disclosure provides for and includes an F1 offspring or part thereof of Animal that matured from an embryo produced by the instant method. In various configurations, the part of the F1 offspring can be a gamete. In various configurations, the gamete can be a sperm.

DETAILED DESCRIPTION

[0021] A goal of a *Bos taurus* breeding program is to combine in a single *Bos taurus* animal an improved combination of desirable traits from the parental germplasm that provides for desirable progeny when used in artificial insemination programs, in vitro fertilization programs, Embryo transfer programs, or a combination thereof. Improved *Bos taurus* inseminate varieties are useful for various artificial breeding techniques, including artificial insemination ("AI") and embryo transfer ("ET"). Improved *Bos taurus* germplasm, varieties, oocytes, embryos, and inseminates prepared therefrom, are desirable.

[0022] The present disclosure provides for, and includes, an improved elite germplasm obtained from a multigenerational breeding program. The germplasm is unique and readily distinguishable from germplasm present in non-selected cattle. Indeed, in the absence of continued selection, the germplasm reverts to heterogeneity and diversity. As provided herein, the germplasm of the present disclosure is identifiable using standard methods and the germplasm can be readily identified in progeny generations. Indeed, as few as 800 SNP markers are sufficient to identify parentage with greater than 99% accuracy. See McClure et al., Frontiers in Genetics 9(84):1-14 (2018). As provided here, the tens of thousands of sequences provide for tracking and selecting animals through multiple generations. Breeding with the germplasm provided herein, combined with the selection of suitable mates, will maintain the desirable germplasm in subsequent generations. Moreover, genetic testing allows for the removal of progeny having germplasm that lacks that set of desired loci for the improvement of cattle herds.

[0023] It is to be understood that the disclosure is not

The disclosure is capable of other aspects or of being practiced or carried out in various ways.

Definitions

[0024] "Animal" as used herein, refers to cells, animals, or particularly gametes and embryos of *Bos taurus* HO840003210132823. The terms "Animal" and "HO840003210132823" are used interchangeably without any change in their meaning.

[0025] "Diploid Genotype" as used herein refers to a genotype containing two copies of each chromosome. This genotype is generally present in somatic cells.

[0026] "Haploid Genotype" as used herein, refers to a genotype containing one copy of each chromosome. Therefore, relative to a diploid genotype for a given individual, it will contain half of the loci comprising a diploid chromosome. This term can refer to the genotype of a haploid cell, or to one half of the genotype of a diploid cell. This genotype is generally present in gametes.

[0027] "Homozygous Genotype" as used herein refers to the loci of a diploid genome wherein both chromosomes have the same allele at a given locus.

[0028] "Heterozygous Genotype" as used herein, refers to the loci of a diploid genome wherein each chromosome has a different allele at a given locus.

[0029] "Animal Genotype" as used herein, refers to the genotype of Animal and is characterized by the SNPs and flanking regions recited in SEQ ID NOs: 1 to 41546.

[0030] "Animal Homozygous Genotype" as used herein, refers to the loci in Animal that are homozygous and is characterized by the SNPs and flanking regions recited in SEQ ID NOs: 1 to 28548.

[0031] "Animal Heterozygous Genotype" as used herein, refers to the Heterozygous Genotype of Animal and is characterized by the SNPs and flanking regions recited in SEQ ID NOs: 28549 to 41546.

[0032] "Animal Haploid Genotype", as used herein, refers to a Haploid Genotype derived from Animal and comprises at least one copy of the SNPs and flanking regions of the Animal Homozygous Genotype and, on average, at least one copy of 50% of the SNPs and flanking regions of the Animal Heterozygous genotype. This can refer to the genotype of a haploid cell, or to one half of the genotype of a diploid cell.

[0033] "Animal F1 Genotype," as used herein, refers to the genome of an F1 offspring derived from Animal and a second parent. This offspring has a set of chromosomes comprising a pair of chromosomes for each chromosome (1-29 and a sex chromosome): one member of the pair inherited from Animal, the other member of the pair inherited from the second parent.

[0034] "Deposit" as used herein, refers to a representative sample of cells of Animal deposited under ATCC Accession No. TBD on TBD. The Deposit contains cells obtained from Animal as described in Example 3. The Deposit was made under the Budapest Treaty with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, Va. 20110-2209 USA. Upon issuance, all restrictions on the availability to the public of the deposit will be irrevocably removed consistent with all of the requirements of the Budapest Treaty and 37 C.F.R. §§ 1.801-1.809. Applicant does not waive any infringement of rights granted under this patent.

