

Cattle Conservation in the 21st Century: A Mini Review

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Abstract

Cattle are arguably the most important livestock species. Since the beginning of this century, the loss of genetic diversity within this species has been a major concern as this could have serious consequences on the ability of this species to respond to future production constraints. Genetic diversity has traditionally been accessed from pedigree, however, with the advances in molecular genetics new opportunities have emerged. We examined different methods for accessing genetic diversity and estimating genetic diversity parameters at the genomic level. We also examined criteria and strategies for the conservation of animal genetic resources. Finally, we examined genomic studies on some endangered cattle breeds.

Keywords

Genetic Diversity, Conservation, Genetic Diversity Parameters, Cattle

1. Introduction

Arguably the biggest threat to biodiversity across the planet is habitat degradation, resulting from direct or indirect human activities [1] [2]. As the human population increases rapidly, we modify the landscape to meet our increasing need for the resources to sustain our modern lifestyles. Presently, the world's

population is currently growing at 1.5 percent per annum [3]. Corresponding to this increase is a rise in energy consumption that is driving climate change across the globe. The rapid pace of the changing climate will outpace the natural ability of some species to respond to survival [4].

A temporal analysis reported by [5] suggests that loss of biodiversity indicates that the Earth is on the path to its sixth mass extinction event, with the rate of extinction in the last century conservatively estimated to be 22 times faster than the historical baseline rate as reported by [6]. It is even more austere when we analytically examine population declines, rather than the complete loss of species, as 32% of known vertebrate species are showing substantial population deterioration [7]. The continuous increase in the human population coupled with rising incomes and urbanization necessitates the need to conserve the genetic potential of animals to avoid or lower the consequences of biodiversity loss.

During the spread of livestock species across Europe, Asia, and Africa, numerous geographically separate populations of livestock species emerged, where each of these populations is expected to have adapted to their respective environment. Mutation, random genetic drift due to geographical separation, population size bottleneck, and genetic reshuffling (recombination); also, adaptation to a diverse selection pressure imposed by climate, nutritional factors, disease, and parasites are natural agents that discern the species and breeds of livestock [8]. Over the years, human-imposed breeding strategies led to the development of essentially closed populations—with members distinguished based on visible phenotypic traits; described as breeds [9].

Consequently, conservation of farm animal breeds had been for several reasons, for example, to retain potentially useful genes and gene combinations such as the Booroola fecundity gene in sheep [10], the exploitation of heterosis, and to overcome selection plateaus, in addition to cultural motivations, research, and food security. Sustaining genetic diversity also offers indemnification against climate change, disease, changing availability of feedstuffs, social change, selection errors, and unexpected catastrophic events such as the Chernobyl where many local breeds' diversity became threatened [11].

A large number of wild species and domesticated breeds of animals now require human intervention to maintain their survival and to study the diversity within the genome which is an important aspect of conservative genetics [12] [13] [14] [15]. As stated by [16], the conservation of genetic diversity is, however, a form of guarantee against possible unexpected environmental conditions because it is a means of maintaining potential adaptation abilities. Hence, the conservation of biological (genetic) diversity in animals has attracted a great deal of attention.

Cattle are important livestock species for human survivability, but certain breeds are at the risk of extinction [17]. According to a report by [18] on the state of animal genetic resources, 17% of the world livestock breeds are on the verge of extinction despite an increasing number of actions to preserve biodiver-

sity. Sequel to the rigorous activities of human-induced selection, highly productive cattle breeds have replaced less productive ones resulting in the extinction of some and numerous others on the verge of extinction [12] [19].

Artificial selection criteria imposed by man (back-crossing of domesticated animals with their wild ancestral species) are factors that have made livestock breeds evolve and have created numerous cattle breeds that display broad phenotypic and genetic variation [20] [21]. As reported by [22], artificial selection has proved to have a rapid and significant effect on the phenotype and genome of organisms; and has resulted specifically to diverse cattle breeds that are specialized for either milk or meat production or raised as dual-purpose breeds and consequently the neglect of the inferior ones [23]. Studying the estimation of diversity within these populations is therefore an important aspect in conservation genetics to retain the potential and economic traits of the animals from going into extinction.

Therefore, the objectives of this review are to examine different methods for accessing genetic diversity and estimating genetic diversity parameters at the genomic level. To discuss the criteria and strategies for the conservation of the animal genetic resources and examine genomic studies on some endangered cattle breeds using here strategies.

2. Estimating Genetic Diversity in Livestock Breeds

In livestock science, conservation genetics is based on estimating diversity by pedigree relatedness and choosing those animals that maximize genetic diversity [24]. One of the main research priorities for the threatened species includes the characterization of genetic diversity and population structure which leads to more effective conservation and management strategies that are necessary to estimate genetic diversity [25] [26]. Also, the identification of the genomic regions affected by natural and artificial selection is a relevant research niche in livestock population genetics and useful in genetic diversity conservation [27].

