Reproductive Biotechnology-Mediated Rescue of Threatened Polish Livestock Breeds

Subjects: Biodiversity Conservation

Contributor: Monika Trzcińska , Marcin Samiec , Małgorzata Duda

This entry was to provide biological, biotechnological and agricultural insights into the research aimed at the generating the bioreservoirs of cryopreserved somatic and stem cell lines and cryopreserved or lyophilized germplasm-based resources of selected livestock species, with the particular scientific emphasis on the pivotal role of the National Research Institute of Animal Production (NRIAP) in Poland in this aspect. To increase implementation in agricultural biotechnology, biomedicine, and pharmacological industry, the extensive efforts are required to be undertaken to improve the overall effectiveness of the investigations focused on the creation of the bioreservoirs comprised of somatic/stem cell lines and germplasm-carrying bioresources suitable for modern assisted reproductive technologies (ARTs).

ex situ conservation | cryogenic protection | lyophilization | assisted reproductive technologies

1. The Biological Background for In Situ and Ex Situ Conservation of Genetic Resources in Farm Animals, with Emphasis on Selected Polish Livestock Breeds

1.1. Genetic, Agricultural and Zootechnical Foundations of Sustainability in Preserving the Biodiversity in Livestock Species and Breeds

Intensive animal production and exploitation of resources stemming from anthropogenic agricultural ecosystems frequently leads to either collapse or disappearance of populations of rare livestock breeds around the world, including in Poland [1][2]. Such populations of livestock breeds are particularly vulnerable to:

(1) Highly intensified bottleneck effects resulting from tremendously impaired genetic drift;

(2) Rapid genetic erosion resulting in a significant reduction in intra-population and inter-individual genotypic variance;

(3) Subsequent drastic quantitative alleviation of population size [3][4].

At present, the number of breeds is decreasing worldwide, which is related to the selection for a particular group of animals with certain traits (e.g., high milk yield, high meat content, species specificity, and adaptability) and the fact that local breeds with more primitive traits are less common or disappear at the expense of high-yielding, widely

distributed production breeds. The main causes of declining animal biodiversity are environmental changes associated with the expansion of cultivated areas, as well as the introduction of human-preferred species into the environment [5][6]. For these reasons, it is very important to protect the genetic resources of animals of native breeds, which are highly adapted to local, often harsh environmental conditions, and at the same time show high resistance and good health. The biodiversity of livestock breeds is preserved through the implementation of in situ and ex situ conservation programs [7][8].

Intra- and inter-population genotypic divergence is a sine qua non condition livestock herds to be able to adapt to extensive anthropogenic alterations within agricultural biotopes. Taking the above-mentioned facts into consideration, the livestock herds displaying the largest rates of genetic diversification seem to exhibit the most desirable genetic backgrounds susceptible to the largest extent of adaptation to unfavorable conditions of zootechnical ecosystems. This, in turn, might be reflected in a more accelerated evolutionary progress of livestock breeds [2][5].

Effective population number (N_e) has been designated as a key predictor of genetic diversity in native livestock populations and can be used to estimate the extent of inbreeding [4][9][10][11][12]. N_e is one of the most crucial parameters of population and evolutionary genetics pinpointed for a perfect population that would be deprived of heterozygosity at a frequency of occurrence equivalent to the observed population [2][13]. In other words, genetic drift is negatively correlated with population quantity (a tiny population number is characterized by very high genetic drift, and a large one by diminished genetic sampling error, fluctuating allele frequencies or Sewall Wright effect). A triangle of Sewall Wright effect, mono- or biallelic alteration and gene relocation serve as pivotal components of the mechanisms underlying intra- and inter-population transmissions of genes [14][15]. As a consequence, the N_e parameters predominantly represent lower quantities of specimens than the animal censuses, which count the complete population numbers (N_c) in livestock species or breeds. The frequencies of occurrence noticed for fluctuating alleles are inadequate for the measurements of N_c, but are rather compatible with quantifications specific to N_e. Only a condition related to the occurrence of perfect populations, which are comprised of completely fertile and fecund specimens, allows for the existence of equality between the parameters of N_e and N_c. It is beyond any doubt that a condition of perfect population is achieved when there are equivalent subgroups of specimens representing both sexes that do not fail to reach and perpetuate sexual and reproductive maturity. Moreover, in perfect populations, each specimen displays the same probability of generating progeny, and the percentage of progeny propagated by all the specimens does not vary to a larger extent than randomly anticipated. The next sine qua non condition is the incidence of total randomization for pairing among the opposite-sex individuals, and furthermore an occurrence of dynamic inter-generation homeostasis (i.e., sustainable balance) between the intra-population numbers of sexually and reproductively active specimens [16][17].

The vast majority of divergences in the above-indicated traits will contribute to the reduction of N_e. It is worth highlighting the crucial differences between two categories of N_e termed as effective population extent of variance and effective population extent of inbreeding. The first one denotes the high probability of alterations in inter-allelic parental and progeny-specific variability, which results in the appearance of biodiversification of genetic background and phenotypic traits observed between herds of livestock breeds. The second one is related to

genotypic divergence arising from maternal and paternal heterozygosity, which gives rise to intensification of inbreeding in livestock herds [4][18]. Furthermore, not without significance is the fact that the N_e displays a negative correlation with the extent of inbreeding. Critically endangered status and the requirement of in situ and ex situ conservation is assigned to livestock breeds with $N_e \leq 50$, while endangered status and the simultaneous necessity of in situ and ex situ conservation is allotted to the livestock breeds exhibiting the parameters of N_e ranging from 50 to 200 [4][19][20]. In turn, the status of vulnerable livestock breed, which is reflected in a prerequisite of in situ and ex situ conservation, is adequate for a more wide range of N_e oscillating between 200 and 1200 [4][21].

In an era of high economic pressure on the profitability of livestock breeding, the protection of native breeds that cannot meet high breeding requirements is needed to preserve unique genotypes. The uncompetitiveness of older, less productive breeds necessitates the creation of programs and projects that make it possible to preserve biodiversity. Conservation of endangered breeds is therefore a very important activity, contributing to the preservation of unique genotypes in breeding [2]. Another important task related to the preservation of biodiversity is the collecting and preserving of biological material derived from high-yielding breeds currently being bred in the country, as well as hybrids produced for specific production purposes. In this regard, measures have been taken to reduce the decline in biodiversity and genetic resources of animals [22][23]. These include a number of in situ protection methods that bring about the preservation of livestock species and breeds in normal production systems. This type of livestock preservation involves breeders whose animal subpopulations are maintained within protected ecosystems, including habitats protected under the European Union's Natura 2000 Network Program [24].

1.2. Biological and Genetic Assumptions of In Situ Conservation in Livestock Species and Breeds

In situ conservation is undertaken to establish animal breeding farms and perpetuate them within their production systems and under the conditions of native ecology. The outcome and final efficacy of these efforts is dependent on the participation of the farmer, for which support and incentives are necessary. The major obstacle for livestock in situ conservation is the number of animals selected and maintained in the niches of agricultural ecosystems. While establishing the percentage for preservation of a livestock breed, the cost of maintenance, availability of specimens of both sexes (males and females) and rate of inbreeding must be taken into consideration [1][25]. In small populations, the effective population size shrinks and the genetic structure of the population is biased by the extent of inbreeding and random drift. A wide variety of strategies are now available which can reduce inbreeding to a minimum. Nevertheless, long-term random drift and disadvantageous crosstalk between genotypic background and environmental components might contribute to the generation of a secondary population, which genotypically varies from its primary counterpart to a large degree. To avoid adverse impacts of inbreeding and random drift on intra-population genetic structure, the Food and Agriculture Organization of the United Nations (FAO) recommends a mating ratio of 5 males and 25 females for protection programs for small populations. A ratio of 50 males and 250 females is preferable for populations characterized by phenotypic traits with low heritability [23].

