THE ROLE OF REPRODUCTIVE TECHNOLOGIES AND CRYOPRESERVATION OF GENETIC MATERIALS IN THE CONSERVATION OF ANIMAL GENETIC RESOURCES

Aweke Engdawork*, Teklewold Belayhun, and Tesfalem Aseged

Ethiopian Biodiversity Institute, P. O. Box: 30726, Addis Ababa, Ethiopia.

ABSTRACT: The conservation of animal genetic resources guarantees the continual existence of diversified nature and thereby ensures the achievement of valuable economic, ecological, social, and cultural demands. However, animal genetic resources are currently facing several threats, such as climate change, habitat loss, pollution, invasive species, and infectious diseases. Animals can be conserved in situ in their natural habitat, ex-situ in zoos and farms, and in vitro in gene banks. Thus, the main objective of this review is to provide a comprehensive overview of the role of reproductive technologies and cryopreservation of genetic materials in the conservation of animal genetic resources. The cryopreservation of genetic materials is capable of generating new offspring and has critical importance in the conservation of threatened animal species. Advanced reproductive technologies, including artificial insemination, in-vitro fertilization, embryo transfer, and nuclear transfer, greatly promoted efficiencies in animal production and conservation. Cryopreservation of genetic materials, including semen, oocytes, embryos, and somatic cells, and assisted reproductive technologies are advantageous in the conservation of genetic resources. Reproductive technologies have been successful in reconstituting animal populations and recovering endangered and extinct animals from cryopreserved genetic materials across the world. In Ethiopia, the application of reproductive and cryopreservation technologies is mainly restricted to livestock genetic improvement. Semen collection and preservation have started for the conservation of threatened cattle breeds and the endangered Ethiopian wolf. Therefore, reproductive and cryopreservation technologies should be extensively applied for sustainable animal conservation. Special emphasis should be placed on the development and application of cryopreservation and reproductive technologies.

Keywords: Animal genetic resource, Conservation, Cryopreservation, Reproductive technologies, Threatened

INTRODUCTION

Animal genetic resources refer to the diverse species, breeds, and strains of animals that serve important purposes for humans and their environment, including economic, ecological, scientific, and cultural roles (EBI, 2016; FAO, 2023). Animal biodiversity has multilateral contributions, and the demand for animal

*Corresponding author: engdaworkaweke@gmail.com

genetic resources is definitely certain for human-contented livelihoods as they provide a variety of animal products and services (Pilling et al., 2020). Animals develop survival and adaptation traits for their ecology and production systems. However, the loss of animal genetic resources is currently a global concern. They are facing multiple threats that endanger their survival which include changes in land use, exploitation, climate change, pollution, invasive species, inbreeding, and infectious diseases (Givens, 2010; Engdawork, 2019). Therefore, the conservation of animals is crucial to ensure the sustainability and diversity of animal genetic resources. Conserving animal genetic resources involves maintaining genetic diversity to conserve, restore, and improve animal breeds and species (Mmassy, 2011).

The various approaches for the conservation of animal genetic resources, include in-situ conservation on farms, ex-situ in-vivo conservation in farms or zoos, and ex-situ in-vitro conservation in gene banks (Andrabi and Mawxell, 2007). Assisted reproductive technologies (ARTs) and cryopreservation of genetic materials play significant roles in the conservation of animal genetic resources. The collection and preservation of genetic materials, such as embryos, semen, oocytes, somatic cells, and tissues, have the potential to reconstitute live animals (Mazur et al., 2007). Ex-situ conservation of genetic materials enables effective protection of genetic resources, along with in-situ conservation of animals (Hiemstra et al., 2011). Advanced reproductive technologies, such as germplasm collection and cryopreservation, multiple ovulation and embryo transfer, sperm and embryo sexing, in-vitro fertilization, nuclear transplantation, and cloning, are great support for animal conservation efforts (Herrick, 2019).

Assisted reproductive technologies (ARTs) can be successfully used for the conservation and restoration of species and breeds in danger of extinction (Paiva et al., 2016). The preservation of biomaterials, such as semen, oocytes, embryos, tissues, or DNA in animal gene banks (AnGB), is essential to the conservation of animal genetic resources (Andrabi and Mawxell, 2007). Animal gene banks allow the collection and storage of genetic material for many years without deterioration through cryopreservation and create wide opportunities for the restoration of a particular animal population after disasters or epidemic events

(Comizzoli et al., 2000). Reproductive technologies encourage the utilization of frozen germplasm stored for extended periods of time, thereby maintaining the genetic diversity of a species or breed (Pukazhenthi and Wildt, 2004; Bolton et al., 2022).

Assisted reproductive technologies consist of various techniques, such as cryopreservation of gametes and embryos, artificial insemination (AI), in-vitro fertilization (IVF), and embryo transfer (ET). ARTs greatly promote efficiencies in animal reproduction and serve as a significant tool for conserving domestic and wildlife populations that are threatened with extinction (Swanson, 2006; Bolton et al., 2022). In Ethiopia, the use of reproductive technologies is primarily focused on improving cattle productivity through genetic improvements. Semen collection and artificial insemination are the most widely used reproductive technologies (Azage et al., 2016). However, advanced reproductive and cryopreservation technologies have not been well implemented in the conservation of endangered and threatened animal species and breeds. As such, animal conservation strategies need to adopt advancements in cryopreservation and reproductive technologies. Therefore, the main objective of this review is to provide a comprehensive overview of the cryopreservation of animal genetic materials and the role of reproductive technologies in the conservation of animal genetic resources.

CRYOPRESERVATION OF ANIMAL GENETIC RESOURCES

Cryopreservation is an advanced biotechnology that allows germplasm to be stored for long periods of time in small, confined areas. It is a procedure where gametes, somatic cells, and tissues are gradually frozen using liquid nitrogen to achieve extremely low temperatures (Weathers and Prien, 2014). The most commonly preserved samples include semen, embryos, oocytes, somatic cells, nuclear DNA, and other types of biomaterials such as blood and serum (Mazur et al., 2007). This process enables the preservation of the genetic material of both maternal and paternal cells for use in assisted reproductive technologies and maintains viability over extended periods. Therefore, it can be applied as a contingency plan when a particular animal species or breed needs to be restored when it has become extinct or for breed improvement purposes (Mendelsohn, 2003; Jinadu et al., 2020).

Cryopreservation of animal genetic resources is necessary for the utilization of genetically merited animal breeds for desired traits, the preservation of genetically and phenotypically diverse populations, and the conservation of animal breeds and species threatened with extinction (Danchin-Burge et al., 2011). The diversity of livestock and wildlife has huge contributions to sustainable agricultural development, food security, and ecological balance, as they are adapted to variable environmental conditions and diseases (Herrick, 2019). Cryogenically preserved genetic materials are used for the conservation and rehabilitation of threatened breeds with low populations, reviving animal species or breeds that are endangered or extinct, and genetic improvements (McEvoy et al., 2000; Larsen, 2021).