These diversities are unravelled by statistical techniques and the knowledge about the relationship between the animals. Generally, half of the total genetic variation within species is estimated to be between breeds, hence the loss of breeds can substantially reduce genetic variation within species [28]. The genetic diversity observed within breeds is, therefore, defined as the allelic variation in a group of individuals. Genetic diversity of domestic cattle can be divided into two components; differences occurring between species from different breeds and those differences between individuals within a single breed measured as differences in their DNA [15] [29] which has a significant contribution to the sustainability of animal production [30]. Remarkably, genetic diversity is usually related with heterozygosity in population genetics [31].

Previously, the selection of breeds for conservation has been difficult until a more operational theoretical framework was developed by [32]. This analytical framework was adopted by animal geneticists using a diversity function to eva-

luate the biochemical polymorphism of 19 European cattle breeds including 18 French breeds and the British Shorthorn Cattle breeds [33]. More recently, the development of molecular biological techniques for the detection of variation in the DNA facilitates a more accurate description of genetic variation. Repetitive sequences of genomic DNA have opened a great means to estimate the genetic information associated with a particular organism [34]. It becomes easier to study genetic diversity with the help of these repetitive sequences. Molecular markers offer numerous advantages over conventional phenotype-based alternatives as they are stable and detectable in all tissues despite growth, differentiation, discrimination, defence, or development status of cells. The greater the genetic diversity within species, the greater the chances of long-term survive for such species [35].

Furthermore, mitochondrial DNA analysis, Nuclear DNA (Y-chromosomal haplotypes, and autosomal), and microsatellites analysis provide more viable alternatives as compared to the conventional blood groups, enzyme polymorphisms, transplantation antigens, and RFLP analysis. The collection of molecular data using microsatellite markers is a useful way of investigating the genetic diversity and differentiation among species and breeds of cattle [18]. These methods have also been used to investigate the genetic diversity of breeds in developing countries such as in Ankole longhorn breeds [36] [37] and, Afrikaner cattle breeds [38]; as well as several indigenous cattle breeds in Mozambique [39], Cuba [15], Cameroon [40] and India [41]. More recently, Single Nucleotide Polymorphisms (SNPs) [19], Copy Number Variants, Whole Genome Sequencing Analysis are the newly adopted techniques to study genetic diversity.

3. Parameters for Estimating Genetic Diversity

3.1. Effective Population Size

The primary factor responsible for the rate of loss of genetic diversity as well as the rate of increase of inbreeding and genetic drift in a biological population is the effective population size (N_e) [42] [43] [44].

As defined by Wright [45], N_e is defined as the size of an idealized population undergoing the same rate of genetic drift as the population under study. The effective population size is not usually equal to the census size (N) of the population. Discrepancies can sometimes be extreme, for instance in the case of the Holstein cattle breed [46]. Despite being a worldwide dairy cow breed with a census size of millions of individuals, the N_e of the whole population is about 100 [47]. This implies that the Holstein breed accumulates inbreeding at the same rate as an ideal population of only 100 individuals [46]. Fluctuations in census population size, breeding sex ratio, and variance in reproductive success are the factors that influence the size of N_e [48].

Estimation of N_e can be achieved using approaches that fall into three methodological categories which are demographic, pedigree-based, or marker-based [49]. Demographic method occasionally provides crucial N_e estimate which is

based on simplified population models and limited population data [49] The pedigree-based approach provides a complete population description [49] but has a problem with incomplete pedigree records [48] [49]. However, the use of genomic information (*i.e.* marker-based approach) has been proposed to overcome the challenge of incomplete pedigree [48] by estimating N_e from information on linkage disequilibrium [50] [51]. N_e is an important measure to preserve diversity and it explains genetic variability [52]. It is also an important parameter to control unfavourable events such as inbreeding and bottleneck effects in a population [53] [54] N_e can be used to evaluate the threat status of a population and to design breeding programs for both wild/captive populations and autochthonous livestock breeds [46] As a “rule of thumb”, it has been suggested that a minimum N_e of 50 is required to avoid the effects of inbreeding depression in the short-term [55], and larger than 500 if the interest is to maintain the evolutionary potential of the population over the long term [56].