1.3. Biotechnological Assumptions of Ex Situ Conservation in Livestock Species and Breeds

Another method for protecting biodiversity, which is complementary to the methods of in situ conservation, includes activities related to the collection of bioresources in the form of somatic and stem cells or germplasm-carrying biological materials encompassing male and female gametes (i.e., sperm cells and oocytes, respectively), and embryos within the framework of biobanks or bioreservoirs. Such approaches to protecting genetic resources are referred to as ex situ conservation methods [3][22]. This assumes the cryogenic preservation or freeze-drying (lyophilization) of somatic and stem cells, spermatozoa, oocytes and embryos propagated by assisted reproductive technologies (ARTs), including in vivo (e.g., artificial insemination) and in vitro production biotechniques (cloning by somatic cell nuclear transfer and in vitro fertilization by gamete coincubation or intracytoplasmic sperm microinjection) [8][26]. Modern ARTs are essential for perpetuating long-term ex situ preservation of the biological diversity of near-threatened, endangered, and extinct livestock breeds. While the current programs of ex situ conservation are mediated by the strategies of cryogenic protection, semen doses collected from a total of 25 unrelated males must be preserved, and embryos originating from 35 different matings are indispensable for the purpose of embryo cryoconservation [27][28].

The use of novel approaches to ARTs is highly warranted, mainly taking into consideration the endangered and critically endangered mammalian species indexed within the Red List of Threatened Species, the last variant of which was reported by the World Conservation Union (IUCN) in 2019. In turn, the Polish issue of the Red List was released for the first time in 1992. As indicated in FAO-based compilations, the rate of threatened breeds oscillated around 17% in relation to the overall quantity of nearly 8800 species of farm animals [29][30].

1.4. A Range of Worldwide to Country-Specific Fundamentals and Challenges of Protecting the Biological Diversity in Livestock Breeds, with a Particular Emphasis on Poland

Many countries participating in public and non-public biodiversity conservation companies have undertaken efforts which targeted the protection of the genetic resources of their indigenous livestock breeds. Wood et al. [6] reported that attempts to preserve in situ rare indigenous livestock breeds should be predictive, and so it is necessary to incorporate changes in environmental conditions into strategies related to the conservation of bioresources. Both intensification of production and propagation of new lines or breeds of livestock species with high genetic merit or high productivity can significantly destabilize the global diversity of domesticated animals [2][28][31][32]. Therefore, countries, including Poland, are developing adequate procedures aimed at the protection of livestock breeds. Polish native breeds undergo in situ preservation, which is financed and promoted by the government. The programs focused on in situ protection are especially intended for the restoration and reintroduction of endangered herds of livestock breeds, which expedites their inter-generation and long-term adaptation to biocenotic alterations occurring in anthropogenic biotopes and agricultural ecosystems [3][9].

Rare Polish indigenous breeds of sheep involve the Old Type of Merino, Blackhead, Polish Heath and Olkuska, whose genetic resources are subjected to in situ and ex situ protection. Their N_e parameters oscillate between 277 and approximately 1107. Therefore, the formerly indicated sheep breeds display the highly diminished extent of inbreeding and attenuated resilience to ecological perturbations resulting from intensive systems of zootechnical

production [10]. On the other hand, the N_e parameters pinpointed for in situ/ex situ protected Polish Red cattle and Carpathian goats have reached the levels of 170 and 199, respectively. For these reasons, cattle and goat breeds exhibit vulnerability to ecosystemic changes and are associated with a relatively high inbreeding rate [10]. In addition, the N_e parameters noted for Polish autochthonic breeds of pigs undergoing in situ and ex situ preservation range from approximately 80 (the Złotnicka Spotted breed) to a level higher than 144 (the Puławska breed). Therefore, these pig breeds qualify for the status of endangered livestock. It is noteworthy that such a status results in their relatively high inbreeding rates [10].

Cumulatively, the preferred model for the conservation of animal genetic resources in Poland and around the world is in situ conservation, in which a species is preserved in its natural habitat with limited use of breeding methods, while maintaining a number of animals that ensures minimal genetic variability and adaptability to changing environmental conditions. The strategies of ex situ conservation, on the other hand, underlie the use of biotechnological methods for the acquisition and long-term storage of germplasm-, somatic cell- and stem cell-based bioreservoirs derived from different species and breeds of farm animals. Efforts focused on the long-term preservation and storage of such bioreservoirs are undertaken by using the bioprotection methods related to cryopreservation or freeze-drying (i.e., lyophilization). Recovery of bioresources allows for the deposition followed by the use of unique genetic material after the death of the donor. The cryogenically or lyophilizogenically protected bioresources collected from random representatives originating from populations comprising endangered livestock species and breeds will enable their future restoration [7][33][34].

2. The Implementation of Modern Reproductive Biotechnology Strategies Based on Somatic Cell Cloning to Ex Situ Conservation of Genetic Resources in Selected Polish Livestock Breeds

2.1. Biological Justification for Applicability of Somatic or Stem Cell Banking to Cloning-Mediated Reproductive Biotechnology of Farm Animals

As part of a biodiversity conservation strategy, creating bioreservoirs of somatic and stem cell lines through cryogenic preservation can provide a reliable and feasible approach applied to regenerate or restore the biocenotic sustainability among herds of livestock breeds followed by reintroduction of vanishing breeds of farm animals into reconstituted niches of zootechnical biotopes [35][36]. Moreover, the establishment and subsequent cryoconservation of ex vivo-expanded permanent somatic or stem cell lines, which have been established from various tissue bioptates, serves as a promising tool independent of freezing or vitrification methods used for gametes and embryos [37][38][39].

Successfully devised and optimized approaches that enable cryogenic protection of somatic and/or stem cell-based bioresources are a indispensable prerequisite for the propagation and multiplication of farm animals with the aid of intra- or interspecies cloning by somatic cell nuclear transfer (SCNT) [40][41]. The latter represents one of the

innovative ARTs that is necessary for long-term maintenance of ex situ protection of the biological diversity of near-threatened, endangered, and even extinct livestock breeds in Poland [3][4][39].

Research directed at the precise identification of the determinants of proliferative capability, genetic stability, cytokinetic aging, and programmed cell death in nuclear donor cell lines (NDCLs) expanded ex vivo is needed. Identification of the above factors can lead to ameliorated and expedited epigenomic reprogrammability of somatic cell nuclei in SCNT-generated embryos [42][43]. The goal of such procedures is to consolidate the enhanced inheritability of superior genotypic traits and relieve the extent of intra-population inbreeding, thereby intensifying the divergence in the genetic background among threatened native livestock breeds. Finally, the progression of processes that lead to increased inter-specimen genetic diversity can enhance physiological and immunological resistance to drastic environmental collapse, climatic perturbations and infectious disease spreads [44].

2.2. The Efforts of the NRIAP Focused on Collecting the Ex Situ Protected Somatic Cell- and Stem Cell-Based Bioresources for the Purposes of Cloning-Mediated ARTs in Selected Livestock Species and Breeds

It is significant that effective methods have been developed at the NRIAP for the successful ex vivo expansion and cryopreservation of excellent quality adult dermal fibroblast cell lines (ADFCLs) of such endangered Polish livestock breeds as Polish Red cattle, Złotnicka Spotted and Puławska pigs, Carpathian goats, Old Type of Merino, Romanowska and Polish Heath sheep. As a result, bioresources of cryogenically protected somatic cell lines of the selected endangered indigenous breeds have been established within the framework of the NRIAP and additionally enriched with bioresources of adult mesenchymal stem cell lines (MSCLs) originating from the bone marrow (BM) and adipose tissue (AT) of the Polish Landrace pig. A comparative analysis of these bioreservoirs is shown in **Table 1**.

Table 1. The NRIAP bioreservoirs of cryopreserved somatic and stem cells derived from Polish indigenous breeds of selected livestock species.