Semen collection and cryopreservation

Semen is collected from animals for different purposes, including assessing reproductive performance, artificial insemination, and conservation of animal breeds and species (Coloma et al., 2010). Semen collection and preservation is the most commonly used biotechnique for germplasm preservation and conservation of threatened animal species due to its ease of application. Semen can be collected from animals using artificial vaginas, electro-ejaculation, and digital or manual massage (Thongphakdee et al., 2022). Semen is most commonly collected using artificial vaginas, particularly in domestic animals, and this method is relatively the best method for bringing good-quality semen (Wulster-Radcliffe et al., 2001). Electro-ejaculation is used in animals that cannot mount or are fractious for management, such as wild animals (Palmer et al., 2005). Semen collection by rectal massage of the seminal vesicles and ampulla is possible whenever both of the above procedures are not suitable to apply (Thongphakdee et al., 2022). The suitability of a particular method depends on the type and condition of the animal.

The cryopreservation of semen involves semen collection from the donor animal, evaluation, extension, and gradually freezing in liquid nitrogen for long-term storage (Vongpralub et al., 2015). Semen collection and preservation is a necessary prerequisite to the development of assisted reproductive technologies such as artificial insemination, in-vitro fertilization, and embryo transfer and can be applied to several animal species (Rodriguez-Martinez and Vega, 2013). Semen collection is practiced throughout the world for genetic improvement through cross-breeding, conservation of threatened animals, and restoration of endangered or extinct animal species and breeds. Accordingly, global trends indicate that semen can be collected from most domestic and wild animal species.

Cryopreservation of embryos

The collection and cryopreservation of embryos is a potential biotechnological development for the purpose of protecting population integrity and conserving endangered animal species. Even if it is a complex and expensive procedure, the embryo conserves the full genetic complement of both dam and sire and maintains heterozygosity (Pukazhenthi and Wildt, 2004). Embryos develop through various morphological stages following fertilization. During embryonic development, the number of blastomeres increases as they migrate through the female reproductive tract. Thereby, the embryos can be collected from donor females by flushing the reproductive tract using a physiological flushing medium. The superovulation technique using follicle-stimulating hormone is usually performed in order to have a female release more oocytes than usual (Pomar et al., 2005).

The cryopreservation of embryos requires the necessary freezing processes and techniques. Vitrification is the preferred technique of the embryo freezing protocol, as it produces good and viable embryos (Rezazadeh et al., 2009). It is the practice of freezing embryos, which is achieved with an extreme increase in cryoprotectant thickness and ultra-rapid cooling to solidify the cell into a glass-like state without intracellular or extracellular ice crystal formation. Applying a higher concentration of the medium during vitrification enables the germplasm to be frozen more rapidly than with slow freezing (Vajta, 2000; Loutradi et al., 2008). As embryos contain the full genetic make-up of animals, cryopreservation of embryos is technically a more reliable method for the restoration of extinct animals and the conservation of threatened animal species.

Collection and cryopreservation of Oocytes

Oocytes are another type of germplasm that can be cryopreserved for the conservation of animal genetic resources. In contrast to semen and embryos, oocytes are extremely sensitive to chilling due to the fact that they typically have a low permeability to cryoprotectants (Woods et al., 2004). Several progresses have been made towards the successful collection and cryopreservation of oocytes from different animal species (Andrabi and Maxwell, 2007). Cryoprotective solutions achieve cellular dehydration by avoiding intracellular ice crystal formation during freezing. Vitrification is considered the best-suited freezing technique for the preservation of oocytes (Huijsmans et al., 2023). Oocytes can be collected by harvesting ova from ovaries in dead animals and by ova pick-up in live animals using the transvaginal ultrasound-guided oocyte collection method (Carter et al., 2002; Farsi et al., 2016). The cooling of ovaries during harvesting dramatically reduces the success rate of embryo production.

In a conventional way, oocytes are often harvested from slaughterhouse ovaries immediately after slaughter and can be an option for cryopreservation. The conventional procedure of oocyte collection has a significant advantage for preserving the genetic resources of females that unexpectedly die or are incapable of being bred (Farsi et al., 2016). The cryopreservation of oocytes provides higher possibilities for the collection and storage of oocytes from dead animals and is one of the best ways to preserve female genetic material postmortem. Moreover, technically, oocytes can be collected and preserved for most mammalian species.

Cryopreservation of somatic cells

Cryopreservation of somatic cells is an alternative means of conserving genetic resources, particularly in situations where semen and oocytes cannot be collected and preserved. The collection and preservation of tissues, DNA, blood, and other biomaterials enable the storage and usage of genetic resources (Di Lecce et al., 2022). The conservation of somatic cells is related to somatic cell nuclear transfer (SCNT), which enables the restoration of threatened or extinct animal species. The collection of tissues other than gametes and embryos can also be used for applied and developmental research to get genetic and health-associated information (Li et al., 2009). Cryopreserved cells and tissues can be stored for long periods of time without deterioration and provide opportunities for the conservation of animal genetic resources.

Somatic cells for somatic cell nuclear transfer and DNA study can be collected and cryopreserved from the tissues of live and dead animals within a short period of time. Live animal tissue can be taken from the ear or other peripheral tissues, while tissue can also be easily obtained from dead animals immediately after death (Araujo et al., 2006). The collected tissue samples are preserved through different cryopreservation techniques, including slow freezing and vitrification. Slow freezing of cells or tissues allows the specimens to be frozen below the melting point with liquid nitrogen in a medium. The vitrification technique is achieved by applying a higher concentration of solute so that the water leaves the cells through osmosis (Loutradi et al., 2008).

The role of reproductive technologies in animal conservation

The development and application of ARTs to enhance reproductive performance have valuable importance in ensuring the sustainable conservation of animal genetic resources (Herrick, 2019). Nowadays, many vertebrate species face variable threats and extinctions. The major significance of reproductive technologies is that they ex-situ resuscitate threatened and endangered animals, thereby conserving valuable and rare alleles (Mastromonaco and Songsasen, 2020). Assisted reproductive techniques help to recover small, endangered, and fragmented populations of wild animal species or domestic breeds (Figure 1). ARTs create an important opportunity for producing many offspring from a desired genitor in the way they minimize inbreeding problems (Comizzolia et al., 2000; Bolton et al., 2022).