3.2. Linkage Disequilibrium

Linkage disequilibrium (LD) is the non-random assortment of alleles at different loci [57] [58], which occurs when chromosomes are mosaics, and this occurrence depends on recombination rate, mutation rate, population size, and natural selection. A better understanding of LD is very important for genetic studies [59]

The information on the degree of LD in livestock populations is important to determine the minimum distance between the markers required for effective coverage when conducting genome-wide association studies (GWAS), marker-assisted selection (MAS) and genomic studies [60], and gene mapping and evolutionary inferences [58]. Furthermore, the pattern of LD is a powerful indicator of the genetic forces shaping a population [58]. However, the terms linkage and LD are puzzled.

LD is population-specific and is difficult between populations depending on the demographic history of the populations [61] [62] [63]. Also, the LD of genome markers is related to various evolutionary forces such as inbreeding, non-random mating, population bottleneck, drift, recombination, and mutation [64].

Considering the economic importance of domesticated animals, the knowledge of the dimension of LD enables the planning and execution of successful genomic breeding programs to achieve global food security.

3.3. Hardy-Weinberg Equilibrium

In 1908, the British mathematician Godfrey H. Hardy and German physician Wilhelm Weinberg independently discovered the relationship between gene and genotype frequencies, known as the Hardy-Weinberg principle, or Hardy-Weinberg equilibrium (HWE) [65] [66]. This discovery marked the beginning of the field of population genetics and has become a powerful research tool in both theoretical and applied research in population and quantitative genetics [67].

HWE is the relationship between the allelic and genotypic frequency that holds for most genetic markers. Also, HWE is not simply a theoretical law; deviations can signal important problems, errors, or peculiarities in the analyzed data sets [68] [69].

The theory of HWE has played a critical and powerful role in the development of population genetics and has frequently been used as a basis for genetic inferences [70]. Tests for differences from Hardy-Weinberg proportions are often used to confirm random mating in populations, and the deviations from the expected frequency of homozygotes are used to evaluate inbreeding coefficients [71]. Moreover, the validity of genetic association studies depends considerably on the use of appropriate controls such as HWE [72]. In recent decades, there has been a greater interest in retrieving and conserving local breeds, especially their capacity to adapt in marginal areas and for their importance as a reservoir of genetic diversity [64].

3.4. Heterozygosity

One of the parameters commonly used as an estimate of genetic diversity is Heterozygosity—Observed and Expected heterozygosity. In a population at Hardy-Weinberg equilibrium, Expected Heterozygosity can be defined as the proportion of heterozygous individuals in that population with equal allelic frequencies as that observed in the population [25]. It is estimated with the formula: $H_e = 1 - \sum P^2$, where P is the frequency of alleles at the locus. The higher the H_e value is, the more genetically diverse the population.

Because the populations we work with are usually finite and not at Hardy-Weinberg equilibrium, expected heterozygosity usually varies from the Observed heterozygosity. Observed heterozygosity is the actual proportion of heterozygotes in the population. Microsatellite marker [73] and SNP chips have been used to estimate heterozygosity in cattle [74].

3.5. Homozygosity

Homozygosity increases in a population where closely related animals are mated. It is estimated with a parameter called runs of Homozygosity (ROH). ROH is defined as a continuous stretch of the genomic segment without heterozygosity in the diploid animal [75]. Concerning effective population size, it has been observed that smaller populations tend to have a higher number of ROH and vice versa [75]. In terms of inbreeding, populations with a high inbreeding coefficient are associated with longer ROHs segment [75]. ROH can be estimated with the use of microsatellites [75] and SNP array [76].

3.6. Genetic Distance and Population Differentiation

The standard genetic distance (D) is often used as a common parameter for classification and evolutionary studies [77]. It is the accumulated number of gene differences per locus and across the genome. The expression that estimates the

genetic distance between two randomly mating diploid populations (X and Y) in which multiple alleles segregate at a locus is given as; $D = -\log_e I$, where $I = J_{XY} / \sqrt{J_X J_Y}$; J_X , J_Y , and J_{XY} are the arithmetic mean of j_X , j_Y , and j_{XY} respectively.

However, the above formula holds if the rate of gene substitution per locus is the same for all loci. Rather data on amino acid substitution in some proteins indicates that the rate of substitution varies considerably among loci [78]. So, when the rate of gene substitution varies among locus, a more appropriate measure of genetic distance [79] is given as; $D' = -\log_e I'$, where $I' = J'_{XY} / \sqrt{J'_X J'_Y}$ in which J'_X , J'_Y , and J'_{XY} are the geometric means of j_X , j_Y , and j_{XY} respectively. The formula can also be expressed as; $D' = \left(\sum_{j=1}^n d_j \right) / n$, where d_j is the value of $-\log_e I_j$ at the j th locus and n is the number of loci examined.

