CONSERVATION OF ANIMAL GENETIC RESOURCES IN MALAYSIA: DEPARTMENT OF VETERINARY SERVICES' EMPHASIS ON SEMEN CRYOPRESERVATION

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ABSTRACT. The Department of Veterinary Services (DVS) is the country's primary custodian of the livestock industry. It has a global responsibility to ensure the growth and development of a strong animal industry, including the control of animal diseases, the production of animal products for human consumption, animal welfare, as well as the conservation of animal genetic resources. The Malaysian livestock industry is currently facing a high demand for livestock products. Concerns over food security have led to the application of artificial breeding and there will be a risk of deterioration of indigenous breed genetic makeup. DVS advocates a policy of *ex-situ* and *in-situ* conservation of animal genetic resources through the establishment of *Institut Biodiversiti Veterinar Kebangsaan* (IBVK) and *Pusat Ternakan Haiwan*. At present, about 40 breeds of various livestock species are being maintained at various DVS nucleus herds throughout the country. The species include cattle (*Bos taurus*), goats (*Capra hircus*), buffaloes (*Bubalus bubalis*), deer (*Cervidae*), and sheep (*Ovis aries*). A nucleus herd serves as a place for maintaining, preserving, and enhancing specific breeds of livestock. This study is carried out to distribute information about the efforts and strategies of DVS in conserving and utilizing animal genetic resources, with a particular focus on semen cryopreservation. Research on the conservation of livestock semen has intensified in recent years. However, there is a lack of research on the development of techniques to preserve animal genetic diversity and efforts to restore and reintroduce breeds in nature for food security. Current technology and practices that promote the industry's sustainability are also highlighted, together with information on species of animals that are available in IBVK semen bank.

Keywords: animal genetic resources, biodiversity, conservation, food security

INTRODUCTION

Malaysia is blessed with a wide biodiversity of natural resources. Considering the importance of preserving animal genetic diversity, the Department of Veterinary Services (DVS) advocated a strategy of preserving animal genetic resources through *in-situ* and *ex-situ* conservation. The former is where the animals are maintained in their adaptive environment, while the latter involves maintaining animal genetic resources outside their native habitat either in the form of living semen, ova or embryos. The *in-situ* conservation of cattle, goats, deer, sheep, and buffaloes is being carried out by DVS Livestock Centre (*Pusat Ternakan Haiwan*) located in various states within Malaysia whereas e*x-situ* conservation is centred at *Institut Biodiversiti Veterinar Kebangsaan* (IBVK), Jerantut, Pahang. DVS also has imported several exotic breeds for breeding purposes which are Belgian Blue, Wagyu, Limousin, Charolais, Nellore, Holstein-Friesian, and Murrah.

The native Malaysian breeds, Kedah-Kelantan cattle and Malin sheep, are purposely kept for meat production. Both of these breeds are well-adapted (Islam *et al.*, 2021; Zain *et al.*, 2013). According to Hufana-Duran & Duran (2020), indigenous breeds have a high level of adaptation to the most common issue for that specific region either climate, disease or nutrition-induced. The livestock industry is currently facing challenges as the demand for livestock products rises. Concerns over food security issues give rise to the development of artificial breeding, with the emphasis to genetically improve the available species. As a result, there is a risk that indigenous breeds will deteriorate due to the infusion of exotic blood and the use of inferior sires, which will directly replace the purebred genotypes.

The animal semen bank in IBVK acts as a form of *ex-situ* conservation where valuable germplasm from various animal breeds is kept somewhere other than its original environment. IBVK was mandated with the following tasks: (1) produce high-quality dairy and meat frozen semen to meet the needs of the country's livestock industry, (2) implement a program to improve the genetic quality of ruminant livestock in selected department farms and private farms, (3) provide expert services to the department's customers in the field of breeding technology, breeding, and livestock biodiversity, (4) carry out conservation and exploitation programs of genetic resources of ruminant livestock, and (5) conduct applied research in the field of ruminant breeding biotechnology. Bank Semen IBVK (Figure 4) currently holds 171,447 semen from various selected livestock species.