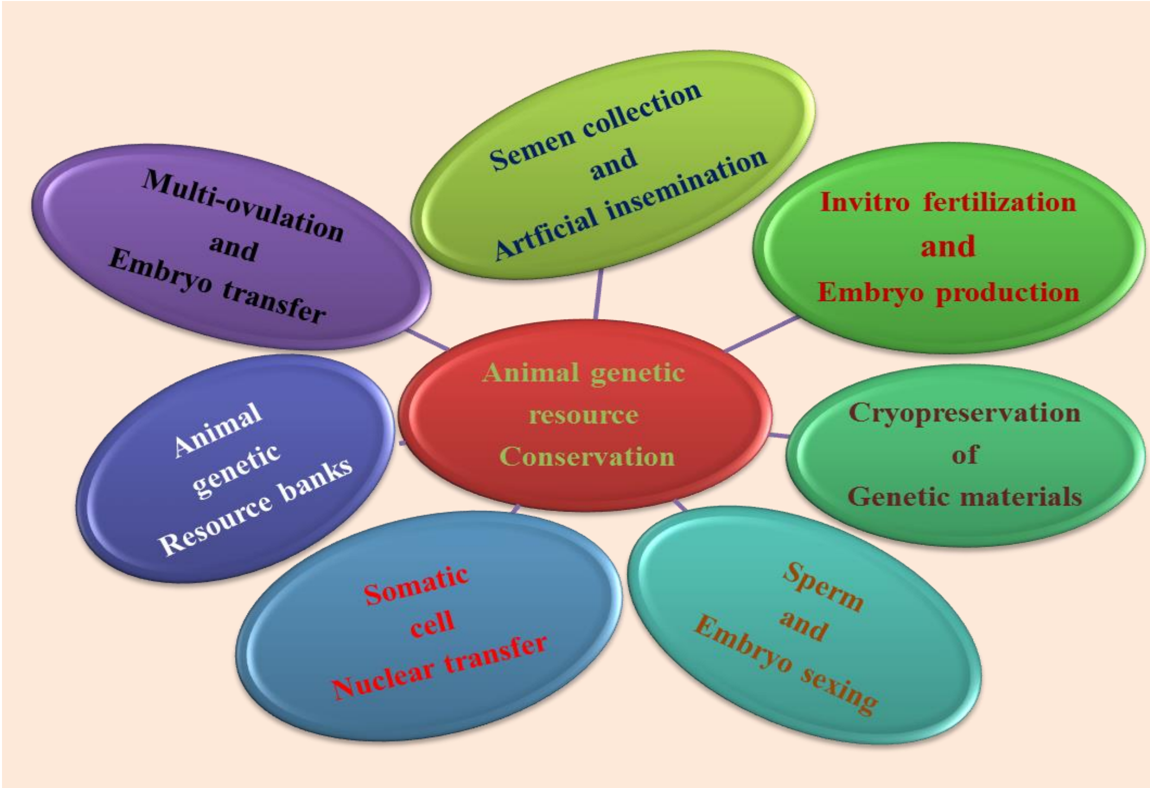

Figure 1. The role of reproductive technologies and cryopreservation of genetic materials in the conservation of animal genetic resources.

Artificial Insemination (AI)

Artificial insemination (AI) is the physical placement of semen into the female reproductive tract by a method other than natural mating. AI is the most commonly used reproductive technology, which is less complex and expensive, and consequently the main choice in the conservation and breeding programs for threatened wild and domesticated animal species and breeds (Durrant, 2009). The conservation of animal populations located in fragmented sites can be enhanced through the application of AI, thereby avoiding genetic depression (Pukazhenthi and Wildt, 2004). A female animal from a small population can be inseminated with semen from a male animal of the species from another site and sent back to their habitat. Male animals can also be easily captured from their freely living environment and semen collected for insemination of females held in captivity (Bolton et al., 2022).

Artificial insemination techniques can be applied to most mammalian species, playing a considerable role in the conservation of variable domestic and wild animal species. The availability of infrastructure and simple techniques for semen collection, evaluation, and preservation attracts the use of frozen and extended semen in animal conservation and breeding, and AI has become a widely applied reproductive technology (Zuidema et al., 2021). There are differences among species in insemination techniques and the success rate of pregnancy with the use of frozen semen and AI (Vazquez et al., 2005). The ease of the technique and its applicability make AI a very suitable reproductive technology for animal breeding and conservation. AI is practiced for most mammalian species, birds, and reptiles.

Multiple Ovulation and Embryo Transfer (MOET)

Multiple ovulation and embryo transfer are very important reproductive technologies that can significantly support efforts in animal production and conservation. MOET is a procedure in which fertilized eggs are removed from a female donor and transferred to multiple female receivers that are genetically unrelated (Hasler, 2004). Even if it is an advanced assisted reproductive technology, the application of the MOET technique is not widespread in most animal populations. However, MOET is a key biotechnology in conservation strategies in which multiple eggs are released from a single dam and embryos are transferred from endangered species to non-threatened recipients (Jainudeen et al., 2000). Synchronization and super-ovulatory protocols are commonly applied to multiple ovulations and embryo transfer techniques. Superovulation and embryo transfer programs have become successful in conserving and restoring several endangered animal species, such as baboons, wild sheep, eland antelope, Grant's zebra, and Przewalski’s horse (Bettencourt et al., 2008).

The stimulation of ovarian follicle growth by induction of follicular stimulating hormones, the initiation of estrus through luteolytic treatment, the preparation of insemination, and the collection of embryos are the basic procedures during the application of the MOET technique in animal production and conservation (Mikkola and Taponen, 2017). The MOET technique can also be applied for the treatment of infertile females due to disease, injury, or aging. The other importance of this technology is to decrease disease transmission risk across species and geographically (Shenk et al., 2006). Collecting and transferring embryos is a relatively challenging and complex procedure, and careful techniques should be utilized to successfully collect and transfer embryos (D'Angelo et al., 2022). In principle, the surgical technique of embryo transfer is almost possible in all mammalian animal species. However, the non-surgical embryo transfer technique is possible in some animal species, like cattle, horses, and pigs, while it may be ineffective or impossible in other species (Robertson, 2014).

In-vitro Fertilization and Embryo Production (IVP)

In-vitro embryo production (IVP) is the collection, fertilization, and maturation of oocytes in an artificial environment (outside the female reproductive tracts). In-vitro fertilization (IVF) of mature oocytes and culturing of embryos is performed at the level at which the embryo's developmental stage is suitable for the embryo transfer to the recipient female (Freitas and Melo, 2010). IVF and embryo production are usually accompanied by multi-ovulation, oocyte collection, and the use of fresh or frozen extended semen, which makes it a valuable way of conserving threatened or endangered animal species. The application of this technique in the conservation strategy is basically based on knowledge of the reproductive physiology of the animal species (Herrick, 2019).

In-vitro fertilization and embryo production constitute sequential steps, including oocyte recovery and their in-vitro maturation (IVM), in-vitro capacitation of sperm, in-vitro fertilization, and the growth of the embryos until they are ready for embryo transfer (Comizzoli et al., 2000). Utilization of in-vitro fertilization and IVP techniques for conservation of threatened species has been performed in Armenian red sheep, Sika deer, and European mouflon (Comizzoli et al., 2001; Ptak et al., 2002), African elephant (Loxodonta africana), mink whale (Meintjes et al., 1997), gorilla (Gorilla gorilla), and zebra (Tetsuka et al., 2004). The success and viability are confirmed by the gestations and births of normal offspring after transfer to recipient animals (Locatelli et al., 2006).

Somatic Cell Nuclear Transfer (SCNT)

Somatic Cell Nuclear Transfer (SCNT), also called cloning, is the technique where the DNA (nucleus) is extracted from a donor cell and transferred to an enucleated recipient cell, thereby creating a strictly similar genetic makeup of the donor cell (Andrabi and Maxwell, 2007). SCNT technology has been considered in the conservation and population increment of threatened breed populations since the first success in the cloning of mammals (Wilmut et al., 1997). There are trends in the restoration of extinct animal species, such that the extinct bucardo (*Capra pyrenaica pyrenaica*) has become a good exemplary animal species born with success by nuclear transfer of somatic cells (Table 1). Nowadays, the application of SCNT for the re-establishment of threatened animal species occurs mainly through interspecies nuclear transfer (Folch et al., 2009).

Reproductive cloning is applicable in several species, but irrespective of the animal species, there are three basic procedures to apply: enucleation, injection or fusion, and activation. SCNT involves the collection, culturing, and in-vitro development of the ovum, removing the nucleus from the ovum, and inserting somatic cell nuclei into the enucleating ovum. Thereby, an embryo is cultured and finally transferred to the recipient female of the same or highly related species (Matoba and Zhang, 2018). Successful offspring have been achieved through somatic cell nuclear transfer in a number of mammalian species, including sheep, cattle, mice, pigs, goats, horses, rabbits, and cats (Table 1).