Concisely, a measure of the genetic distance (D) based on the identity of genes between populations is expressed as $D = -\log_e I$, where I is the normalized identity of genes between two populations which is equivalent to protein identity. If the rate of gene substitution per year is constant, it is linearly related to the divergence time between populations under sexual isolation. It is also linearly related to geographical distance or area in some migration models. It is important to note that population differentiation is estimated as the genetic distance between isolated populations and the distance between incompletely isolated populations. Further clarifications as to how genetic distance can be estimated in these forms of populations can be found in Nei's (1972) report. Since D is a measure of the accumulated number of codon differences per locus, it can also be estimated from data on amino acid sequences in proteins even for a distantly related species. Thus, if enough data is available, the genetic distance between any pair of organisms can be measured in terms of D . This measure applies to any kind of organism without regard to the ploidy or mating scheme [79].

There had been several variations of D that have been proposed and used over time, for example, D_C [80], D_A , D_m [77], D_{SW} [81], and D_{LR} [82]. A comparison between some conventional parameters of genetic distance [e.g., D , F_{ST} , R_{ST} , and $(\delta\mu)^2$], and newly introduced ones [e.g., D' , D_R , D_{CSD} and D_{SA}] have also been conducted [62] [83].

4. Setting Priorities for Conservation

Though conservation decisions rely upon a range of information including the degree of endangerment, adaptation to a specific environment, possession of traits of current or future economic importance, possession of unique traits of scientific interest, and the cultural or the historical value of the breed. Moreover, accurate assessment of populations concerning their contribution to national and overall genetic diversity is an important step in determining priorities for conservation [84].

It is imperative to note that, to retain or maximize among-breed diversity, it

might be predictable that genetically divergent breeds are given the highest priority. However, when objective measures of genetic relationships among breeds are obtainable, other criteria for setting priorities may be classified as; 1) risk of breed extinction, 2) measure of “merit” of the breed, 3) within breed variation [85] [86]. Breeds at the peril of extinction are mostly classified as being threatened, based on their current population numbers (numbers of males and females), alterations in population size, and the degree of crossbreeding with other breeds [87]. The probability of extinction for each breed can be estimated by simply relating the current population size to a critical effective size and accounting for other demographic and environmental risk factors. These risk factors may include a decreasing number of breeding females; reduced population size; high level of crossbreeding; alteration in the production environment and so on [9].

The measure of traits unique to each breed (which may include adaptive, production, and other quality traits of sociocultural and ecological value) may be classified as breed merits. Ideally, objective information about these phenotypic traits should be available, nevertheless, subjective information relating to the adaptation or unique features from the local animal owners should be considered. Besides, a decision-making system that takes both the phenotypic merit and measures between and within breeds’ genetic diversity into account was developed by [88]. Though sociocultural and ecological values may be difficult to define, therefore, it is important to consider the opinions of indigenous owners.

While it is important to measure genetic variability among populations, the measure of within-population variability would not be out of place in priority decision-making. The measure of within-population variability has been described above. Though there are differences in opinions of which method best measures the within-population variability, in the context of conservation and minimizing loss of diversity, “the number of alleles per locus” is most appropriate [89]. A more detailed analogy of these steps can be found in a report published by [18].

5. Categories/Strategies for Farm Animal Genetic Resources Conservation

5.1. *In Situ* Conservation

At large, *in situ* conservation or conservation by utilization is an ideal mechanism to conserve breeds, in a way that diversity is optimally utilized in the short-term and maintained for the long-term [18]. A breed has to evolve and acclimatize to changing environments and efforts to fashion a need for products or functions of the breed should be encouraged. However, continuous conservation deprived of further development of the breed or without expected future use is not an essential strategy. This approach is expensive, and unless the breed can be used for production, it is not likely to succeed [90]. Activities relating to *in*

situ conservation may include performance recording, schemes, development of breeding programs, and management of diversity within populations. It also includes steps taken to ensure sustainable management of ecosystems [91] [92] concluded that the conservation of traditional systems may depend on the conservation of the breeds that resides in them.

Furthermore, in addition to in situ conservation, practices to maintain live animals outside their production or natural environment (*ex-situ* live) or through cryopreservation of germplasm (*ex-situ*) are established to preserve germplasm of rare breeds as well as the more commonly used commercial breeds in the case of cattle and other livestock species [93] [94]. Besides, cryopreservation of germplasm is the current state of the art in *ex-situ* strategy and an integral part of conservation strategies employed to conserve existing allelic diversity for future use [95].

5.2. Ex-Situ Conservation

5.2.1. Ex-Situ in Vivo Conservation

Occasionally, there might be uneconomical reasons for conservation and management may however be demanding. Nevertheless, cultural, historical, or ecological values may exist. Breeds in this category can rationalize roles in zoos, farm parks, and natural parks. Since the cost is relatively low, breeds are kept out of their production environment to which they are adapted [28]. In the context of domestic animal diversity, *ex-situ* conservation means conservation away from the habitat and production systems where the resource is developed. This category includes both the maintenance of live animals (*in vivo*) and preservation of their genetic properties through cryopreservation of their reproductive organs (*in vitro*).