Species	Breed Name	The Type and Provenance of Somatic and Stem Cell Lines	Number of Somatic/Stem Cell Lines	Number of Female Donors	Number of Male Donors	Quality Parameters			
						Post-Freeze/Thaw Survivability *	Attachment to the Substratum and Enzymatically Assisted Detachment *	Mitotic Lifespan and Resistance to Morphological Senescence *	Capacity to Reach a Total Confluency and Undergo Cell Multiplication *
Cattle	Polish Red	Mitotically stable cell lines of adult dermal fibroblasts	45	5	-	++++	++++	++++	++++
Pigs	Puławska		71	10	1				
	Złotnicka Spotted		22	6	-				

Species	Breed Name	The Type and Provenance of Somatic and Stem Cell Lines	Number of Somatic/Stem Cell Lines	Number of Female Donors	Number of Male Donors	Quality Parameters			
						Post-Freeze/Thaw Survivability *	Attachment to the Substratum and Enzymatically Assisted Detachment *	Mitotic Lifespan and Resistance to Morphological Senescence *	Capacity to Reach a Total Confluency and Undergo Cell Multiplication *
Sheep	Polish Heath		15	3	3				
	Romanowska		32	3	3				
	Old Type of Merino		22	3	3				
Goats	Carpathian		13	3	3				
Pigs	Polish Landrace	Mitotically stable cell lines of bone marrow-derived mesenchymal stem cells	25	10	-	[45]			
		Mitotically stable cell lines of adipose tissue-derived mesenchymal stem cells	14	10	-	[46][47][48]			

* Quality marked with "+/+/-" symbol indicates that the percentage of somatic/stem cells exhibiting desired parameters was higher than or equal to 95%.

nuclear-
%) noted
ilar to or
embryos
complete
at either
analogous or considerably increased levels as compared to those achieved by other researchers [49][50][51].

2.3. The Factors Determining the Outcome of Cloning-Mediated Reproductive Biotechnology and Their Usefulness for a Broad Spectrum of Research Disciplines

The effectiveness of SCNT-based cloning in different livestock species is dependent, to a large extent, on the provenance and quality of somatic or stem cell lines [52][53][54][55] and the incidence of apoptotic symptoms in NDCLs and nuclear-transferred embryos [42][54][56]. Another factor that determines the SCNT efficiency is the capability of donor cell nuclei to be epigenetically reprogrammed in the blastomeres of cloned embryos [43][57][58]. Genomic, transcriptomic and proteomic interplays between nuclear and mitochondrial compartments have also been shown to impact the developmental ability of nuclear-transferred embryos [59][60][61].

Cumulatively, at the current stage of research, the efficiency of SCNT-based cloning in mammals remains extremely low, oscillating between 0.5% and 5% of offspring produced relative to the overall quantity of oocytes reconstituted using various types of somatic and stem cells. Taking this fact into account, thoroughly unravelling the genomic, epigenomic, transcriptomic and proteomic signatures giving rise to the capability of donor cell nuclei to be faithfully and completely reprogrammed is undoubtedly needed, and would lead to considerable enhancements of the developmental outcomes for cloned embryos, conceptuses and progeny in different mammalian species [54][62]. Such a condition is also required for successful combination of somatic cell cloning and transgenesis to efficiently generate the gene/genome-edited progeny utilized in such interdisciplinary research areas as agricultural ecology, livestock species/breed-related biodiversification, animal production, nutria-biotechnology, human dietetics,

biopharmacy, regenerative and reconstructive biomedicine and immunological and medical xenotransplantology [43][63][64][65][66].

3. The Implementation of Modern Reproductive Biotechnology Strategies Based on Cryogenic Protection, Lyophilization and In Vitro Embryo Production in the Ex Situ Conservation of Genetic Resources in Selected Polish Livestock Breeds

3.1. Justification for the Ex Situ Conservation of Germplasm-Based Bioresources

Long-term cryopreservation or lyophilization of germplasm-carrying biological materials (spermatozoa, embryos and oocytes) expedites the use of bioresources recovered from non-existing specimens to restore, sustainably re-establish and perpetuate the previously disappeared biodiversity in currently existing subpopulations of livestock species and breeds. The utilization of the formerly lost genetic resources may result in genetic gain for breeds that are currently undergoing intensive zootechnical and biotechnological improvement [8][22].

3.2. Biological and Biotechnological Determinants of Cryogenically Protecting the Spermatozoa

Cryopreservation of semen is of tremendous importance for the management and conservation of genetic resources in different species and breeds of farm animals. The methods applied to cryopreserve the germplasm-carrying biological materials (spermatozoa and embryos) derived from cattle are characterized by the highest advancement and efficacy. Therefore, they are used extensively in ARTs and ex situ conservation programs intended for livestock. In recent years, the NRIAP has conducted comprehensive investigations focused on increasing the effectiveness of cryopreservation of semen originating from other livestock species [67][68]. To enable the high quality of cryogenically protected sperm cells collected from males representing a variety of livestock species, the semen probes pre-selected for cryopreservation undergo a multifaceted biological evaluation. The significance of research aimed at the identification and thorough assessment of low-temperature ultrastructural and functional biodegradation of intracellular organelles and compartments and plasmalemma-related cytoskeleton, as well as detection of proapoptotic and/or pronecrotic symptoms in sperm cells, cannot be overestimated [25][69]. The occurrence of the intra-spermatozoon processes triggered by cryogenically induced biodestruction can be mitigated or relieved by enrichment of cryopreservation media with ectopic scavengers of oxygen-derived free radicals (ODFRs) [2][67][70].

3.3. Biological and Biotechnological Determinants of Lyophilizogenically Protecting the Spermatozoa

In recent years, there has been increased interest in a new technique for preserving mammalian semen: freeze-drying, also known as lyophilization or cryodesiccation. This technique serves as an alternative to cryopreserving

the semen in liquid nitrogen. The biophysical mechanism underlying this technique is a low-temperature dehydration process that involves freezing the semen samples and lowering pressure, which together lead to the elimination of the intracellular ice crystals formed during the freezing process through sublimation [33][34][71][72][73].

Previous research has demonstrated that lyophilized (cryodesiccated) spermatozoa can undergo the processes of biodestruction within plasma membrane and/or genomic DNA that had been separately or collectively damaged due to the occurrence of oxidative stress and impairments occurring in anti-oxidative events. The latter can be, in turn, prompted by bioaccumulating reactive oxygen species (ROS) and diminishing the concentration or attenuating the enzymatic activity of intrinsic water-soluble isoforms for potent scavengers of ODFRs [74][75]. Attempts to decrease the incidence of ROS-induced DNA biodegradation during lyophilization of spermatozoa have targeted the enhancement of anti-oxidative activity of endogenous biocatalytic ODFR scavengers, which can be accomplished via enrichment of the freeze-drying media with exogenous water-soluble phenolic (polyphenol) antioxidants such as rosmarinic acid (RosA; an ester of caffeic acid and 3,4-dihydroxyphenyl lactic acid), ethylenediaminetetraacetic acid (EDTA) or ethyleneglycol-*bis*(β-aminoethyl)-N,N,N′,N′-tetraacetic acid (EGTA) [76][77][78].

It is worth highlighting that aberrations in such sperm quality parameters as viability, motility and even DNA stability do not exclude the usefulness of the lyophilized and subsequently rehydrated spermatozoa for intracytoplasmic sperm injection (ICSI)-mediated procedures carried out to in vitro fertilize metaphase II (MII)-stage oocytes in livestock species [33][34][74]. Nevertheless, maintenance of the conditions facilitating the alleviation of DNA biodegradation or internucleosomal fragmentation during freeze-drying of spermatozoa might largely improve the applicability of ICSI to in vitro fertilization (IVF), even if the male gametes exhibit a large amount of not only ultrastructural and biophysical impairments in the sperm intracellular organelles and compartments, but also hyperpermeabilization or biochemical destabilization of plasmalemma integrity [72][75].

Thus far, the application of ICSI-mediated in vitro fertilization (IVF) of meiotically matured oocytes by using lyophilized sperm cells followed by rehydration of formerly freeze-dried spermatozoa has contributed to the propagation of viable progeny in a wide variety of mammalian species, including livestock. These species include hamsters [79], mice [80], rats [81], rabbits [82] and horses [83].

In contrast to the above-indicated studies, the attempts to obtain the ICSI-generated offspring by using freeze-dried/rehydrated spermatozoa failed in cattle [84], sheep [85] and pigs [77]. All these approaches gave rise to the production of the embryos at the blastocyst stage under in vitro culture conditions.