Table 1. Application of somatic cell nuclear transfer (SCNT) and the successful production of new offspring in mammalian species.

Types of cloning	Donor species	Donor cell type	Recipient oocyte species	References
Intraspecies	Pig	Fetal fibroblasts	Pig	(Onishi et al., 2000)
Interspecies	Gaur (*Bos gaur*)	Preserved adult skin cells	Cow (*Bos taurus*)	(Lanza et al., 2000)
Intraspecies	Pig	Cultured adult granulosa cells	Pig	(Polejaeva et al., 2000)
Interspecies	Mouflon (*Ovis orientalis musimon*)	Adult granulosa cells	Sheep (*Ovis aries*)	(Loi et al., 2001)
Interspecies	Zebu (*Bos indicus*)	Morula stage blastomere	Cow (*Bos taurus*)	(Meirelles et al., 2001)
Intraspecies	Rabbit	Adult transgenic cumulus cell	Rabbit	(Chesne et al., 2002)
Intraspecies	Cat	Adult cumulus cells	Cat	(Shin et al., 2002)
Intraspecies	Mule	Fetal fibroblasts	Horse	(Woods et al., 2003)
Intraspecies	Horse	Adult skin fibroblasts	Horse	(Galli et al., 2003)
Intraspecies	Rat	Fetal fibroblasts	Rat	(Zhou et al., 2003)
Interspecies	African Wildcat (*Felis lybica*)	Adult skin fibroblasts	Cat (*Felis catus*)	(Gomez et al., 2004)
Interspecies	Banteng (*Bos javanicus*)	Cryopreserved adult fibroblasts	Cow (*Bos taurus*)	(Janssen et al., 2004)
Intraspecies	Dog (*Afghan hound*)	Adult skin fibroblasts	Dog (golden retriever)	(Lee et al., 2005)
Intraspecies	Ferret	Adult cumulus cells	Ferret	(Li et al., 2006)
Interspecies	Gray wolf (*C. lupus*)	Adult ear fibroblasts	Dog (*C. familiaris*)	(Kim et al., 2007)
Intraspecies	Red deer	Adult antlerogenic cells	Deer	(Berg et al., 2007)
Intraspecies	Buffalo	Fetal fibroblasts and adult granulosa cells	Buffalo	(Shi et al., 2007)
Interspecies	Pyrenean ibex (*Capra pyrenaica*)	Cryopreserved skin fibroblasts	Goat (*Capra aegagrus hircus*)	(Folch et al., 2009)
Intraspecies	Camel	Adult cumulus cells	Camel	(Wani et al., 2010)
Interspecies	Coyote (*C. latrans*)	Neonatal/adult fibroblasts	Dog (*C. familiaris*)	(Hwang et al., 2013)
Interspecies	Bactrian camel (*Camelus bactrianus*)	Adult skin fibroblasts	Dromedary camel (*C. dromedaries*)	(Wani et al., 2017)
Intraspecies	Cynomolgus monkey	Fetal fibroblast	Cynomolgus monkey	(Liu et al., 2018)

Sperm and Embryo Sexing

Sperm and embryo sexing is an advanced development for effectively managing the sex of offspring in multi-ovulation and embryo transfer technology. Embryo sexing is a useful technique as a conservation tool for managing the sex ratio in endangered animal species. Population analysis should be thoroughly conducted before embryo sexing to ensure a balanced sex ratio in at-risk groups. Sex prediction was 100% achieved in 58 bovine embryos when the blastomeres dissociated from a morula exceeds three (Zoheir and Allam, 2010). Similar achievements have been recorded in the sex determinations of 43 goat embryos of single blastomere at the blastula stage. This technique can be effectively applied to the sex determination of bovine and ovine embryos (Tsai et al., 2011).

There are two different approaches to the sexing of embryos, which are categorized as non-invasive and invasive methods depending on the requirement for a biopsy of embryonic tissue. Invasive methods, such as karyotyping and identification of sex chromatin, do not maintain embryonic integrity, while non-invasive methods (detection of X-linked enzymes and H-Y antigen) maintain integrity and cause less damage to the embryo (Wakchaure et al., 2015). Each of the methods has its own advantages and disadvantages in the sexing procedures. During the selection of embryo sexing techniques, the percentage accuracy and viability of the embryo produced must be taken into consideration. Among the available methods, polymerase chain reaction is taken to be a simple, effective, extremely reliable, and precise procedure for sexing embryos (Lakshmy et al., 2018).

Application of reproductive technologies for animal conservation in Ethiopia

Reproductive technologies in animal conservation

The application of reproductive technologies in Ethiopia is mainly restricted to the genetic improvement of livestock production. Semen collection and artificial insemination are the first and most commonly applied reproductive technologies in the livestock industry (Figure 2). AI is widely practiced throughout the country by agricultural offices, dairy industries, and institutions (Chencha and Kefyalew, 2012). Reproductive technologies such as oestrous synchronization, multi-ovulation, mass artificial insemination, and embryo transfer have been practiced in livestock to a limited extent by agricultural and research institutions in the country (Azage et al., 2016). Artificial insemination has been practiced in cattle, sheep, and goats for crossbreeding, mostly on commercial dairy farms.

Figure 2. Mass oestrous synchronization and artificial insemination in cattle in Ethiopia (Ndambi et al., 2017)

There are few practices in the application of reproductive technologies for the conservation of animal genetic resources in the country. Semen is collected and cryopreserved from some indigenous cattle breeds, such as Sheko, Begait, Fogera, Irob, and Borana, at the initiation of the Ethiopian Biodiversity Institute (EBI, 2016). Interestingly, semen collection trials were conducted for the endangered Ethiopian wolf. However, it was a big challenge to obtain good-quality semen as Ethiopian wolves have a restricted and narrow window of breeding time within the year. The presence of a strict hierarchy for mating affects the production of viable sperm good enough for storage with adequate fertility in adult males other than the alpha male (Farstad, 2017).

Opportunities for application of reproductive

The collection and preservation of genetic resources and the application of assisted reproductive technologies significantly improve the conservation of endangered animals, along with in-situ conservation efforts. The availability of threatened and highly endangered species of animals in the Ethiopia presents good opportunities for the application and expansion of reproductive technologies. The extensive use of AI and current developments in practicing other reproductive technologies (multi-ovulation, in-vitro fertilization, and embryo transfer) by research institutions can be a huge support for conservation and farm animal improvement (Desalegn et al., 2009). Different stakeholders, including non-governmental organizations, are likely interested in taking part in the development and use of reproductive technologies in the conservation of threatened animal species (EBI, 2016).

The presence of several threats, such as global climate change and recurrent drought in pastoral and agropastoral areas of Ethiopia, inbreeding's in endangered domestic and wild animals, crossbreeding and breed dilution in the central highlands, and habitat loss, bring huge opportunities for the application of reproductive and cryopreservation technologies. Herewith, the current infrastructural development and capacity of experts allow the application of assisted reproductive technologies in the conservation of animal genetic resources. Semen can be collected from almost all farm animals and endangered wild animals for use in AI and embryo transfer. Female gametes and embryos can also be collected, preserved, and transferred, particularly in highly endangered mammalian species.

