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Genetic Cryopreservation of Rare Breeds of Domesticated North American Livestock: Smithsonian & SVF Biodiversity Preservation Project

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Abstract: Modern agriculture has responded to the growing pressure for animal-protein consumption in the global human population by selecting for specific production traits, which, over the last fifty years, has resulted in a loss of genetic diversity. Most rare and endangered breeds of livestock have not experienced the same selection pressures for production and therefore may contain useful genetic traits not found within modern breeds. In an effort to maintain biodiversity of livestock breeds, the SVF Foundation, a non-profit organization founded to preserve the genetic diversity of food and fiber livestock, has established an ex situ repository of genetic material from endangered North American cattle, sheep, and goats. This genetic material includes in vivo and in vitro produced embryos, semen, fibroblasts, serum, and whole blood DNA cards. The majority of samples in the SVF repository are cryopreserved, creating a genome resource bank for future use. Through the Smithsonian and SVF Biodiversity Preservation Project, this repository will be maintained at the Smithsonian's Front Royal, VA, facility. This effort represents an excellent model for understanding and sustaining the genetic diversity of rare breeds in the US and in other countries.

Keywords: biorepository; biodiversity; livestock gene bank; cryopreservation; germplasm

1. Introduction

At least 17% of the world's domesticated livestock breeds, which have evolved in response to natural and artificial selection pressures over the last 10,000 years, are currently threatened with extinction, with an alarming 58% of livestock breeds having an unknown stability status [1]. Endangered livestock breeds are well suited to thrive in a variety of ecosystems, climates and production systems, but most are not well adapted for the environments or production systems which have emerged with the recent industrialization of commercial agriculture. The United Nations Food and Agriculture Organization (FAO) reports that 99 documented breeds were lost to extinction between 2000 and 2014 due to insufficient breeding efforts or crossbreeding [1].

In response to this rapid loss of genetic diversity, the SVF Foundation, a privately-funded nonprofit based in Newport, RI, USA, was founded in 2002. In collaboration with the Cummings School of Veterinary Medicine at Tufts University, the SVF has established an ex situ repository of genetic material from endangered breeds of North American livestock, including cattle, sheep, and goats. The majority of genetic material is cryopreserved with a goal of creating a genome bank for potential use at an undetermined time in the future.

Examples of genetic advantages present within endangered breeds include the slick-hair phenotype for heat tolerance in certain tropical bovine breeds [2,3], and natural resistance to gastrointestinal parasitism in Gulf Coast Native sheep [4]. Recent advances in gene-mapping technology are providing the means to identify unique loci and to validate maintenance of these rare-breed populations as more genetic advantages are identified within each breed.

2. Materials and Methods

Endangered livestock breeds are selected for preservation based on the total estimated global population and stability of each breed. Global populations are estimated using periodic census data that is collected every three–five years, either by individual breed associations or the Livestock Conservancy, Pittsboro, North Carolina [5]. Animals are sourced from herds located throughout the United States and strict biosecurity guidelines dictate a panel of health testing for each animal prior to transportation to the SVF facility, Newport, RI, USA.

Samples collected include semen, fibroblasts, serum, whole blood, and embryos from endangered ovine, caprine, and bovine breeds.

In vivo and in vitro produced embryos are collected from super-stimulated donors that are naturally bred or artificially inseminated. Embryos are produced, evaluated and cryopreserved following standard methods approved by the International Embryo Technology Society (IETS) [6].

Small ruminant semen is collected on-site using an artificial vagina, and extended with a commercially available soy-based extender Bioxcell® (IMV International, Maple Grove, MN, USA) prior to cryopreservation. All samples are evaluated at the time of collection, after extending and after thawing using a computer-assisted sperm analysis (CASA) system (Sperm Vision®v. 3.5; Minitube of America, Verona, WI, USA) [7].

Additional units of semen are included in the repository from off-site collection of donor males. Sources include donated samples, contracted services from mobile semen collection units and purchased semen from commercial bull studs.

Fibroblasts are cultured from dermal biopsies and stored in cryovials prior to cryopreservation.

The majority of semen and embryos are stored in liquid phase nitrogen, while fibroblasts and quarantined samples are stored in vapor phase liquid nitrogen. Whole blood samples are applied to DNA storage cards (FTA®, Whatman®; GE Healthcare, Waukesha, WI, USA) and desiccated in multi-barrier pouches at room temperature. Serum samples are stored at −80 °C.

In addition to a required panel of health testing and quarantine processes for incoming germplasm donors, access to the animal and laboratory facilities is strictly monitored in an effort to prevent the introduction of any bacteria or virus that may impact either the livestock or germplasm stored in the collection. A vapor phase quarantine tank is maintained for storage of cryopreserved samples obtained from donors without documented health testing, as disease transfer is a higher risk factor within liquid phase vs. vapor phase liquid nitrogen [6].

Detailed records are maintained on all donors, including animal origin, pedigree, breed history and characteristics, phenotype, anecdotal history, health testing results, and animal husbandry records. Data points recorded on samples in the repository include donor (interfacing with the previously mentioned database), date of collection, method of collection, method of processing, recommended method of thawing, assigned quality (embryo grade, semen motility, etc.), unique sample identification, and storage location within the repository.

The target number of donors represented in the collection and amount of genetic material to be collected per breed is based on mathematical models developed in 2005 [8].