3.4. The State of the Art and a Wide Range of Biotechnological and Physicochemical Factors Affecting Cryoconservation of Oocytes and Embryos

Females of mammalian species, including farm animals, are born with their gametogenic reservoirs of oocytes accumulated in ovarian follicles. These reservoirs are sufficient for the whole period of their lifespan related to reproductive activity during adult ontogenesis. In contrast to male gonads (testes), ovaries do not generate new

gametes throughout the reproductive period of female ontogenetic development. Maintenance of the female genotypic background is also possible to achieve with the aid of the methods applied to cryogenically preserve oocytes and embryos. The oocytes can be recovered and cryopreserved at any stage of meiotic maturation, including oocytes retrieved from primordial, preantral or antral ovarian follicles [86][87][88]. Nonetheless, cryogenic protection of oocytes is tremendously limited due to the extremely low efficiency of this biotechnique. This is a major hindrance to its practical applicability to such modern ARTs as complex in vitro embryo production (IVP), including in vitro oocyte maturation (IVM), gamete co-incubation- or ICSI-mediated IVF, or SCNT-based cloning and in vitro culture (IVC) of IVF- or SCNT-derived embryos in different livestock species [89][90].

Two basic strategies are frequently utilized for the cryopreservation of oocytes and embryos in liquid nitrogen. The first strategy involves the method of slow freezing, in which germplasm-carrying bioresources are sequentially subjected to treatment with relatively low concentrations of cryoprotectants displaying a high capability to permeabilize the plasma membranes [91][92][93]. Some of the most efficient, most potent and most permeable cryoprotectants are dimethyl sulfoxide (DMSO), glycerol, ethylene glycol (EG) and propylene glycol, while their non-permeable counterparts are monosaccharides, e.g., glucose and fructose, and disaccharides, e.g., sucrose [88][90][94]. The second strategy involves vitrification, in which a combination of different cryoprotectants is frequently applied to enhance the biophysical parameters of viscosity of the cryopreservation medium, increase the glass transition temperature and finally decrease the excitotoxicity. It is worth highlighting that there is an erroneous comparative determination of the biophysical mechanisms underlying slow freezing and vitrification, because a slow cooling rate and low concentrations of cryoprotectants are not always used in slow cooling, while vitrification does not always use a high cooling rate or high concentrations of cryoprotectants. In contrast, an efficient vitrification process can take place via onset of a very low cooling rate and very low concentrations of cryoprotectants [95][96][97]. In terms of biophysical characterization, slow freezing induces crystallization of extracellular water. This gives rise to the generation of an osmotic gradient that drives the water flux from the intracellular microenvironment until the intracellular vitrification is terminated. In turn, vitrification can largely affect both intra- and extracellular microenvironments after intracellular dehydration has been completed. For all the above-mentioned reasons, the terms “freezing” and “thawing” are related to the processes of slow freezing [98][99][100], whereas the terms “cooling” and “warming” are associated with the processes of vitrification. It is also noteworthy that, considering the vitrification-mediated cryopreservation of oocytes, the warming rate appears to play a more important role than the cooling rate. Such a finding can be explained by the fact that the viability of murine oocytes that had been subjected to vitrification under the conditions of a very slow cooling rate and high warming rate increased to a large extent, as compared to the viability of oocytes undergoing vitrification characterized by a combination of highly rapid kinetics of cooling and subsequent slow kinetics of warming [86][87][100][101]. Nevertheless, such a negative correlation has not been confirmed in the investigations aimed at the vitrification of oocytes and embryos in selected livestock species (rabbits, pigs and cattle), which exhibit a particular oversensitivity to cooling rates related to cryopreservation procedures [92][99][101][102].

3.5. The Applicability of Ex Situ Conservation Methods to Modern ARTs—The Importance of NRIAP Bioreservoirs of Embryos and Semen for Sustainable Maintenance of Biodiversity in Selected Livestock Species and Breeds in Poland

The strategies elaborated in the NRIAP to cryopreserve or lyophilize semen derived from native wide-spread pig breeds (Polish Landrace and Polish Large White) accelerate, to a large extent, the utilization of semen for other goals. These goals involve the biotechnological rescue of germplasm-based bioresources, and programs designed for perpetuating the desirable genotypic and phenotypic diversification and immunological resilience to epidemic and pandemic events in endangered Polish pig breeds, including Złotnicka Spotted, Puławska and Złotnicka White.

The cryogenically or lyophilizogenically protected semen recovered from rare indigenous Polish pig breeds (Złotnicka White, Złotnicka Spotted and Puławska) can be utilized for a broad panel of the ARTs based on the IVP procedures. The latter is comprised of three consecutive steps, as follows: (1) in vitro maturation of metaphase II (MII)-stage oocytes; (2) IVF or SCNT-mediated oocyte reconstitution; and (3) IVC of IVF- or SCNT-derived embryos [46][103][104]. Analogous to standard artificial insemination, to successfully perform repeatable IVF experiments, cryogenically protected male gametes can be used either for conventional co-incubation of MII-stage oocytes with motile spermatozoa or for ICSI-mediated IVF [105][106][107][108]. Alternatively, ICSI-based IVF can be achieved with the aid of the freeze-dried (lyophilized) and subsequently rehydrated sperm cells for microsurgical deposition into a cytoplasm of MII-stage oocytes [34][72]. For ICSI-fertilized oocytes, the artificial activation of their embryonic developmental program, which has been prompted by extrinsic factors (e.g., electrostimulation, ionomycin-mediated intraooplasmic iontophoretic transport of extracellular calcium sources) also plays a pivotal role [109][110]. Furthermore, epigenomic reprogrammability of oocyte- and spermatozoon-inherited chromosomes brings about the pre- and postimplantation developmental competences of IVF- or ICSI-derived embryos [111][112][113]. Finally, transcriptomic and proteomic crosstalk between nuclear and mitochondrial DNA fractions [114][115] and the initialization of necrotic, apoptotic or autophagic processes [116][117][118] are undoubtedly significant for successfully promoting the ex vivo- and in vivo- developmental capabilities of IVF- and ICSI-generated embryos in a wide variety of farm animal species and breeds.

Collectively, to protect a variety of biological materials ex situ, including those recovered from Polish indigenous breeds of the selected livestock species (cattle, pigs and sheep), the long-term efforts efficiently undertaken by NRIAP have resulted in accumulation of ex situ protected bioreservoirs encompassing a broad spectrum of genetic resources. The latter include germplasm-based materials (cryopreserved and lyophilized spermatozoa and cryopreserved embryos), whose quantitative distribution is presented in **Table 2**.

Table 2. The NRIAP bioreservoirs of germplasm-based biological materials derived from selected native livestock breeds.

Species	Breed	The Category of Germplasm-Carrying Biological Materials	The Approach to Ex SITU Conservation	Number of Protected Samples	Number of Female Donors	Number of Male Donors
Cattle	Polish Red	Embryos	Cryopreservation	1200	125	-
Pigs	Polish Landrace	Semen	Cryopreservation	645	-	6

Species	Breed	The Category of Germplasm-Carrying Biological Materials	The Approach to Ex SITU Conservation	Number of Protected Samples	Number of Female Donors	Number of Male Donors
Sheep	Polish Large White	Semen	Lyophilization	160		4
			Cryopreservation	720	-	7
	Polish Heath			587	-	8
	Romanowska			968	-	5
	Olkuska			3519	-	8
	Blackhead			788	-	4