[illegible]

chromosomes as the cell line comprising the Deposit. As used herein, two cells may have the same genotype even when read errors or mutations make the measured DNA sequences of the two cells not completely identical. While the cell line comprising the Deposit is a diploid line, a plurality of haploid Cells of Type Animal can be genotyped and can have the same genotype (allowing for read errors or mutations) as the cells comprising the Deposit. Multiple haploid Cells of Type Animal have the same average genotype as the cell line comprising the Deposit. For any given cell, meiotic recombination may result in chromosomes that have a different arrangement of alleles than the parent, but a plurality of haploid cells from an animal with an Animal cell type can be genotyped and will have the same genotype as the cells comprising the Deposit. The germplasm of Animal inherently comprises this genotype, and therefore can be defined by a cell of type Animal.

[0036] The phrase "producing an embryo from an animal" as used herein refers to the process of fertilization. With regard to the bull, this refers to contact of the bull's sperm cells with a *Bos taurus* ovum resulting in fertilization of the ovum to produce an embryo. Regarding the cow or heifer, this refers to contact of an ovum of the cow or heifer with *Bos taurus* sperm cells. Contacting a sperm and an ovum can comprise natural service, artificial insemination, in vitro fertilization or any other method wherein an ovum and sperm are brought into proximity such that they can join to form an embryo.

[0037] "Sire" as used herein, refers to the sire of Animal, *Bos taurus HO*840003141494670.

[0038] "Dam" as used herein, refers to the dam of Animal, *Bos taurus* HO840003143328234.

[0039] "Paternal Grand-Sire" as used herein, refers to the paternal grand sire of Animal, *Bos taurus* HO840003129038181.

[0040] "Paternal Grand-Dam" as used herein, refers to the paternal grand dam of Animal, *Bos taurus* HO840000074258448.

[0041] "Maternal Grand Sire" as used herein, refers to the maternal grand sire of Animal, *Bos taurus* HO840003128557482.

[0042] "Maternal Grand Dam" as used herein, refers to the paternal grand dam of Animal, *Bos taurus* HO840003133318719.

[0043] Offspring, as used herein, refers to the progeny of an animal created through natural service, artificial insemination, somatic cell nuclear transfer, or in vitro fertilization (IVF).

[0044] As used herein, "germplasm" includes intact genomes comprising chromosomes present in cells or nuclei. The term "germplasm" may include any gamete or germ cell, or any somatic cell from which an animal can be cloned.

[0045] As used herein the term "about" refers to ±10%.

[0046] As used herein, the singular forms "a," "an," and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a compound" or "at least one compound" may include a plurality of compounds, including mixtures thereof.

[0047] Throughout this application, various embodiments of this disclosure may be presented in a range format. It should be understood that the description in range format is

disclosure. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as "from 1 to 6" should be considered to have specifically disclosed subranges such as "from 1 to 3," "from 1 to 4," "from 1 to 5," "from 2 to 4," "from 2 to 6," "from 3 to 6," etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

[0048] Whenever a numerical range is indicated herein, it is meant to include any cited numeral (fractional or integral) within the indicated range. The phrases "ranging/ranges between" a first indicate number and a second indicate number and "ranging/ranges from" a first indicate number "to" a second indicate number are used herein interchangeably and are meant to include the first and second indicated numbers and all the fractional and integral numerals there between.

[0049] For the purposes of this disclosure, the term "semen" means seminal fluid which may contain sperm (also referred to as "spermatozoa") secreted by the gonads of a male animal which can be collected from the male animal by any method known to those in the animal breeding arts.

[0050] As used herein, "locus", or plural "loci", refers to a physical site or location of a specific gene or marker on a chromosome. Loci of the present disclosure include, and are identifiable by, a SEQ ID NO, each SEQ ID NO providing the identity of the polymorphism at a single nucleotide polymorphic (SNP) site and the adjacent 100 base pairs.