5.2.2. Cryoconservation or Cryopreservation

Ex-situ (in vitro) conservation programs of livestock genetic resources have focused efforts on cryopreservation of gametes, embryos, and somatic cells as well as the testis and ovarian tissues, effectively lengthening the genetic lifespan of individuals in a breeding program even after their death [96] [97]. It also gives us the ability to reconstitute live animals at a later date. One of the major issues surrounding genome banks is the amount and type of material that needs to be stored, which is a function of the intended future use of the material [13]. To avoid inbreeding, a gene bank of male and female genetics formed from the largest number of individuals would be ideal [98].

1) Gonadal Cryopreservation

Testicular tissue cryopreservation is a possibility in livestock production. There are reports of successful cryopreservation of immature testicular tissues in goat [99], pig [100] [101], cattle [102], and sheep [103]. However, it is important to note that the developmental stage of the testis plays an important role in the cryopreservation and subsequently on the semen product [104]. Successful cryopreservation of gonadal tissue is an important factor that guarantees fertility

preservation [93]. With the advent of the Assisted Reproductive Technique (ART) and an improved understanding of cryobiology, strategies have been developed to allow the long-term storage of gametes and embryos [105] which would help in conserving animals with genetic potentials. Similar reports have been documented in Ovarian tissues in sheep [106] [107], rabbit [108], bovine [109], and in porcine [110].

2) Sperm

Systematic cryopreservation and storage of male gametes from threatened species skirt the problem of homozygosis in secluded populations by introducing new genetic material across populations and facilitates genetic interactions between captive areas/zoos/research centers or countries [111]. Sperm is one of the most practical means of storing germplasm due to its abundance and ease of application [112]. Preserved frozen-thawed semen of superior males of endangered livestock could be reintroduced into the population either through *in vitro* fertilization (IVF) or artificial insemination (AI). It has the potential to retain existing diversity and maintain heterozygosity while minimizing live animal transport [111]. Semen from most mammalian and few avian species have been successfully frozen in the past [112].

However, [113] contested the efficiency of sperm cryopreservation due to physiological damage leading to loss of fertility after freezing and thawing sustained by a large number of sperm cells. [114] concisely state the stressors influencing the cells during cooling and freezing stages as follows: 1) exposure to harmful effects while cooling like metabolism decoupling, ionic imbalance, activation of proteases, cellular acidosis, deprivation of energy, membrane phase transition, destabilization of the cytoskeleton and production of free radicals or reactive oxygen species (ROS); 2) during freezing, sperm cell are predisposed to effects of ice crystal formation, hyperosmolarity, alteration in the cell volume and protein denaturation [115]. These and other factors form the hindrance to the full exploitation of sperm cryopreservation in farm animal species.

However, an appealing success is being recorded in the cattle AI industry, where bulls are selected for “freezing ability” of their semen, exhibiting good post-thaw semen quality, ranging from 50% - 70% motile spermatozoa. Pregnancy or calving rate is similar to that of fresh semen provided that higher sperm dosages are used for frozen sperm [116]. This depicts the prospect and efficacy that cryopreserved sperm cells can contribute to the conservation of genetic diversity in this industry.

3) Oocytes

Female gametes (ova) can be obtained through follicle puncture, ovarian tissue biopsies, unilateral or bilateral ovariectomy, or ovary collection immediately after an animal's death, irrespective of its age [117]. Abattoir harvested ovaries are at the germinal vesicle (GV) stage in which the genetic material is confined within the nucleus. Since this stage has no spindle present, GVs are assumed to be less prone to chromosomal and microtubular damage during cryopreserva-

tion. However, oocytes can also be cryopreserved at the metaphase II (MII) stage of maturation. During the MII stage, the cumulus cells surrounding the oocyte are expanded, microfilaments of actin are involved in cell shape and movements, and microtubules form the spindle apparatus [118].

Oocytes collected by *in vivo* pickup or at slaughter, ovarian tissue, isolated follicles, and mature or immature oocytes [119] can all be stored by cryopreservation for extended periods for subsequent *in vitro* fertilization (IVF) to produce embryos. Oocyte banks would enlarge the gene pool, facilitate several assisted reproductive procedures, salvage female genetics after an unexpected death, and avoid the controversy surrounding the preservation of embryos [120] [121]. Like semen, oocyte cryopreservation is beneficial for the international exchange of germplasm, as it avoids injury and sanitary risks involved in live animal transportation [122]. Oocytes are extremely sensitive to chilling, and the technique is not as established as in semen or embryos, because oocytes tend to have large cells that have a low surface-to-volume ratio and a low permeability coefficient, both of which hinder the migration of water and cryoprotectants (CPAs) through the cell [90] [123].