Constraints in the application of reproductive technologies for animal conservation

The major constraints to the development and utilization of reproductive technologies in Ethiopia are related to the lack of good infrastructural and financial support. The lack of skilled manpower and technicians is one of the main challenges. There are also difficulties related to access, affordability, and community awareness of the extensive application of reproductive technologies. Moreover, the complexity and cost-demanding nature of advanced technologies like in-vitro production and embryo transfer impose greater constraints. Thus, there is low efficiency in the application of reproductive technologies (Adane, 2009). The limitations in our understanding of the reproductive physiology of threatened animal species reduce the success rate of reproductive technologies. Effective application of ARTs requires adequate knowledge of the reproductive status, seasonality, and behaviors of the particular species (Wildt and Wemmer, 1999).

Technical advances in reproductive science allow the development and effective application of ARTs in the conservation of threatened animal species (Le Gac et al., 2021).

CONCLUSION AND RECOMMENDATIONS

The development and application of reproductive technologies and cryopreservation of genetic materials play significant roles in realizing the sustainability and conservation of animal genetic resources. The most commonly cryopreserved genetic materials include semen, oocytes, embryos, and somatic cells. Reproductive technologies have made significant achievements in strengthening in-situ conservation programs, establishing threatened animal populations, and rehabilitating extinct animal species. Artificial insemination, multi-ovulation and embryo transfer, in-vitro fertilization and embryo production, somatic cell nuclear transfer, and embryo sexing are the most important reproductive technologies for animal conservation. In Ethiopia, many animal species and breeds are threatened due to climate change, recurrent drought, inbreeding, crossbreeding, and habitat loss. However, the application of reproductive technologies is limited to the genetic improvement of livestock and the conservation of a few threatened cattle breeds. Therefore, cryopreservation of genetic materials and assisted reproductive technologies should be extensively applied to the sustainable conservation of animal genetic resources. Special emphasis should be given to facilitate the global transfer of knowledge and resources in the development and application of cryopreservation and reproductive technologies.

ACKNOWLEDGEMENT

We thank all staff of Ethiopian Biodiversity Institute, Animal Biodiversity Research Directorate for their technical and material support.

REFERENCES

Adane, A. 2009. Review Agricultural biotechnology research and development in Ethiopia. *African Journal of Biotechnology*, **8(25):7196-7204**. http://www.academicjournals.org/AJB

Andrabi, S.M. and Maxwell, W.M. 2007. A review on reproductive biotechnologies for conservation of endangered mammalian species. *Animal Production Science,* **99(3-4):223-43**. DOI: 10.1016/j.anireprosci.2006.07.002

Araujo, A.M., Guimaraes, S., Machado, T., Lopes, P.S., Pereira, C.S., da Silva, F.L.R., Rodrigues, M.T., Columbiano, V.S. and Fonseca, C.G. 2006. Genetic Diversity between Herds of Alpine and Saanen Dairy Goats and the Naturalized Brazilian Moxoto Breed. *Genetics and Molecular Biology*, **29(1):67-74**. DOI: 10.1590/S1415-47572006000100014

Azage, T., Hoekstra, D., Berhanu, G. and Solomon, G. 2016. History and experiences of hormonal oestrus synchronization and mass insemination of cattle for improved genetics in Ethiopia: From science to developmental impact. International Livestock Research Institute (ILRI). https://searchworks.stanford.edu/view/12133723

Berg, D.K., Li, C., Asher, G., Wells, D.N. and Oback, B. 2007. Red deer cloned from antler stem cells and their differentiated progeny. *Biology of Reproduction* **77(3):384-94**. DOI: 10.1095/biolreprod.106.058172

Bettencourt, E.M., Bettencourt, C.M., Chagase Silva, N., Ferreira, P., Manito, C.I., Matos, C.M., Romão, R.J. and Rocha, A. 2008. Effect of season and gonadotrophin preparation on superovulatory response and embryo quality of Portuguese Black Merino ewes. *Small Ruminant Research*, **74 (1-3):134-139**. https://doi.org/10.1016/j.smallrumres.2007.05.001

Bolton, R.L., Mooney, A., Pettit, M.T., Bolton, A.E., Morgan, L., Drake, G.J., Appeltant, R., Walker, S.L., Gillis, J.D. and Hvilsom, C. 2022. Resurrecting biodiversity: advanced assisted reproductive technologies and biobanking. *Reproduction & fertility*, **3(3):121–146**. https://doi.org/10.1530/RAF-22-0005

Carter, J.A., Bellow, S., Meintjes, M., Perez, O., Ferguson, E. and Godke, R. 2002. Transvaginal Ultrasound-guided Oocyte Aspiration for Production of Embryos in Vitro. *Archiv Fur Tierzucht*, **45(1):99-108**. https://eurekamag.com/research/003/992/003992794.php

Chencha, C. and Kefyalew, A. 2012. Trends of Cattle Genetic Improvement Programs in Ethiopia: Challenges and Opportunities. *Livestock Research for Rural Development,* **24(7)**. http://www.lrrd.org/lrrd24/7/cheb24109.htm

Chesné, P., Adenot, P.G., Viglietta, C., Baratte, M., Boulanger, L., Renard, J.P. 2002. Cloned rabbits produced by nuclear transfer from adult somatic cells. *Nature Biotechnology*, **20(4):366-9**. DOI: 10.1038/nbt0402-366

Coloma, M.A., Toledano-Díaz, A., López-Sebastián, A. and Santiago-Moreno, J. 2010. The influence of washing Spanish ibex (Capra pyrenaica) sperm on the effects of cryopreservation in dependency of the photoperiod. *Theriogenology*, **73(7):900-8**. DOI: 10.1016/j.theriogenology.2009.11.014

Comizzoli, P., Mermillod, P. and Mauget, R. 2000. Reproductive biotechnologies for endangered mammalian species. *Reproduction Nutrition Development,* **40(5):493-504**. DOI: 10.1051/rnd:2000113

Comizzoli, P., Mermillod, P., Cognié, Y., Chai, N., Legendre, X. and Mauge, R. 2001. Successful in vitro production of embryos in the red deer (*Cervus elaphus*) and the sika deer (*Cervus nippon*). *Theriogenology*, **55(2):649-59**. DOI: 10.1016/s0093-691x(01)00433-2

Danchin-Burge, C., Hiemstra, S.J. and Blackburn, H. 2011. Ex situ conservation of Holstein-Friesian cattle: Comparing the Dutch, French, and US germplasm collections. *Journal of Dairy Science,* **94(8):4100-08**. https://doi.org/10.3168/jds.2010-3957

D'Angelo, A., Panayotidis, C., Alteri, A., Mcheik, S. and Veleva, Z. 2022. Evidence and consensus on technical aspects of embryo transfer. *Human Reproduction Open,* **2022(4):hoac038**. DOI: 10.1093/hropen/hoac038

Desalegn, G., Bekana, M., Tegegne, A. and Blihu, B. 2009. Status of Artificial Insemination Service in Ethiopia. A paper presented at the 17th Annual Conference of the Ethiopian Society of Animal Production (ESAP), held at the Head Quarters of the Ethiopian Institute of Agricultural Research (EIAR), Addis Ababa, Ethiopia, **Pp:87-104**.