[0051] Loci may also be characterized as either 'A' loci, 'B' loci, or heterozygous 'A/B' loci. The identification of loci as either 'A' or 'B' loci is determined according to the top (TOP) and bottom (BOT) designations based on the polymorphism itself, or the contextual surrounding sequence as developed by ILLUMINA®, Inc. (San Diego, Calif.). Methods for determining the designation of a polymorphic site as 'A' or 'B' are known in the art, for example as provided by ILLUMINA®'s Technical Note entitled ""TOP/BOT" Strand and 'A/B' Allele", available on the internet at www(dot)illumina(dot)com/documents/products/technotes/technote_topbot.pdf. The NCBI's dbSNP database adopted the TOP/BOT nomenclature in 2005 and the designation is well known to those of skill in the art. As shown in the Examples, thorough sequence comparison extensive information about each locus is available to a person of ordinary skill in the art, including, but not limited to, dbSNP identifier, sources, chromosomal location, genes, transcripts, linkage to genes or quantitative trait loci (QTL), and interactions.

[0052] In diploid cells, the germplasm is characterized by the presence of sequences representing the sequences at polymorphic sites (e.g., SNPs) that are homozygous or heterozygous. As used herein, the germplasm of the present disclosure is characterized by the homozygous loci. In haploid cells (e.g., ova or sperm), each of the sequences at each homozygous locus in the parent are present in each cell. In contrast, heterozygous loci are present in haploid germplasm according to random assortment. Accordingly, the genotype at heterozygous loci in haploid cells varies from haploid cell to haploid cell, while the sequences of the

[0053] During breeding, to maintain and improve the germplasm, selections of a second parent may be an elite second parent having superior traits. As demonstrated in the examples, such select crosses result in progeny that retain significant numbers of homozygous loci present in each elite parent and further result in additional loci becoming homozygous. Thus, select crosses result in some or all of the homozygous loci of the *Bos taurus* cells of the present disclosure being retained in progeny generations. Generally, locus homozygosity is maintained when the second parent is an elite parent with high trait values as described below. Similarly, the improved germplasm of the present disclosure can be maintained by selecting superior breeding partners in the F1 and later progeny generations. By suitable selection of the second parent, the germplasm can be maintained at the homozygous loci. Even further, careful selection of elite breeding partners results in increased numbers of homozygous loci as the preferred allele at the heterozygous loci of the parent become homozygous in elite progeny.

[0054] As used herein, "gamete" refers to a haploid germ cell and includes either a sperm or an ovum and may be used interchangeably. Generally, the identity as a sperm or an ovum can be determined by the context as bulls produce sperm and cows produce ova. For the purposes of this disclosure the term "sperm" means the haploid cell that is the gamete of a male animal which may join an egg (also referred to as "ovum") to produce a zygote and broadly encompasses infertile sperm, sperm having a comparably lesser or a comparably greater fertility between a first amount of sperm obtained from a first animal and a second amount of sperm obtained from a second animal and which may be obtained in the form of a raw ejaculated semen, frozen semen, as sperm separated from the semen and contained in an extender or diluent, or as sex-selected sperm.

[0055] As used herein, the term "inseminate" is intended to broadly encompass an amount of sperm whether contained in semen alone or together with a cryoprotectant, extender, or other diluent. Inseminates may optionally include one or more "extenders" and diluents which can be utilized to fertilize the eggs of a female animal whether in vitro or in vivo. As used herein, inseminates can further include sex-selected sperm compositions.

[0056] As used herein, the term "sex-selected sperm" means sperm which have been separated, regardless as to the method of separation, into subpopulations containing X-chromosome bearing sperm and Y-chromosome bearing sperm having a purity in the range of about 70 percent ("%"), 80%, 90%, and about 100%.

[0057] As used herein, the term F1 refers to the first filial generation—progeny born from the gametes of a particular parent. Therefore, as used herein, an F1 Animal is a direct genetic reproduction descendent of Animal. Because cattle reproduce by meiosis, this term refers to an animal arising from a gamete of the parent—in this case, from a gamete from Animal. Therefore, for each of Animal's chromosome pairs, their offspring, an F1 Animal, will receive one copy of the chromosome carrying one of Animal's alleles for each locus. In general, this means that for each locus, an F1 Animal will receive one allele from Animal and one from their other parent. An F1 Animal, therefore, will receive one copy of each of Animal's homozygous alleles, and, for each locus, one copy of one of Animal's heterozygous alleles.

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