3. Results

106,109 units of germplasm from 39 breeds have been included in the SVF cryo-repository as of August 2019. A summary of the collection is presented Table 1:

Table 1. SVF Foundation Germplasm Repository Summary.

Species/Breed	Semen (Donors)	Embryos (Donors)	Fibroblasts (Donors)	Serum (Donors)	Whole Blood (Donors)
Bovine					
Ancient White Park	2985 (29)	329 (18)	95 (19)	39 (20)	19 (19)
Ayrshire	4196 (23)				
Beef Devon	448 (1)				
Belted Galloway	5 (3)				
Canadienne	3231 (12)	230 (10)	70 (14)	28 (14)	14 (14)
Dexter	1549 (10)	25 (1)	10 (2)	4 (2)	2 (2)
Dutch Belted	3568 (28)	232 (9)	54 (10)	22 (11)	1 (1)
Florida Cracker	1490 (5)	31 (4)	8 (4)	20 (4)	4 (4)
Guernsey	838 (12)				
Kerry	3133 (16)	257 (18)	94 (18)	31 (18)	18 (18)
Lincoln Red	1231 (15)	220 (11)	18 (9)	45 (9)	9 (9)
Milking Devon	6469 (16)	248 (16)	119 (22)	51 (27)	12 (12)
Milking Shorthorn-Native	3525 (32)	293 (14)	40 (8)	18 (9)	8 (8)
Pineywoods	5773 (26)	81 (9)	55 (11)	26 (13)	12 (12)
Randall	8189 (17)	259 (17)	95 (19)	39 (20)	19 (19)
Red Poll	2423 (22)	273 (16)	45 (9)	18 (9)	9
Caprine					
Arapawa	4196 (23)	266 (26)	220 (49)	98 (49)	49 (49)
Golden Guernsey	1541 (9)		45 (9)	18 (9)	10 (10)
Myotonic	2401 (21)	231 (18)	233 (42)	87 (43)	7 (7)
Oberhasli	1526 (35)	44 (6)	45 (9)	18 (9)	9 (9)
San Clemente	2933 (12)	289 (23)	230 (46)	97 (60)	1 (1)
Spanish	3844 (18)	199 (24)	279 (52)	104 (50)	52 (52)
Equine					
American Cream			15 (3)	10 (5)	
Exmoor Pony			25 (5)	40 (20)	20 (20)
Ovine					
Black Welsh Mountain	1650 (8)	179 (22)	220 (49)	98 (49)	49 (49)
Clun Forest	703 (4)	102 (11)	30 (15)	85 (17)	15 (15)
Cotswold	2234 (8)	212 (26)	168 (33)	86 (38)	1 (1)
California Vaerigated Mutant	3031 (11)	299 (26)	227 (46)	92 (46)	45 (45)
Gulf Coast Native	3520 (18)	395 (39)	327 (67)	172 (80)	12 (12)
Hog Island	3684 (19)	323 (24)	244 (47)	98 (49)	47 (47)
Horned Dorset	2775 (12)	318 (28)	225 (42)	99 (57)	39 (39)
Jacob	649 (4)	279 (23)	147 (30)	62 (35)	26 (26)
Leicester Longwool	3659 (17)	213 (22)	102 (51)	250 (50)	51 (51)
Navajo Churro	1737 (8)	253 (27)	200 (39)	84 (39)	42 (42)
Santa Cruz	2880 (11)	403 (36)	348 (70)	116 (74)	15 (15)
St. Croix	1359 (5)	319 (27)	220 (44)	88 (44)	44 (44)
Tunis	1991 (7)	335 (30)	265 (53)	108 (54)	53 (53)
Porcine					
British Saddleback	2 (597)				
Tamworth			45 (9)		
Five Species/39 Breeds	93,673 (525)	7235 (617)	3371 (665)	1418 (739)	412 (412)

4. Conclusions/Future Implications

The wide range of biological samples collected and maintained by SVF from a variety of threatened domestic livestock populations over the last 17 years demonstrate the success of this program in creating a repository of endangered livestock breed genetics. One of the ways modern agriculture has responded to the continual pressure to provide higher levels of animal protein for consumption by a growing human population is by selecting for a narrower pool of genetics. However, gains in production rates have been offset by an unintended consequence of a narrower genetic base and concomitant loss of livestock biodiversity. [9] Using some of the same advanced reproductive technologies that have led to a loss of genetic variability, SVF is working to capture the remaining diversity of selected livestock genetics by creating a rare breed gene bank. In 2014, the SVF established a partnership with the Smithsonian, ensuring long-term curation of this repository with an organization well-recognized for cutting edge research and conservation of biodiversity on a global scale. Information about samples will be accessible to anyone interested in livestock conservation.

Although gene mapping technology is advancing rapidly, we cannot yet identify all genes and gene combinations which could provide vital traits for domestic species in the future. Continual adaptation to both natural and artificial pressures is a constant process within biological systems. By establishing and maintaining a bio-secure and bio-diverse genome resource bank, the Smithsonian and the SVF Biodiversity Preservation Project is providing a potential means by which species may adapt to future pressures from sources such as climate change. Replicating similar local banking efforts in other regions and countries will enable food production models that are more sustainable and more resilient to environmental changes [10].

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