Di Lecce, I., Sudyka, J., Westneat, D.F. and Szulkin, M. 2022. Preserving avian blood and DNA sampled in the wild: A survey of personal experiences. *Ecol. Evol.*, **12(8):e9232**. DOI: 10.1002/ece3.9232

Durrant, B.S. 2009. The importance and potential of artificial insemination in CANDES (companion animals, non-domestic, endangered species). *Theriogenology*, **71(1):113-22**. DOI: 10.1016/j.theriogenology.2008.09.004

EBI. 2016. Ethiopian national strategy and plan of action for conservation, sustainable use and development of animal genetic resources. Ethiopian Biodiversity Institute (EBI). http://www.ebi.gov.et

Engdawork, A. 2019. Types of Animal Diseases and Their Potential Threats to Sustainability of Animal Biodiversity. *Journal of animal and Veterinary Advances,* **18:227-238**. DOI: 10.36478/javaa.2019.227.238

FAO. 2023. Animal Genetic Resources and their Contribution to Livestock Sustainability. Food and Agriculture Organization (FAO) of the United Nations: Commission on Livestock Development for Latin America and the Caribbean, Ecuador, 11-13 July 2023.

Farsi, M.M., Kamali, N. and Pourghasem, M. 2013. Embryological aspects of oocyte in vitro maturation. *International Journal of Molecular and Cellular Medicine*, **2(3):99-109**. PMCID: PMC3920538

Farstad, W. 2017. Ethiopian Wolf Semen Banking Expedition 2014-2017. Institute for Breeding Rare and Endangered African Mammals (IBREAM). https://ibream.org/

Folch, J., Cocero, M.J., Chesné, P., Alabart, J.L., Domínguez, V., Cognié, Y., Roche, A., Fernández-Arias, A., Martí, J.I., Sánchez, P., Echegoyen, E., Beckers, J.F., Bonastre, A.S. and Vignon, X. 2009. First birth of an animal from an extinct subspecies (*Capra pyrenaica pyrenaica*) by cloning. *Theriogenology*, **71(6):1026-34**. DOI: 10.1016/j.theriogenology.2008.11.005

Freitas, V.J. and Melo, L.M. 2010. In vitro embryo production in small ruminants. *Revista Brasileira de Zootecnia*, **39:409-413**. DOI: 10.1590/S1516-35982010001300045

Galli, C., Lagutina, I., Crotti, G., Colleoni, S., Turini, P., Ponderato, N., Duchi, R. and Lazzari, G. 2003. Pregnancy: a cloned horse born to its dam twin. *Nature*, **424(6949):635**. DOI: 10.1038/424635a

Givens, D.I. 2010. Milk and meat in our diet: good or bad for health? *Animal*, **4(12):1941-52**. DOI: 10.1017/S1751731110001503

Gómez, M.C., Pope, C.E., Giraldo, A., Lyons, L.A., Harris, R.F., King, A.L., Cole, A., Godke, R.A. and Dresser, B.L. 2004. Birth of African Wildcat cloned kittens born from domestic cats. *Cloning Stem Cells*, **6(3):247-58**. DOI: 10.1089/clo.2004.6.247

Hasler, J.F. 2004. Factors influencing the success of embryo transfer in cattle. Proceedings of the WBC Congress, Québec, Canada. Corpus ID: 53345442

Herrick, J.R. 2019. Assisted Reproductive Technologies for Endangered Species Conservation: Developing sophisticated protocols with limited access to animals with unique reproductive mechanisms. *Biology of Reproduction* **100(5):1158-1170**. https://doi.org/10.1093/biolre/ioz025

Hiemstra, S., Lende, T. and Woelders, H. 2011. The Potential of Cryopreservation and Reproductive Technologies for AnGR Conservation Strategies. *Cryobiology*, **63(3):316-17**. https://doi.org/10.1016/j.cryobiol.2011.09.042

Huijsmans, T.E.R.G., Hassan, H.A., Smits, K. and Van Soom, A. 2023. Postmortem Collection of Gametes for the Conservation of Endangered Mammals: A Review of the Current State-of-the-Art. *Animals (Basel)*, **13(8):1360**. DOI: 10.3390/ani13081360

Hwang, I., Jeong, Y.W., Kim, J.J., Lee, H.J., Kang, M., Park, K.B., Park, J.H., Kim, Y.W., Kim, W.T., Shin, T., Hyun, S.H., Jeung, E.B. and Hwang, W.S. 2013. Successful cloning of coyotes through interspecies somatic cell nuclear transfer using domestic dog oocytes. *Reproduction, Fertility and Development*, **25(8):1142-8**. DOI: 10.1071/RD12256

Jainudeen, M.R., Wahid, H. and Hafez, E.S.E. 2000. Ovulation induction, embryo production and transfer. In: B. Hafez, E.S.E Hafez (ed.). *Reproduction in Farm Animals*, **Pp:405-430**. DOI: 10.1002/9781119265306.CH29

Janssen, D., Edwards, M., Koster, J., Lanza, R. and Ryder, O. 2004. Postnatal management of chryptorchid Banteng calves cloned by nuclear transfer utilizing frozen fibroblast cultures and enucleated cow ova. *Reproduction, Fertility and Development,* **16:224-224**. DOI: 10.1071/RDV16N1AB206

Jinadu, K.B., Akingbade, A.O., Olona, J.F., Adekanmbi, O.A., Popoola, M.A., Agboola, T.B., Olagbaju, O.T., Olufayo, O. and Oladele-Bukola, M.O. 2020. Semen characteristics and scrotal size of pubertalWest African dwarf rams fed diets containing (Bitter kola) Seed Meal. *Nigerian Journal of Animal Production*, **45(3):147–157**. https://doi.org/10.51791/njap.v45i3.406

Kim, M.K., Jang, G., Oh, H.J., Yuda, F., Kim, H.J., Hwang, W.S., Hossein, M.S., Kim, J.J., Shin, N.S., Kang, S.K. and Lee, B.C. 2007. Endangered wolves cloned from adult somatic cells. *Cloning Stem Cells*, **9(1):130-7**. DOI: 10.1089/clo.2006.0034

Lakshmy, K.V., Manimegalai, J. and Lambe, U. 2018. Different methods of embryo sexing: A review. *The Pharmal Innovation Journal,* **7(10):170-172**.

Lanza, R.P., Cibelli, J.B., Diaz, F., Moraes, C.T., Farin, P.W., Farin, C.E., Hammer, C.J., West, M.D. and Damiani, P. 2000. Cloning of an endangered species (*Bos gaurus*) using interspecies nuclear transfer. *Cloning*, **2(2):79-90**. DOI: 10.1089/152045500436104

Larsen, J.W. 2021. Artificial Insemination in Sheep. MSD Vet Manual, Reviewed/Revised May 2021/Modified Oct 2022. https://www.msdvetmanual.com/

Le Gac, S., Ferraz, M., Venzac, B. and Comizzoli, P. 2021. Understanding and Assisting Reproduction in Wildlife Species Using Microfluidics. *Trends in Biotechnology,* **39(6):584-597**. https://doi.org/10.1016/j.tibtech.2020.08.012

Lee, B.C., Kim, M.K., Jang, G., Oh, H.J., Yuda, F., Kim, H.J., Hossein, M.S., Kim, J.J., Kang, S.K., Schatten, G. and Hwang, W.S. 2005. Dogs cloned from adult somatic cells. *Nature*, **436(7051):641**. DOI: 10.1038/436641a