1. Perry, S.M.; Mitchell, M.A. Reptile assisted reproductive technologies: Can ART help conserve 300 million years of evolution by preserving extant reptile biodiversity? Reprod. Fertil. Dev. 2022, 34, 385–400.
2. Bolton, R.L.; Mooney, A.; Pettit, M.T.; Bolton, A.E.; Morgan, L.; Drake, G.J.; Appeltant, R.; Walker, S.L.; Gillis, J.D.; Hvilsom, C. Resurrecting biodiversity: Advanced assisted reproductive technologies and biobanking. Reprod. Fertil. 2022, 3, R121–R146.
3. Caroli, A.M.; Pizzi, F. Livestock biodiversity: From genes to animal products through safeguard actions. Theor. Biol. Forum. 2012, 105, 71–82.
4. Leroy, G.; Gicquel, E.; Boettcher, P.; Besbes, B.; Furre, S.; Fernandez, J.; Danchin-Burge, C.; Alnahhas, N.; Baumung, R. Coancestry rate's estimate of effective population size for genetic variability monitoring. Conservation Genet. Resour. 2020, 12, 275–283.
5. Comizzoli, P.; Holt, W.V. Breakthroughs and new horizons in reproductive biology of rare and endangered animal species. Biol. Reprod. 2019, 101, 514–525.
6. Wood, K.A.; Stilman, R.A.; Hilton, G.M. Conservation in a changing world needs predictive models. Anim. Conserv. 2018, 21, 87–88.
7. Jacques, A.; Leroy, G.; Rognon, X.; Verrier, E.; Tixier-Boichard, M.; Restoux, G. Reintroducing genetic diversity in populations from cryopreserved material: The case of Abondance, a French local dairy cattle breed. Genet. Sel. Evol. 2023, 55, 28.
8. Mara, L.; Casu, S.; Carta, A.; Dattena, M. Cryobanking of farm animal gametes and embryos as a means of conserving livestock genetics. Anim. Reprod. Sci. 2013, 138, 25–38.
9. Ryder, O.A.; Onuma, M. Viable Cell Culture Banking for Biodiversity Characterization and Conservation. Annu. Rev. Anim. Biosci. 2018, 6, 83–98.
10. Polak, G.; Krupiński, J.; Martyniuk, E.; Calik, J.; Kawęcka, A.; Krawczyk, J.; Majewska, A.; Sikora, J.; Sosin-Bzducha, E.; Szyndler-Nędza, M.; et al. The risk status of Polish local breeds under

conservation programmes - new approach. Ann. Anim. Sci. 2021, 21, 125–140.

11. Long, J.A. Reproductive biotechnology and gene mapping: Tools for conserving rare breeds of livestock. Reprod. Domest. Anim. 2008, 43 (Suppl. 2), 83–88.
12. Kikuchi, K.; Kaneko, H.; Nakai, M.; Somfai, T.; Kashiwazaki, N.; Nagai, T. Contribution of in vitro systems to preservation and utilization of porcine genetic resources. Theriogenology 2016, 86, 170–175.
13. Son, Y.B.; Jeong, Y.I.; Jeong, Y.W.; Yu, X.; Cai, L.; Choi, E.J.; Hossein, M.S.; Tinson, A.; Singh, K.K.; Rajesh, S.; et al. Vitrification of camel skin tissue for use as a resource for somatic cell nuclear transfer in Camelus dromedarius. In Vitro Cell. Dev. Biol. Anim. 2021, 57, 487–492.
14. Dua, S.; Sharma, P.; Saini, M.; Rawat, N.; Rajendran, R.; Bansal, S.; Wakil, A.M.; Beniwal, M.; Parashar, A.; Bajwa, K.K.; et al. Cryobanking of primary somatic cells of elite farm animals - A pilot study in domesticated water buffalo (Bubalus bubalis). Cryobiology 2021, 98, 139–145.
15. Soglia, D.; Sartore, S.; Lasagna, E.; Castellini, C.; Cendron, F.; Perini, F.; Cassandro, M.; Marzoni, M.; Iaffaldano, N.; Buccioni, A.; et al. Genetic Diversity of 17 Autochthonous Italian Chicken Breeds and Their Extinction Risk Status. Front. Genet. 2021, 14, 715656.
16. Liu, C.; Guo, Y.; Guan, W.; Ma, Y.; Zhang, H.H.; Tang, X. Establishment and biological characteristics of Luxi cattle fibroblast bank. Tissue Cell 2008, 40, 417–424.
17. Chen, F.; Zhao, C.; Zhao, Y.; Li, L.; Liu, S.; Zhu, Z.; Guan, W. The biological characteristics of sheep umbilical cord mesenchymal stem cells. Can. J. Vet. Res. 2018, 82, 216–224.
18. Elyasi Gorji, Z.; Farzaneh, P.; Nasimian, A.; Ganjibakhsh, M.; Izadpanah, M.; Farghadan, M.; Vakhshiteh, F.; Rahmati, H.; Shahzadeh Fazeli, S.A.; Khaledi, H.; et al. Cryopreservation of Iranian Markhoz goat fibroblast cells as an endangered national genetic resource. Mol. Biol. Rep. 2021, 48, 6241–6248.
19. Bai, C.; Wang, D.; Li, C.; Jin, D.; Li, C.; Guan, W.; Ma, Y. Establishment and biological characteristics of a Jingning chicken embryonic fibroblast bank. Eur. J. Histochem. 2011, 55, e4.
20. León-Quinto, T.; Simón, M.A.; Cadenas, R.; Martínez, A.; Serna, A. Different cryopreservation requirements in foetal versus adult skin cells from an endangered mammal, the Iberian lynx (Lynx pardinus). Cryobiology 2014, 68, 227–233.
21. Silyukova, Y.L.; Stanishevskaya, O.I.; Dementieva, N.V. The current state of the problem of in vitro gene pool preservation in poultry. Vavilovskii Zhurnal Genet. Selektsii 2020, 24, 176–184.
22. Comizzoli, P.; Holt, W.V. Recent advances and prospects in germplasm preservation of rare and endangered species. Adv. Exp. Med. Biol. 2014, 753, 331–356.
23. Huijsmans, T.E.R.G.; Hassan, H.A.; Smits, K.; Van Soom, A. Postmortem Collection of Gametes for the Conservation of Endangered Mammals: A Review of the Current State-of-the-Art. Animals

2023, 13, 1360.

24. Thiermann, I.; Bittmann, T. Should I stay or should I go? The impact of nature reserves on the survival and growth of dairy farms. J. Environ. Manage. 2023, 328, 116993.
25. Comizzoli, P. Biobanking efforts and new advances in male fertility preservation for rare and endangered species. Asian J. Androl. 2015, 17, 640–645.
26. Eynard, S.E.; Windig, J.J.; Hulsegge, I.; Hiemstra, S.J.; Calus, M.P.L. The impact of using old germplasm on genetic merit and diversity-A cattle breed case study. J. Anim. Breed Genet. 2018, 135, 311–322.
27. De Oliveira Silva, R.; Cortes Gardyn, O.; Hiemstra, S.J.; Marques, J.G.O.; Tixier-Boichard, M.; Moran, D. Ex situ and in situ data for endangered livestock breeds in Spain. Data Brief 2021, 35, 106805.
28. Lauvie, A.; Audiot, A.; Couix, N.; Casabianca, F.; Brives, H.; Verrier, E. Diversity of rare breed management programs: Between conservation and development. Livest. Sci. 2011, 140, 161–170.
29. Rege, J.E.O.; Gibson, J.P. Animal genetic resources and economic development: Issues in relation to economic valuation. Ecol. Econ. 2003, 45, 319–330.
30. Blackburn, H.D. The National Animal Germplasm Program: Challenges and opportunities for poultry genetic resources. Poult. Sci. 2006, 85, 210–215.
31. Woelders, H.; Windig, J.; Hiemstra, S.J. How developments in cryobiology, reproductive technologies and conservation genomics could shape gene banking strategies for (farm) animals. Reprod. Domest. Anim. 2012, 47, 264–273.
32. Sun, Y.; Li, Y.; Zong, Y.; Mehaisen, G.M.K.; Chen, J. Poultry genetic heritage cryopreservation and reconstruction: Advancement and future challenges. J. Anim. Sci. Biotechnol. 2022, 13, 115.
33. Comizzoli, P.; Wildt, D.E. Mammalian fertility preservation through cryobiology: Value of classical comparative studies and the need for new preservation options. Reprod. Fertil. Dev. 2013, 26, 91–98.
34. Keskintepe, L.; Eroglu, A. Preservation of Mammalian Sperm by Freeze-Drying. Methods Mol. Biol. 2021, 2180, 721–730.
35. Men, H.; Walters, E.M.; Nagashima, H.; Prather, R.S. Emerging applications of sperm, embryo and somatic cell cryopreservation in maintenance, relocation and rederivation of swine genetics. Theriogenology 2012, 78, 1720–1729.
36. Gavin-Plagne, L.; Perold, F.; Osteil, P.; Voisin, S.; Moreira, S.C.; Combourieu, Q.; Saïdou, V.; Mure, M.; Louis, G.; Baudot, A.; et al. Insights into Species Preservation: Cryobanking of Rabbit Somatic and Pluripotent Stem Cells. Int. J. Mol. Sci. 2020, 21, 7285.