The relationship between animal preservation and their use in the natural sciences suggests that there are parallels between enhancing animal productivity and protecting animal germplasm. Freezing and long-term storage of germplasm of selected livestock species will allow the preservation of genetic diversity for future users, enabling agricultural modernisation to proceed. Consequently, ongoing efforts must be made in order to conserve material records of animal genetic diversity while identifying conservation strategies in the way that people expect to use these materials in the future. It is vital for that strategy to focus on increasing animal productivity and enhancing germplasm quality for future use.

MATERIALS AND METHOD

Semen Collection

Semen was routinely collected using an artificial vagina (Figure 1) by trained technicians. The bull was clean prior to collection. New artificial vagina was used for each mounting after successive ejaculates were collected.

Semen Quality

Figure 1. Artificial vagina used for semen collection. It consists of an inner liner, AV tube, funnel, and calibrated tube

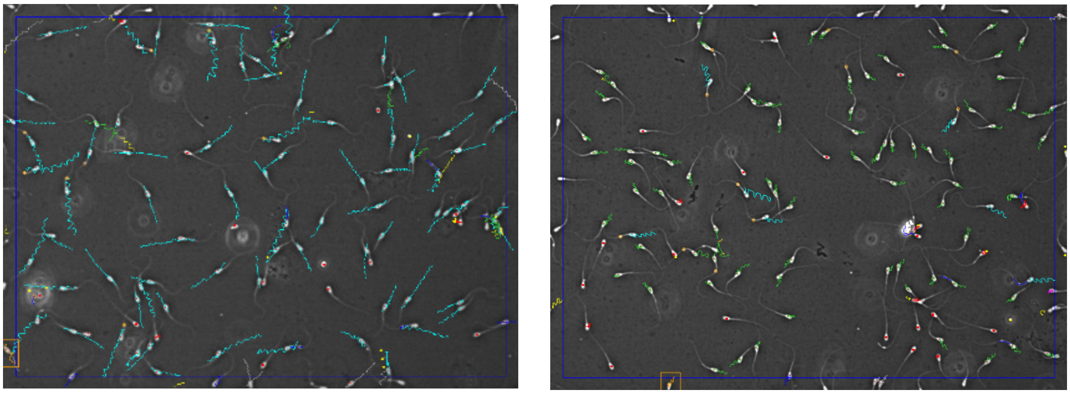

Figure 2. The spermatozoon cells are labelled with the corresponding colour; green - motile, red - progressive between 50 to 70, and blue - progressive

The fresh semen samples were immediately examined after each ejaculation. Quantitative measurements of sperm including motility, forward progression, and velocity were assessed using computer-assisted semen analysis (CASA) (Figure 2). 30 µl of fresh sample was subjected to a spectrophotometer to determine the concentration of the semen. The progressive motility of fresh semen must exhibit above 60 % for cryopreservation.

Semen Cryopreservation

Commercial semen extender was added to the analysed semen and kept in semen straws. The straws were frozen down in the automatic semen freezer. (Figure 3) After 14 days of semen collection, the straws were subjected to microbiological screening and survival test prior to cryopreservation. For microbiological screening, IBVK cooperates with *Makmal Veterinar Zon Timur* (MVZT), Pahang to perform bacterial screening through routine culture to detect the presence of microorganism in the sample. For the survival test, morphology of the sperm cells was evaluated manually by trained technicians.