Li, X.C., Yue, H., Li, C.Y., He, X.H., Zhao, Q.J., Ma, Y.H., Guan, W.J. and Ma, J.Z. 2009. Establishment and characterization of a fibroblast cell line derived from Jining Black Grey goat for genetic conservation. *Small Ruminant Research,* **87(3):17-26**. https://doi.org/10.1016/j.smallrumres.2009.09.028

Li, Z., Sun, X., Chen, J., Liu, X., Wisely, S.M., Zhou, Q., Renard, J.P., Leno, G.H. and Engelhardt, J.F. 2006. Cloned ferrets produced by somatic cell nuclear transfer. *Developmental Biology*, **293(2):439-48**. DOI: 10.1016/j.ydbio.2006.02.016

Liu, Z., Cai, Y., Wang, Y., Nie, Y., Zhang, C., Xu, Y., Zhang, X., Lu, Y., Wang, Z., Poo, M. and Sun, Q. 2018. Cloning of Macaque Monkeys by Somatic Cell Nuclear Transfer. *Cell*, **172(4):881-887**. DOI: 10.1016/j.cell.2018.01.020

Locatelli, Y., Vallet, J.C., Huyghe, F.P., Cognie, Y., Legendre, X. and Mermillod, P. 2006. Laparoscopic ovum pick-up and in vitro production of sika deer embryos: Effect of season and culture conditions. *Theriogenology*, **66(5):1334-42**. https://doi.org/10.1016/j.theriogenology.2006.05.005

Loi, P., Ptak, G., Barboni, B., Fulka, J., Cappai, P. and Clinton, M. 2001. Genetic rescue of an endangered mammal by cross-species nuclear transfer using post-mortem somatic cells. *Nature Biotechnology*, **19:962-964**. https://doi.org/10.1038/nbt1001-962

Loutradi, K.E., Kolibianakis, E.M., Venetis, C.A., Papanikolaou, E.G., Pados, G., Bontis, I. and Tarlatzis, B.C. 2008. Cryopreservation of human embryos by vitrification or slow freezing: a systematic review and meta-analysis. *Fertility and Sterility*, **90(1):186-93**. DOI: 10.1016/j.fertnstert.2007.06.010

Mastromonaco, G.F. and Songsasen, N. 2020. Reproductive technologies for the conservation of wildlife and endangered species. *Reproductive Technologies in Animals*, **Pp:99-117**. https://doi.org/10.1016/B978-0-12-817107-3.00007-2

Matoba, S. and Zhang, Y. 2018. Somatic Cell Nuclear Transfer Reprogramming: Mechanisms and Applications. *Cell Stem Cell*, **23(4):471-485**. DOI: 10.1016/j.stem.2018.06.018

Mazur, P., Leibo, S.P. and Seidel, G.E. 2008. Cryopreservation of the germplasm of animals used in biological and medical research: importance, impact, status, and future directions. *Biology of Reproduction*, **78(1):2-12**. DOI: 10.1095/biolreprod.107.064113

McEvoy, T.G., Coull, G.D., Broadbent, P.J., Hutchinson, J.S. and Speake, B.K. 2000. Fatty acid composition of lipids in immature cattle, pig and sheep oocytes with intact zona pellucida. *Journal of Reproduction and Fertility*, **118(1):163-70**. PMID:10793638

Meintjes, M., Bezuidenhout, C., Bartels, P., Visser, D.S., Meintjes, J., Loskutoff, N.M., Fourie, F.L., Barry, D.M. and Godke, R.A. 1997. In vitro maturation and fertilization of oocytes recovered from free-ranging Burchell's zebra (*Equus burchelli*) and Hartmann's zebra (*Equus zebra hartmannae*). *Journal of Zoo and Wildlife Medicine*, **28(3):251-259**. PMID:9365936

Meirelles, F.V., Bordignon, V., Watanabe, Y., Watanabe, M., Dayan, A., Lôbo, R.B., Garcia, J.M. and Smith, L.C. 2001. Complete replacement of the mitochondrial genotype in a *Bos indicus* calf reconstructed by nuclear transfer to a *Bos taurus* oocyte. *Genetics,* **158(1):351-356**. https://doi.org/10.1093/genetics/158.1.351

Mendelsohn, R. 2003. The Challenge of Conserving Indigenous Domesticated Animals. *Ecological Economics*, **45(3): 501–10**. https://doi.org/10.1016/S0921-8009(03)00100-9

Mikkola, M. and Taponen, J. 2017. Embryo yield in dairy cattle after superovulation with Folltropin or Pluset. *Theriogenology*, **88:84-88**. DOI: 10.1016/j.theriogenology.2016.09.052

Mmassy, B. 2011. Animal genetic resource conservation and biotechnology. Slideshare. https://www.slideshare.net/

Ndambi, O.A., Van der Lee, J., Ulfina, G.G., Berhanu, T. and Andeweg, K. 2017. Improving private AI services to dairy farmers through AI technician refresher training: lessons from Ethiopia. Practice Brief Dairy BISS project. *Wageningen Livestock Research, Wageningen University & Research, Wageningen*, 1-6.

Onishi, A., Iwamoto, M., Akita, T., Mikawa, S., Takeda, K., Awata, T., Hanada, H. and Perry, A. 2000. Pig cloning by microinjection of fetal fibroblast nuclei. *Science*, **289(5482):1188-1190**. DOI: 10.1126/science.289.5482.11

Paiva, S.R., McManus, C.M. and Blackburn, H. 2016. Conservation of animal genetic resources: A new tact. *Livestock Science*, **193:32-38**. DOI: 10.1016/j.livsci.2016.09.010

Palmer, C.W., Brito, L.F., Arteaga, A.A., Söderquist, L., Persson, Y. and Barth, A.D. 2005. Comparison of electroejaculation and transrectal massage for semen collection in range and yearling feedlot beef bulls. *Animal Reproduction Science*, **87(1-2):25-31**. DOI: 10.1016/j.anireprosci.2004.09.004

Pilling, D., Bélanger, J., Diulgheroff, S., Koskela, J., Leroy, G., Mair, G. and Hoffmann, I. 2020. Global status of genetic resources for food and agriculture: challenges and research needs. *Genetic Resources,* **1(1):4-16**. DOI: https://doi.org/10.46265/genresj.2020.1.4-16

Polejaeva, I.A., Chen, S.H., Vaught, T.D., Page, R.L., Mullins, J., Ball, S., Dai, Y., Boone, J., Walker, S., Ayares, D.L., Colman, A. and Campbell, K.H. 2000. Cloned pigs produced by nuclear transfer from adult somatic cells. *Nature*, **407(6800):86-90**. DOI: 10.1038/35024082

Pomar, F.J., Teerds, K.J., Kidson, A., Colenbrander, B., Tharasanit, T., Aguilar, B. and Roelen, B.A. 2005. Differences in the incidence of apoptosis between in vivo and in vitro produced blastocysts of farm animal species: a comparative study. *Theriogenology*, **63(8):2254-68**. DOI: 10.1016/j.theriogenology.2004.10.015

Ptak, G., Clinton, M., Barboni, B., Muzzeddu, M., Cappai, P., Tischner, M. and Loi, P. 2002. Preservation of the wild european mouflon: the first example of genetic management using a complete program of reproductive biotechnologies. *Biology of Reproduction,* **66(3):796-801**. https://doi.org/10.1095/biolreprod66.3.796

Pukazhenthi, B.S. and Wildt, D.E. 2004. Which reproductive technologies are most relevant to studying, managing and conserving wildlife? *Reproduction, Fertility and Development*, **16(1-2):33-46**. DOI: 10.10371/RD03076