It is, however, important to note that, [124] had stated that immature oocytes at the germinal vesicle stage that is yet to form the spindle lack cortical granules and have a higher membrane permeability, which makes them more resistant to chilling injury than mature metaphase II oocytes. The major differences between oocytes and embryos are the plasma membrane, presence of cortical granules, and spindle formation at the metaphase II (MII) stage of meiosis [125]. However, [126] concluded that cryopreserving ovarian tissue helps to circumvent many restrictions encountered in mature oocyte preservation, from a limited number of mature oocytes available in the ovaries to conceivable lethal effects of its conservation under low temperatures, and the need for a super-ovulation procedure. The main constraint of its use is the difficulty in preserving ovarian tissue, given the diversity of cell types and tissue components in it [127].

Two procedures have been defined for female gametes preservation: slow freezing (SF) and vitrification. Slow freezing or conventional freezing refers to the exposure of the tissues/cells to a low concentration of CPA and cooling them slowly in a programmable freezer [128]. CPA concentration and exposure time before freezing need to be balanced to reach sufficient dehydration; however, it has to be low enough to avoid cytotoxic damages [129]. Although this procedure is widely accepted, however, sophisticated and expensive programmable freezers are required for the cooling procedure, those of which are generally not available [126].

Alternatively, vitrification is considered a cheap technique that can be performed under field conditions with no need for special equipment [130], including after animal death [131]. This method involves the use of high concentrations of CPAs and rapid cooling ($-20,000^{\circ}\text{C}/\text{min}$ $-40,000^{\circ}\text{C}/\text{min}$) to achieve a glass-like highly viscous solution without the formation of ice crystals [132].

Vitrification promotes the viscosity state of the solution, but without water crystallization [133].

To date, there is no consistent oocyte cryopreservation method established in any species, although, there has been significant progress and offsprings have been produced from frozen-thawed oocytes in cattle [134], sheep, and horses [123] [135]. During the process of cryopreservation, oocytes suffer considerable morphological and functional damage, although the extent of cryoinjuries depends on the species and the origin (*in vivo* or *in vitro* produced). The mechanism of cryoinjuries is yet to be fully understood and until more insight is gained, improvement of oocyte cryopreservation will be difficult [122].

4) Embryos

Embryo cryopreservation allows the preservation of the full genetic complement of both dam and sire and has incredible opportunities for preserving heterozygosity and population integrity. However, it is a more complex and costly procedure than semen cryopreservation. Moreover, a large number of embryos would be required for the complete reconstruction of a population and are unlikely to be available from donor females of threatened breeds [98]. Embryos of virtually all mammals have been successfully frozen, thawed, and transferred to synchronized recipient females in the past; however, embryos from species such as swine or equine are much more sensitive to cryopreservation when compared to bovine or ovine embryos [136].

Currently, the widespread use of embryo cryopreservation is limited to cattle, sheep, and goats [137]. In the year 2015, almost 700,000 IVP embryos were produced, surpassing for the first time, the number of bovine embryos produced *in vivo*. In this context, 269,353 bovine OPU IVP embryos were transferred in Brazil alone [138], which is considered the world's largest producer of bovine embryos. An advantage of *in vitro* embryo preservation is that they are more resistant than gametes when subjected to high body temperatures due to thermal stress [139]. Thus, the pregnancy rates are better in embryo transfer (ET) than artificial insemination (AI) throughout the year [140] [141] [142]. Another benefit of this method is the smaller number of viable sperms required for fertilization, therefore, more efficient results are recorded by using sex-sorted semen [143] [144].

Despite the advantages of embryo cryopreservation, the greatest challenge is its low resistance to the cryopreservation process [145] [146] reported that its high sensitivity to cooling is due to the accumulation of lipids in their cells, arranged in the form of cytoplasmic lipid droplets that are constituted mainly of triglycerides [147]. However, indications that this high lipid content is because of the medium in which the embryos cultured has been confirmed [148] [149] reported some strategies for refining post-cryopreservation survival capacity which produce more cryotolerant embryos.