37. Smits, K.; Hoogewijs, M.; Woelders, H.; Daels, P.; Van Soom, A. Breeding or assisted reproduction? Relevance of the horse model applied to the conservation of endangered equids. Reprod. Domest. Anim. 2012, 47, 239–248.
38. Enya, S.; Kawarasaki, T.; Otake, M.; Kangawa, A.; Uenishi, H.; Mikawa, S.; Nishimura, T.; Kuwahawa, Y.; Shibata, M. Preservation and Reproduction of Microminipigs by Cloning Technology. In Vivo 2016, 30, 617–622.
39. Hu, T.; Taylor, L.; Sherman, A.; Keambou Tiambo, C.; Kemp, S.J.; Whitelaw, B.; Hawken, R.J.; Djikeng, A.; McGrew, M.J. A low-tech, cost-effective and efficient method for safeguarding genetic diversity by direct cryopreservation of poultry embryonic reproductive cells. eLife 2022, 25, e74036.
40. Borges, A.A.; Lira, G.P.O.; Nascimento, L.E.; Santos, M.V.O.; Oliveira, M.F.; Silva, A.R.; Pereira, A.F. Isolation, characterization, and cryopreservation of collared peccary skin-derived fibroblast cell lines. PeerJ 2020, 8, e9136.
41. Praxedes, É.A.; Silva, M.B.; Oliveira, L.R.M.; Viana, J.V.D.S.; Silva, A.R.; Oliveira, M.F.; Pereira, A.F. Establishment, characterization, and cryopreservation of cell lines derived from red-rumped agouti (Dasyprocta leporina Linnaeus, 1758) - A study in a wild rodent. Cryobiology 2021, 98, 63–72.
42. Jeong, P.S.; Sim, B.W.; Park, S.H.; Kim, M.J.; Kang, H.G.; Nanjidsuren, T.; Lee, S.; Song, B.S.; Koo, D.B.; Kim, S.U. Chaetocin improves pig cloning efficiency by enhancing epigenetic reprogramming and autophagic activity. Int. J. Mol. Sci. 2020, 21, 4836.
43. Samiec, M.; Wiater, J.; Wartalski, K.; Skrzyszowska, M.; Trzcińska, M.; Lipiński, D.; Jura, J.; Smorąg, Z.; Słomski, R.; Duda, M. The Relative Abundances of Human Leukocyte Antigen-E, α-Galactosidase A and α-Gal Antigenic Determinants Are Biased by Trichostatin A-Dependent Epigenetic Transformation of Triple-Transgenic Pig-Derived Dermal Fibroblast Cells. Int. J. Mol. Sci. 2022, 23, 10296.
44. Leroy, G.; Besbes, B.; Boettcher, P.; Hoffmann, I.; Pilling, D.; Baumung, R.; Scherf, B. Factors and determinants of animal genetic resources management activities across the world. Livest. Sci. 2016, 189, 70–77.
45. Samiec, M.; Skrzyszowska, M.; Witarski, W. Conservation of valuable genetic resources and restitution of endangered livestock breeds and species—Potential targets of somatic cell cloning of mammals in livestock breeding practice (Article in Polish). Przegl. Hod. 2020, 2, 1–4.
46. Glanzner, W.G.; Rissi, V.B.; De Macedo, M.P.; Mujica, L.K.S.; Gutierrez, K.; Bridi, A.; De Souza, J.R.M.; Gonçalves, P.B.D.; Bordignon, V. Histone 3 lysine 4, 9, and 27 demethylases expression profile in fertilized and cloned bovine and porcine embryos. Biol. Reprod. 2018, 98, 742–751.

47. Zhou, C.; Wang, Y.; Zhang, J.; Su, J.; An, Q.; Liu, X.; Zhang, M.; Wang, Y.; Liu, J.; Zhang, Y. H3K27me3 is an epigenetic barrier while KDM6A overexpression improves nuclear reprogramming efficiency. FASEB J. 2019, 33, 4638–4652.
48. Xu, L.; Mesalam, A.; Lee, K.L.; Song, S.H.; Khan, I.; Chowdhury, M.M.R.; Lv, W.; Kong, I.K. Improves the in vitro developmental competence and reprogramming efficiency of cloned bovine embryos by additional complimentary cytoplasm. Cell. Reprogram. 2019, 21, 51–60.
49. Zhang, Y.L.; Liu, F.J.; Zhuang, Y.F.; Wang, X.A.; Zhai, X.W.; Li, H.X.; Hong, Z.Y.; Chen, J.J.; Zhong, L.C.; Zhang, W.C. Blastocysts cloned from the Putian Black pig ear tissues frozen without cryoprotectant at -80 and -196 degrees Celsius for 3 yrs. Theriogenology 2012, 78, 1166–1170.
50. Qu, J.; Wang, X.; Jiang, Y.; Lv, X.; Song, X.; He, H.; Huan, Y. Optimizing 5-aza-2’-deoxycytidine treatment to enhance the development of porcine cloned embryos by inhibiting apoptosis and improving DNA methylation reprogramming. Res. Vet. Sci. 2020, 132, 229–236.
51. Nguyen, V.K.; Somfai, T.; Salamone, D.; Thu Huong, V.T.; Le Thi Nguyen, H.; Huu, Q.X.; Hoang, A.T.; Phan, H.T.; Thi Pham, Y.K.; Pham, L.D. Optimization of donor cell cycle synchrony, maturation media and embryo culture system for somatic cell nuclear transfer in the critically endangered Vietnamese Ỉ pig. Theriogenology 2021, 166, 21–28.
52. Lee, J.; Lee, Y.; Lee, G.S.; Lee, S.T.; Lee, E. Comparative study of the developmental competence of cloned pig embryos derived from spermatogonial stem cells and fetal fibroblasts. Reprod. Domest. Anim. 2019, 54, 1258–1264.
53. Zhang, L.; Zhang, Y.; Han, Z.; Fang, J.; Chen, H.; Guo, Z. Transcriptome analyses reveal effects of vitamin C-treated donor cells on cloned bovine embryo development. Int. J. Mol. Sci. 2019, 20, 2628.
54. Gorczyca, G.; Wartalski, K.; Wiater, J.; Samiec, M.; Tabarowski, Z.; Duda, M. Anabolic Steroids-Driven Regulation of Porcine Ovarian Putative Stem Cells Favors the Onset of Their Neoplastic Transformation. Int. J. Mol. Sci. 2021, 22, 11800.
55. Wiater, J.; Samiec, M.; Wartalski, K.; Smorąg, Z.; Jura, J.; Słomski, R.; Skrzyszowska, M.; Romek, M. Characterization of Mono- and Bi-Transgenic Pig-Derived Epidermal Keratinocytes Expressing Human FUT2 and GLA Genes – In Vitro Studies. Int. J. Mol. Sci. 2021, 22, 9683.
56. Chi, D.; Zeng, Y.; Xu, M.; Si, L.; Qu, X.; Liu, H.; Li, J. LC3-dependent autophagy in pig 2-cell cloned embryos could influence the degradation of maternal mRNA and the regulation of epigenetic modification. Cell. Reprogram. 2017, 19, 354–362.
57. Sampaio, R.V.; Sangalli, J.R.; De Bem, T.H.C.; Ambrizi, D.R.; Del Collado, M.; Bridi, A.; De Ávila, A.C.F.C.M.; Macabelli, C.H.; De Jesus Oliveira, L.; Da Silveira, J.C.; et al. Catalytic inhibition of H3K9me2 writers disturbs epigenetic marks during bovine nuclear reprogramming. Sci. Rep. 2020, 10, 11493.