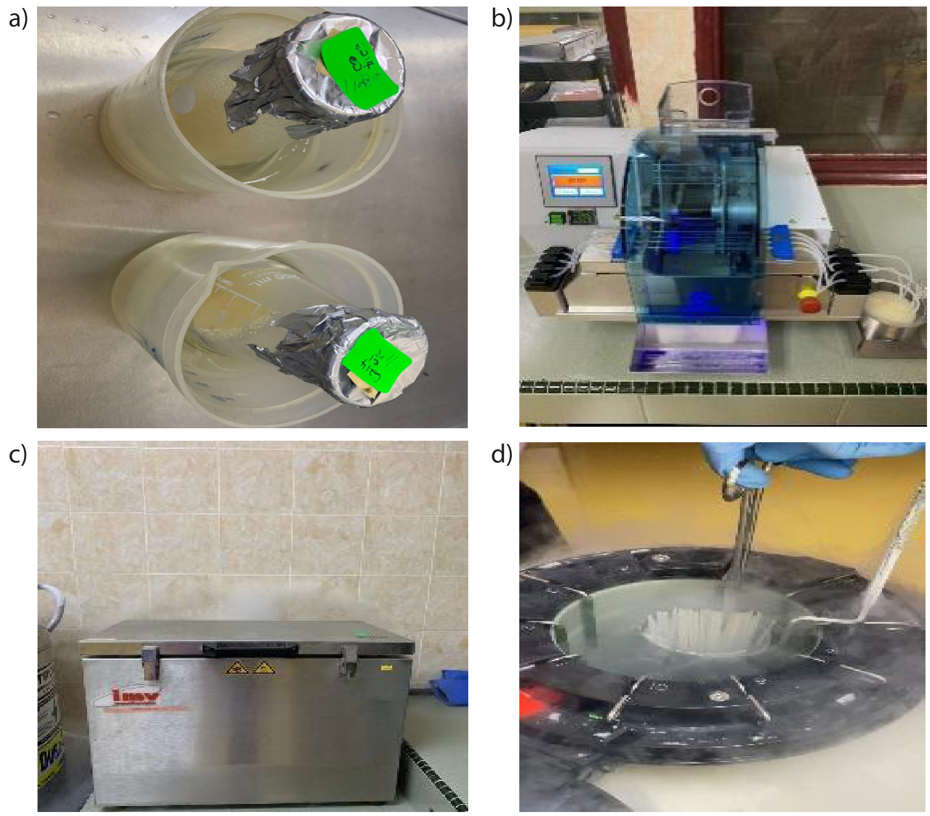

Figure 3. (a) Fresh semen were cooled down to 4 °C in chiller, (b) Semen were packed into semen straw using filling and sealing machine, (c) The straws were frozen down in automatic semen freezer, and (d) Semen straws were transferred into mother tank

Semen Storage in Semen Bank

The frozen semen was reassessed for its quality, with a criterion set at 50 % progressive motility before being stored in the Semen Bank. Mother tanks containing frozen semen were kept in the semen bank and labelled with breed identification number and the date of semen collection in order to facilitate identification and semen retrieval (Figure 4).

Figure 4. Genetic materials (semen and embryo) were kept in Semen Bank, *Institut Biodiversiti Veterinar Kebangsaan*

Data Analysis

Data were gathered from DVS official sources and public materials, a quick analysis was performed to gain information about the available biological material. The primary data source for this study was DVS's livestock census data.

RESULTS AND DISCUSSION

Table 1 shows the number of animal genetic materials available in Semen Bank IBVK. A total of 168,638 semen samples from 103 male donors consisting of various breeds of livestock species were stored in Semen Bank IBVK. 120,307 samples were from cattle, 5,681 samples from goats, and 45,459 from sheep. Common procedures conducted in the process of semen cryopreservation for *ex-situ* conservation include semen collection by skilled technicians and evaluation of the semen quality. For semen quality, both macroscopic factors including volume and colour as well as microscopic parameters such as count, motility, and morphology were analysed manually as well as through automated semen analysis using computer-aided sperm analysis (CASA) system. Study by Finelli *et al*. (2021) mentioned that CASA system is a reliable alternative for assessing semen parameters especially to measure semen concentration and motility.

An artificial vagina (AV) is used for semen collection because it is easier to use and more closely mimics the natural state of the female reproductive tract, as it contributes a threshold of warmth, pressure and friction. This method has several advantages over other methods such as electro-ejaculation, vaginal spoon, and sponge technique. Normal and clear ejaculation can be obtained through AV method and the sterile state of the apparatus ensures disease control. A study by Jiménez-Rabadán *et al*. (2012) found that ejaculates collected by AV have a greater sperm quality after thawing and are more resistant to cold shock than those obtained by electro-ejaculation. Additionally, semen collected through AV is also free from external secretions. However, it is difficult to get males to cooperate with an artificial vagina. Normally, two to three ejaculations can be collected from a single bull. The initial ejaculation usually exhibits high concentration and volume. However, according to a study by Khaki *et al*. (2021), there is no difference observed in semen quality particularly seminal plasma trace elements such as zinc and copper between the first and second ejaculations. Zinc and copper play crucial roles in reproductive development and sexual maturity as highlighted in a study by Geary *et al*. (2021).