6. Genomic Studies on Some Endangered Cattle Breeds

The Wild Gaur (*Bos gaurus*) is a critically endangered species in Vietnam with a

reported population size of just 500 individuals (<http://www.iucnredlist.org>). [150] revealed the genetic status of Vietnamese wild guar by amplifying 130 bovine microsatellite markers in these species. Out of the 130, only 117 markers were efficiently amplified. Sixty-eight of them were polymorphic, which resulted in a total of 264 alleles. It was noted that three cattle Y-chromosome specific microsatellite markers were also highly expressed in the wild gaur. Their result revealed that bovine microsatellites are highly conserved in the wild gaur genome. This indicates the possibility of using bovine microsatellites for genetic characterization and studies in the Wild Guar. Also, low genetic diversity was observed occasioned by a mean Polymorphic Information Content (PIC) of 0.252, Observed Heterozygosity (H_o) of 0.269, Nei's unbiased mean heterozygosity of 0.298. In general, the study obtained genomic information that highlighted the risk of extinction facing the species and the potential to use genomic information from cattle to design breeding strategies that will help preserve these species.

Chikso is a beef cattle breed in Korea and classified as an endangered breed by the [18]. To obtain genomic information that could help in conservation efforts, [151] conducted whole-genome sequencing on the Chikso cattle using the parallel sequencing method of the Illumina HiSeq 2000 sequencing platform. When compared to the bovine reference genome sequence, the authors identified 5,874,026 SNPs and 551,363 insertions/deletions. 45% and 75% of the variations were identified in the autosomes and X-chromosome, respectively, which were previously unknown. In total, 16,273 missense mutations were identified in 7111 genes throughout the genome. With the breed having a very small population at the brink of extinction and the potential of SNPs identified contributing to variation in economically important traits, the information provided by [151] could be beneficial in crafting a genomic breeding program that will facilitate conservation of the cattle breed.

Gayal (Mithan) is another bovine breed with a rapidly dwindling population size that has been classified as endangered (<https://www.iucnredlist.org/>). This bovine specie is found in China, India, and Bangladesh [152]. It is a dual-purpose breed with better body size and meat quality traits than most indigenous cattle breed in those regions [153]. To gain insight into the valuable genetic resource of this species for conservation, Mei *et al.* (2016) sequenced the whole genome of Gayal together with two controls (Red Angus and Japanese Black cattle breeds). When compared with the bovine reference genome, 23,828,562 SNPs were identified, of which 62.24% were novel in Gayal, which is higher than the 2.53% and 5.10% novel SNPs identified in Red Angus and Japanese Black cattle breeds, respectively. 16901 non-synonymous SNPs were identified that might be associated with variation in physical traits in Gayal. The genomic information provided could be a guide to conservationists in the design of breeding programs for Gayal to prevent its extinction.

It is widely known that the knowledge of population size and structure can shed light on the risk status of cattle breeds. In this regard, [154] genotyped eight

Iranian cattle breeds (Sarabi, Kurdi, Najdi, Taleshi, Mazandarani, Pars, Kermani, and Sistani breeds) for 777,962 SNPs. Apart from the Mazandarani breed that had an effective population size of 107, the Mean effective population size for other breeds was 24. The lowest effective population size was 13, found in the Sarabi breed. Runs of homozygosity (ROH) in Sarabi, Pars, and Sistani breeds were of higher proportion. The study revealed the critical status of Sarabi, Sistani, Pars, Teleshi, and Kermani cattle breeds in Iran and suggests the urgent need for conservation efforts to prevent the Sarabi breed from becoming extinct. **Table 1** at the end of this review has a list of some threatened cattle breeds in different locations; information on researches that had been done concerning the molecular markers used, and the genetic parameters calculated to estimate diversity.

Table 1. List of some threatened cattle breeds in different locations.

Country	Breed(s)	Molecular marker(s) used	Genetic parameters estimated	References		
Spain	Betizu	Microsatellite	Allele frequencies	[159]		
	Mallorquina		Population size			
	Menorquina		Nei's genetic distances			
	Monchina		Hardy-Weinberg equilibrium			
	Serrana de Teruel		Linkage disequilibrium			
			Heterozygosity			
			Inbreeding coefficient			
	Berrenda en Negro		rob(1;29) chromosome translocation		Translocation distribution	[160]
	Berrenda en Colorado				Translocation frequencies	
	Cardena Andaluza				F statistic	
Pajuna		Hardy-Weinberg equilibrium				
	Negra Andaluza		Heterozygosity			
Western Pyrenees	Betizu	DNA Microsatellite	Heterozygosity	[161]		
	Terrena		Allele frequencies			
	Monchina		Hardy-Weinberg equilibrium			
			Parentage exclusion power			
			Mean PIC value			
			F statistic			
Germany	Pustertaler–Sprinzen	DNA Microsatellite	Heterozygosity	[162]		
			Allele frequencies			
			Genetic distance			