58. Wang, X.; Qu, J.; Li, J.; He, H.; Liu, Z.; Huan, Y. Epigenetic reprogramming during somatic cell nuclear transfer: Recent progress and future directions. Front. Genet. 2020, 11, 205.
59. Takeda, K. Functional consequences of mitochondrial mismatch in reconstituted embryos and offspring. J. Reprod. Dev. 2019, 65, 485–489.
60. Magalhães, L.C.; Cortez, J.V.; Bhat, M.H.; Sampaio, A.C.N.P.C.; Freitas, J.L.S.; Duarte, J.M.B.; Melo, L.M.; Freitas, V.J.F. In vitro development and mitochondrial gene expression in brown brocket deer (Mazama gouazoubira) embryos obtained by interspecific somatic cell nuclear transfer. Cell. Reprogram. 2020, 22, 208–216.
61. Samiec, M.; Skrzyszowska, M. Extranuclear Inheritance of Mitochondrial Genome and Epigenetic Reprogrammability of Chromosomal Telomeres in Somatic Cell Cloning of Mammals. Int. J. Mol. Sci. 2021, 22, 3099.
62. Zhang, X.; Gao, S.; Liu, X. Advance in the Role of Epigenetic Reprogramming in Somatic Cell Nuclear Transfer-Mediated Embryonic Development. Stem Cells Int. 2021, 2021, 6681337.
63. Li, Z.; He, X.; Chen, L.; Shi, J.; Zhou, R.; Xu, W.; Liu, D.; Wu, Z. Bone marrow mesenchymal stem cells are an attractive donor cell type for production of cloned pigs as well as genetically modified cloned pigs by somatic cell nuclear transfer. Cell. Reprogram. 2013, 15, 459–470.
64. Lee, K.; Uh, K.; Farrell, K. Current progress of genome editing in livestock. Theriogenology 2020, 150, 229–235.
65. Perisse, I.V.; Fan, Z.; Singina, G.N.; White, K.L.; Polejaeva, I.A. Improvements in Gene Editing Technology Boost Its Applications in Livestock. Front. Genet. 2021, 11, 614688.
66. Singh, B.; Mal, G.; Verma, V.; Tiwari, R.; Khan, M.I.; Mohapatra, R.K.; Mitra, S.; Alyami, S.A.; Emran, T.B.; Dhama, K.; et al. Stem cell therapies and benefaction of somatic cell nuclear transfer cloning in COVID-19 era. Stem Cell Res. Ther. 2021, 12, 283.
67. Trzcińska, M.; Bryła, M.; Gajda, B.; Gogol, P. Fertility of boar semen cryopreserved in extender supplemented with butylated hydroxytoluene. Theriogenology 2015, 83, 307–313.
68. Trzcińska, M.; Bryła, M. Apoptotic-like changes of boar spermatozoa in freezing media supplemented with different antioxidants. Pol. J. Vet. Sci. 2015, 18, 473–480.
69. Yeste, M. Sperm cryopreservation update: Cryodamage, markers, and factors affecting the sperm freezability in pigs. Theriogenology 2016, 85, 47–64.
70. Len, J.S.; Koh, W.S.D.; Tan, S.H. The roles of reactive oxygen species and antioxidants in cryopreservation. Biosci. Rep. 2019, 39, BSR20191601.
71. Comizzoli, P.; He, X.; Lee, P.C. Long-term preservation of germ cells and gonadal tissues at ambient temperatures. Reprod. Fertil. 2022, 3, R42–R50.

72. Comizzoli, P.; Amelkina, O.; Lee, P.C. Damages and stress responses in sperm cells and other germplasms during dehydration and storage at nonfreezing temperatures for fertility preservation. Mol. Reprod. Dev. 2022, 89, 565–578.
73. Saragusty, J.; Loi, P. Exploring dry storage as an alternative biobanking strategy inspired by Nature. Theriogenology 2019, 126, 17–27.
74. Thiangthientham, P.; Kallayanathum, W.; Anakkul, N.; Suwimonteerabutr, J.; Santiviparat, S.; Techakumphu, M.; Loi, P.; Tharasanit, T. Effects of freeze-drying on the quality and fertilising ability of goat sperm recovered from different parts of the epididymis. Theriogenology 2023, 195, 31–39.
75. Olaciregui, M.; Luño, V.; Martí, J.I.; Aramayona, J.; Gil, L. Freeze-dried stallion spermatozoa: Evaluation of two chelating agents and comparative analysis of three sperm DNA damage assays. Andrologia 2016, 48, 900–906.
76. Mercati, F.; Domingo, P.; Pasquariello, R.; Dall'Aglio, C.; Di Michele, A.; Forti, K.; Cocci, P.; Boiti, C.; Gil, L.; Zerani, M.; et al. Effect of chelating and antioxidant agents on morphology and DNA methylation in freeze-drying rabbit (Oryctolagus cuniculus) spermatozoa. Reprod. Domest. Anim. 2020, 55, 29–37.
77. Olaciregui, M.; Luño, V.; González, N.; Domingo, P.; de Blas, I.; Gil, L. Chelating agents in combination with rosmarinic acid for boar sperm freeze-drying. Reprod. Biol. 2017, 17, 193–198.
78. Domingo, P.; Olaciregui, M.; González, N.; De Blas, I.; Gil, L. Long-term preservation of freeze-dried rabbit sperm by adding rosmarinic acid and different chelating agents. Cryobiology 2018, 81, 174–177.
79. Muneto, T.; Horiuchi, T. Full-term development of hamster embryos produced by injecting freeze-dried spermatozoa into oocytes. J. Mamm. Ova Res. 2011, 28, 32–39.
80. Kaneko, T.; Whittingham, D.G.; Yanagimachi, R. Effect of pH value of freeze-drying solution on the chromosome integrity and developmental ability of mouse spermatozoa. Biol. Reprod. 2003, 68, 136–139.
81. Hirabayashi, M.; Kato, M.; Ito, J.; Hochi, S. Viable rat offspring derived from oocytes intracytoplasmically injected with freeze-dried sperm heads. Zygote 2005, 13, 79–85.
82. Li, M.W.; Willis, B.J.; Griffey, S.M.; Spearow, J.L.; Lloyd, K.C. Assessment of three generations of mice derived by ICSI using freeze-dried sperm. Zygote 2009, 17, 239–251.
83. Choi, Y.H.; Varner, D.D.; Love, C.C.; Hartman, D.L.; Hinrichs, K. Production of live foals via intracytoplasmic injection of lyophilized sperm and sperm extract in the horse. Reproduction 2011, 142, 529–538.

84. Hara, H.; Tagiri, M.; Hwang, I.S.; Takahashi, M.; Hirabayashi, M.; Hochi, S. Adverse effect of cake collapse on the functional integrity of freeze-dried bull spermatozoa. Cryobiology 2014, 68, 354–360.
85. Palazzese, L.; Gosálvez, J.; Anzalone, D.A.; Loi, P.; Saragusty, J. DNA fragmentation in epididymal freeze-dried ram spermatozoa impairs embryo development. J. Reprod. Dev. 2018, 64, 393–400.
86. Berteli, T.S.; Vireque, A.A.; Borges, E.D.; Da Luz, C.M.; Navarro, P.A. Membrane lipid changes in mouse blastocysts induced by ovarian stimulation, IVF and oocyte vitrification. Reprod. Biomed. Online 2023, 46, 887–902.
87. Tian, C.; Shen, L.; Gong, C.; Cao, Y.; Shi, Q.; Zhao, G. Microencapsulation and nanowarming enables vitrification cryopreservation of mouse preantral follicles. Nat. Commun. 2022, 13, 7515.
88. Hochi, S. Japanese Society for Animal Reproduction: Award for outstanding research 2002. Cryopreservation of follicular oocytes and preimplantation embryos in cattle and horses. J. Reprod. Dev. 2003, 49, 13–21.
89. Paramio, M.T.; Izquierdo, D. Current status of in vitro embryo production in sheep and goats. Reprod. Domest. Anim. 2014, 49, 37–48.
90. Hosseini, S.M.; Asgari, V.; Ostadhosseini, S.; Hajian, M.; Ghanaei, H.R.; Nasr-Esfahani, M.H. Developmental competence of ovine oocytes after vitrification: Differential effects of vitrification steps, embryo production methods, and parental origin of pronuclei. Theriogenology 2015, 83, 366–376.
91. Park, M.J.; Lee, S.E.; Yoon, W.; Park, H.J.; Kim, S.H.; Oh, S.H.; Lee, D.G.; Pyeon, D.B.; Kim, E.Y.; Park, S.P. Effect of supplementation of cryoprotectant solution with hydroxypropyl cellulose for vitrification of bovine oocytes. Cryo Letters 2023, 44, 37–46.
92. Carrascal-Triana, E.L.; Zolini, A.M.; de King, A.R.; Penitente-Filho, J.M.; Hansen, P.J.; Torres, C.A.A.; Block, J. Effect of addition of ascorbate, dithiothreitol or a caspase-3 inhibitor to cryopreservation medium on post-thaw survival of bovine embryos produced in vitro. Reprod. Domest. Anim. 2022, 57, 1074–1081.
93. Fernandez, J.; Bruno-Galarraga, M.M.; Lacau-Mengido, I.M.; Cueto, M.I.; Gibbons, A.E. A successful vitrification technique for goat morulae conservation. Theriogenology 2022, 182, 103–109.
94. Gonzalez-Plaza, A.; Cambra, J.M.; Garcia-Canovas, M.; Parrilla, I.; Gil, M.A.; Martinez, E.A.; Rodriguez-Martinez, H.; Martinez, C.A.; Cuello, C. Cryotop vitrification of large batches of pig embryos simultaneously provides excellent postwarming survival rates and minimal interference with gene expression. Theriogenology 2023, 206, 1–10.