Rezazadeh V.M., Eftekhari-Yazdi, P., Karimian, L., Hassani, F. and Movaghar, B. 2009. Vitrification versus slow freezing gives excellent survival, post warming embryo morphology and pregnancy outcomes for human cleaved embryos. *Journal of Assisted Reproduction and Genetics,* **26(6):347-54**. doi: 10.1007/s10815-009-9318-6

Robertson, E.G. 2014. Embryo collection and transfer. In: John Wiley and Sons, Inc. (Editions), Bovine Reproduction. Editor(s): Richard M, Hopper DVM, Diplomate ACT. Wiley Blackwell, **Pp:703-717**. https://doi.org/10.1002/9781118833971.ch76

Rodriguez-Martinez, H. and Vega, F.J.P. 2013. Semen technologies in domestic animal species. *Animal Frontiers*, **3(4):26-33**. DOI:10.2527/AF.2013-0030

Schenk, J.L., Suh, T.K. and Seidel, G.E. 2006. Embryo production from superovulated cattle following insemination of sexed sperm. *Theriogenology*, **65(2):299-307**. DOI: 10.1016/j.theriogenology.2005.04.026

Shi, D., Lu, F., Wei, Y., Cui, K., Yang, S., Wei, J. and Liu, Q. 2007. Buffalos (Bubalus bubalis) cloned by nuclear transfer of somatic cells. *Biology of Reproduction,* **77(2):285-91**. DOI: 10.1095/biolreprod.107.060210

Shin, T., Kraemer, D., Pryor, J., Liu, L., Rugila, J., Howe, L., Buck, S., Murphy, K., Lyons, L. and Westhusin, M. 2002. A cat cloned by nuclear transplantation. *Nature*, **415(6874):859**. DOI:10.1038/nature723

Swanson, W.F. 2006. Application of assisted reproduction for population management in felids: the potential and reality for conservation of small cats. *Theriogenology,* **66(1):49-58**. DOI: 10.1016/j.theriogenology.2006.03.024

Tetsuka, M., Asada, M., Mogoe, T., Fukui, Y., Ishikawa, H. and Ohsumi, S. 2004. The pattern of ovarian development in the prepubertal antarctic minke whale (*Balaenoptera bonaerensis*). *Journal of Reproduction and Development*, **50(4):381-389**. DOI: 10.1262/jrd.50.381

Thongphakdee, A., Kiatsomboon, S., Noimoon, S., Kongprom, U., Boonorana, I., Karoon, S., Thawnern, J., Sakulthai, A., Sombutputorn, P., Sukmak, M., Punkong, C. and Thongtip. N. 2022. Semen characteristics and second successful artificial insemination of Asian elephant (*Elephas maximus*) in Thailand. *Veterinary World*, **15(5):1246-1255**. DOI: 10.14202/vetworld.2022.1246-1255

Tsai, T.C., Wu, S.H., Chen, H.L., Tung, Y.T., Cheng, W.T., Huang, J.C. and Chen, C.M. 2011. Identification of sex-specific polymorphic sequences in the goat amelogenin gene for embryo sexing. *Journal of Animal Science*, **89(8):2407-14**. DOI: 10.2527/jas.2010-3698

Vajta, G. 2000. Vitrification of the oocytes and embryos of domestic animals. *Animal Reproduction Sciene*, 60-**61:357-64**. DOI: 10.1016/s0378-4320(00)00097-x

Vazquez, J.M., Martinez, E.A., Roca, J., Gil, M.A., Parrilla, I., Cuello, C., Carvajal, G., Lucas, X., Vazquez, J.L. 2005. Improving the efficiency of sperm technologies in pigs: the value of deep intrauterine insemination. *Theriogenology*, **63(2):536-47**. DOI: 10.1016/j.theriogenology.2004.09.043

Vongpralub, T., Chinchiyanond, W., Hongkuntod, P., Sanchaisuriya, P., Liangpaiboon, S., Thongprayoon, A. and Somphol, N. 2015. Cryopreservation of Sambar deer semen in Thailand. *Zoo Biology*, **34(4):335-44**. DOI: 10.1002/zoo.21214

Wakchaure, R., Ganguly, S., Praveen, K. and Mahajan, T. 2015. Methods for Embryos Sexing and Their Applications in Animal Breeding: A Review. *Octa Journal of Biosciences*, **3(2):47-49**.

Wani, N.A., Vettical, B.S. and Hong, S.B. 2017. First cloned Bactrian camel (*Camelus bactrianus*) calf produced by interspecies somatic cell nuclear transfer: A step towards preserving the critically endangered wild Bactrian camels. *PLoS One*, **12(5): e0177800**. DOI: 10.1371/journal.pone.0177800

Wani, N.A., Wernery, U., Hassan, F.A.H., Wernery, R. and Skidmore, J.A. 2010. Production of the First Cloned Camel by Somatic Cell Nuclear Transfer1. *Biology of Reproduction*, **82(2):373-379**. https://doi.org/10.1095/biolreprod.109.081083

Weathers, J.D., and Prien, S.D. 2014. Estimation of Weight and Lipid Composition in Preimplantation Embryos from Jersey and Beef Breeds of Cattle. November, 261–266.

Wildt, D.E. and Wemmer, C. 1999. Sex and wildlife: the role of reproductive science in conservation. *Biodiversity and Conservation*, **8:965-976**. https://doi.org/10.1023/A:1008813532763

Wilmut, I., Schnieke, A.E., McWhir, J., Kind, A.J. and Campbel K.H. 1997. Viable offspring derived from fetal and adult mammalian cells. *Nature*, **385(6619):810-3**. DOI: 10.1038/385810a0

Woods, E.J., Benson, J.D., Agca, Y. and Critser, J.K. 2004. Fundamental cryobiology of reproductive cells and tissues. *Cryobiology*, **48(2):146-56**. DOI: 10.1016/j.cryobiol.2004.03.002

Woods, G.L., White, K.L., Vanderwall, D.K, Li, G.P., Aston, K.I., Bunch, T.D., Meerdo, L.N. and Pate, B.J. 2003. A mule cloned from fetal cells by nuclear transfer. *Science*, **301(5636):1063**. DOI: 10.1126/science.1086743

Wulster-Radcliffe, M.C., Williams, M.A., Stellflug, J.N. and Lewis, G.S. 2001. Technical note: Artificial vagina vs a vaginal collection vial for collecting semen from rams. *Journal of Animal Science*, **79(12):2964-7**. DOI: 10.2527/2001.79122964x

Zhou, Q., Renard, J.P., Le Friec, G., Brochard, V., Beaujean, N., Cherifi, Y., Fraichard, A. and Cozzi, J. 2003. Generation of fertile cloned rats by regulating oocyte activation. *Science*, **302(5648):1179**. DOI: 10.1126/science.1088313

Zoheir, K.M. and Allam, A.A. 2010. A rapid method for sexing the bovine embryo. *Animal Reproduction Science*, **119(1-2):92-6**. DOI: 10.1016/j.anireprosci.2009.12.013

Zuidema, D., Kerns, K. and Sutovsky, P. 2021. An Exploration of Current and Perspective Semen Analysis and Sperm Selection for Livestock Artificial Insemination. *Animals (Basel)*, **11(12):3563**. DOI: 10.3390/ani11123563