Continued

Portugal	Cachena	Microsatelite	Heterozygosities inbreeding coefficient (Fis) Hardy weinberg equilibrium Mean number of allele	[163]
	Garvonesa	Microsatelite	Heterozygosity Total Number of Allele Mean number of allele Hardy weinberg equilibrium	[164]
Italy	Maremmana	Single nucleotide Polymorphism	Heterozygosity Runs of homozygosity Linkage disequilibrium decay Nucleotide diversity	[19]
	Modicana	Single nucleotide Polymorphism	Wright Fixation Index Runs of Homozygosity (ROH) Heterozygosity	[165]
Scotland (UK)	Native Aberdeen Angus	Microsatellites	Expected/observed heterozygosity	[166]
			Gene diversity PIC Allele frequencies homozygosity Nei's genetic distance	[167]
	Chillingham Wild Cattle	SNPs	Fraction of polymorphic loci observed homozygosity Inbreeding coefficient linkage disequilibrium ROHom and ROHet heterozygosity	[168]
Ireland	Irish Moiled (Kerry)	SNPs	Effective population size Runs of Homozygosity Maximum Likelihood	[169]
	Lincoln Red		Effective population size Runs of Homozygosity Maximum Likelihood	[169]
Nigeria	Muturu Cattle	SNPs	Nei's genetic distance Rsb Analysis Homozygosity selection tests Integrated haplotype score	[170]

Continued

Tanzania	Maasai	SNPs	Integrated haplotype score	[171]
	Tarime		runs of homozygosity (ROH)	
	Sukume		Identical by state (IBS)	
			Pairwise distance (FST)	
African	N'dama,		Fixation index (Fst)	[172]
	Ankole	SNPs	linkage disequilibrium	
	Boran		population differentiation and structure,	
	Kenana		maximum likelihood (ML) approach,	
	Ogaden		Allelic frequency	
			Population size	
Senegal	Gobra Zebu,	Autosomal microsatellite markers	Allelic frequencies,	[173]
	Maure Zebu		observed number of alleles per locus	
	Djakore		observed heterozygosity (Ho)	
	N'dama		unbiased expected heterozygosity	
			Gene diversity of Nei's (Hs),	
			Inbreeding coefficient	
			Shannon's information index (I)	
			effective number of alleles (Ne)	
			Genetic distance	
			Hardy-weinberg equilibrium (HWE)	
			Genotypic linkage equilibrium.	
Tanzania	Local Zebu	Polymorphic DNA marker	Interbreed dissimilarities	[174]
South Africa	Nguni		Population structure	[175]
			genetic distance	
		Microsatellite markers	number of alleles	
			Genetic Relationship	
			heterozygosity values	
Bangladesh	Red Chittagong	SNPs	Population Structure	[176]
			Observed Heterozygosity (Ho)	
			Expected Heterozygosity (He)	
			Hardy-Weinberg equilibrium	
Korea	Brindle Hanwoo	SNPs	Linkage disequilibrium	[176]
	Jeju Black Hanwoo	SNPs	Hardy-Weinberg Equilibrium	[177]

SNPs: Single nucleotide polymorphism; Mean PIC value: Mean Polymorphic information Content value.

Crossbreeding is a common technique used in the livestock industry. Although beneficial, it has the potential to cause loss of breed-specific characteristics, thereby threatening the existence of natural genetic resources of certain breeds [155]. To avoid extinction, it is expedient that the original genetic backgrounds of those breeds are recovered [156] by removing exogenous genetic materials in a process called de-introgression.

[157] used the Merino and Poll Dorset sheep breeds as models to investigate a genomic approach to identify and recover chromosome segments of endangered breeds whose genetic materials have been mixed with other breeds. First, the full genomic data was obtained from pure Merino and Poll Dorset breeds, after which the breeds were crossed to produce F1 crossbred. The authors tested the efficiency of two genome-wide methods adapted from [158] and [129] to detect Merino segments and Poll Dorset segments in the F1 crossbred. The methods used are either genomic prediction or identification of breed-specific haplotypes. Their results showed that both approaches could efficiently identify genomic segments belonging to different origins in the admixture. Also, both methods were able to restore the full Merino genetic background in a controlled breeding program. Although the sheep breeds used are not threatened breeds, this study has potential application in breed conservation efforts, by helping to identify and restore genetic resources from endangered cattle breeds that have been indiscriminately crossed with other breeds.

7. Conclusion

Genomics has allowed for genetic diversity study on a wider scope. This has helped to gain better insight into the genetic diversity in cattle which would allow for effective management and conservation of this species.

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Authors' Contribution

BU, AA, RO, and BS conceived ideas. All authors contributed equally during the drafting of the paper. BU, AA, and RO corrected the final draft. All authors read and approved the paper for publication.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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