95. Xingzhu, D.; Qingrui, Z.; Keren, C.; Yuxi, L.; Yunpeng, H.; Shien, Z.; Xiangwei, F. Cryopreservation of Porcine Embryos: Recent Updates and Progress. Biopreserv. Biobank. 2021, 19, 210–218.
96. Liu, J.; Phy, J.; Yeomans, E. Theoretic considerations regarding slow cooling and vitrification during cryopreservation. Theriogenology 2012, 78, 1641–1652.
97. Jia, B.; Xiang, D.; Guo, J.; Jiao, D.; Quan, G.; Hong, Q.; Fu, X.; Wei, H.; Wu, G. Successful vitrification of early-stage porcine cloned embryos. Cryobiology 2020, 97, 53–59.
98. Xu, X.; Hao, T.; Komba, E.; Yang, B.; Hao, H.; Du, W.; Zhu, H.; Zhang, H.; Zhao, X. Improvement of Fertilization Capacity and Developmental Ability of Vitrified Bovine Oocytes by JUNO mRNA Microinjection and Cholesterol-Loaded Methyl-β-Cyclodextrin Treatment. Int. J. Mol. Sci. 2022, 24, 590.
99. Angel-Velez, D.; De Coster, T.; Azari-Dolatabad, N.; Fernández-Montoro, A.; Benedetti, C.; Pavani, K.; Van Soom, A.; Bogado Pascottini, O.; Smits, K. Embryo morphokinetics derived from fresh and vitrified bovine oocytes predict blastocyst development and nuclear abnormalities. Sci. Rep. 2023, 13, 4765.
100. Guo, Y.; Bai, J.; Zhang, Z.; Liu, Y.; Lu, S.; Liu, C.; Ni, J.; Zhou, P.; Fu, X.; Sun, W.Q.; et al. Pregnancy of cryopreserved ovine embryos at different developmental stages. Cryo Letters 2022, 43, 269–275.
101. Somfai, T.; Haraguchi, S.; Dang-Nguyen, T.Q.; Kaneko, H.; Kikuchi, K. Vitrification of porcine immature oocytes and zygotes results in different levels of DNA damage which reflects developmental competence to the blastocyst stage. PLoS One 2023, 18, e0282959.
102. Marco-Jiménez, F.; Garcia-Dominguez, X.; García-Valero, L.; Vicente, J.S. A 3D-Printed Large Holding Capacity Device for Minimum Volume Cooling Vitrification of Embryos in Prolific Livestock Species. Animals 2023, 13, 791.
103. Samiec, M.; Romanek, J.; Lipiński, D.; Opiela, J. Expression of pluripotency-related genes is highly dependent on trichostatin A-assisted epigenomic modulation of porcine mesenchymal stem cells analysed for apoptosis and subsequently used for generating cloned embryos. Anim. Sci. J. 2019, 90, 1127–1141.
104. Nguyen, H.T.; Dang-Nguyen, T.Q.; Somfai, T.; Men, N.T.; Viet Linh, N.; Xuan Nguyen, B.; Noguchi, J.; Kaneko, H.; Kikuchi, K. Selection based on morphological features of porcine embryos produced by in vitro fertilization: Timing of early cleavages and the effect of polyspermy. Anim. Sci. J. 2020, 91, e13401.
105. Salamone, D.F.; Canel, N.G.; Rodríguez, M.B. Intracytoplasmic sperm injection in domestic and wild mammals. Reproduction 2017, 154, 111–124.

106. Fowler, K.E.; Mandawala, A.A.; Griffin, D.K.; Walling, G.A.; Harvey, S.C. The production of pig preimplantation embryos in vitro: Current progress and future prospects. Reprod. Biol. 2018, 18, 203–211.

107. Magata, F.; Tsuchiya, K.; Okubo, H.; Ideta, A. Application of intracytoplasmic sperm injection to the embryo production in aged cows. J. Vet. Med. Sci. 2019, 81, 84–90.

108. Zuo, Z.; Niu, Z.; Liu, Z.; Ma, J.; Qu, P.; Qiao, F.; Su, J.; Zhang, Y.; Wang, Y. The effects of glycine-glutamine dipeptide replaced L-glutamine on bovine parthenogenetic and IVF embryo development. Theriogenology 2020, 141, 82–90.

109. Ashibe, S.; Miyamoto, R.; Kato, Y.; Nagao, Y. Detrimental effects of oxidative stress in bovine oocytes during intracytoplasmic sperm injection (ICSI). Theriogenology 2019, 133, 71–78.

110. Ressaissi, Y.; Anzalone, D.A.; Palazzese, L.; Czernik, M.; Loi, P. The impaired development of sheep ICSI derived embryos is not related to centriole dysfunction. Theriogenology 2021, 159, 7–12.

111. Kropp, J.; Carrillo, J.A.; Namous, H.; Daniels, A.; Salih, S.M.; Song, J.; Khatib, H. Male fertility status is associated with DNA methylation signatures in sperm and transcriptomic profiles of bovine preimplantation embryos. BMC Genomics 2017, 18, 280.

112. Diao, Y.F.; Lin, T.; Li, X.; Oqani, R.K.; Lee, J.E.; Kim, S.Y.; Jin, D.I. Dynamic changes of SETD2, a histone H3K36 methyltransferase, in porcine oocytes, IVF and SCNT embryos. PLoS One 2018, 13, e0191816.

113. Takeda, K.; Kobayashi, E.; Nishino, K.; Imai, A.; Adachi, H.; Hoshino, Y.; Iwao, K.; Akagi, S.; Kaneda, M.; Watanabe, S. Age-related changes in DNA methylation levels at CpG sites in bull spermatozoa and in vitro fertilization-derived blastocyst-stage embryos revealed by combined bisulfite restriction analysis. J. Reprod. Dev. 2019, 65, 305–312.

114. Tsai, T.S.; St. John, J.C. The effects of mitochondrial DNA supplementation at the time of fertilization on the gene expression profiles of porcine preimplantation embryos. Mol. Reprod. Dev. 2018, 85, 490–504.

115. Zuidema, D.; Sutovsky, P. The domestic pig as a model for the study of mitochondrial inheritance. Cell Tissue Res. 2020, 380, 263–271.

116. Jin, Y.X.; Zheng, Z.; Yu, X.F.; Zhang, J.B.; Namgoong, S.; Cui, X.S.; Hyun, S.H.; Kim, N.H. Autophagy and ubiquitin-mediated proteolysis may not be involved in the degradation of spermatozoon mitochondria in mouse and porcine early embryos. Zygote 2016, 24, 31–41.

117. Rodríguez, M.B.; Gambini, A.; Clérico, G.; Ynsaurralde-Rivolta, A.E.; Briski, O.; Largel, H.; Sansinena, M.; Salamone, D.F. Time of first polar body extrusion affects the developmental competence of equine oocytes after intracytoplasmic sperm injection. Reprod. Fertil. Dev. 2019, 31, 1805–1811.

118. Ramos-Ibeas, P.; Gimeno, I.; Cañón-Beltrán, K.; Gutiérrez-Adán, A.; Rizos, D.; Gómez, E. Senescence and apoptosis during in vitro embryo development in a bovine model. Front. Cell Dev. Biol. 2020, 8, 619902.