During semen processing, a lengthening solution called a semen extender is essential because a single ejaculation contains exponentially more sperm than the sperm needed for fertilization. Dilution with extenders will allow multiple insemination and greatly advances the practice of artificial insemination. Other than that, semen extender also provides nutrients for sperm to survive. It protects sperm against damaging elements such as osmotic shock, oxidative stress, and ice crystals during the freezing and thawing process (Bustani & Baiee, 2021).

Cryopreservation of sperm is a consecutive process that includes temperature reduction, cell dehydration, freezing, storage, and thawing (Ugur *et al*., 2019). During cryopreservation, the metabolism of sperm cells is reduced to the bare minimum required for survival without losing their ability to fertilize an egg. Cryopreserved sperm can last almost forever in liquid nitrogen. It can be transported across geographic locations with the purpose of the maintenance of genetic variation. Semen extenders help cryopreserved sperm retain features including morphology, motility, viability, acrosomal, and DNA integrity (Bustani & Baiee, 2021). Apart from that, it is also vitally important to conduct microbiological screening and survival tests because it will determine how the sperm function for future use.

Table 1. The collection of animal genetic materials (semen) originating from native breeds of selected livestock species. Conservation was done by collecting specimens of semen from 14 cattle species, 6 goat species, and 10 sheep species

BANK OF BIOLOGICAL MATERIAL				
Species	**Breed name**	**Material type**	**Number of samples**	**Number of male donors**
Cattle	Bali	Semen	2,809	3
	Belgian Blue		13,907	2
	Brahman		4,057	1
	Jersey		32,098	4
	Kedah-Kelantan		27,175	10
	Limousin		1,507	2
	Low line Angus		500	1
	Mafriwal 90		8,499	2
	Mafriwal 70		6,658	4
	Mafriwal 50		1,288	1
	Mafriwal 25		3,438	1
	Nellore		1,759	2
	Holstein-Friesian		14,014	2
	Wagyu		2,598	1
Total cattle semen straws available			120,307	36
Goat	Anglo Nubian	Semen	568	1
	Boer X Feral		1,480	3
	Feral		2,138	3
	Katjang		444	1
	Red Kalahari		934	2
	Boer X Katjang		117	1
Total goat semen straws available			5,681	11

BANK OF BIOLOGICAL MATERIAL				
Sheep	Barbados Blackbelly	Semen	3,140	11
	Damara		4,374	2
	Dorper		4,865	5
	Malin		1,697	2
	Morada Nova		10,018	7
	Poll Dorset		2,541	4
	Santa Ines		8,336	13
	Siamese Long tail		2,023	1
	Segurena		6,365	7
	Southdown		2,100	4
Total sheep semen straws available			45,459	56
Total			**171,447**	**103**

Towards sustainability in the livestock industry, research in conservation, application of new technology, and strengthening the conservation programme in the community are necessary prerequisites for access to biological resources.

Enhancing Research in Conservation of Animal Genetic Resources

Moving forward to a better environment with a variety of animal species, more planned research is needed to preserve the available genetic resources while also assisting animal species that are on the verge of extinction. The study of the genetic linkage patterns will lead to new discoveries and taxonomic revisions. In addition, it is also important to evaluate the risks of genetic impoverishment and inbreeding in order to maintain genetic variability. Molecular research related to inbreeding should be conducted as inbreeding depression promotes the expression of more recessive genes (Doekes *et al*., 2021). Loss of genetic variations and genetic erosion equates to the loss of potentially essential information for humans that could not be recovered (Hoffmann, 2010). Moreover, by conducting research, scientists lay the foundation for cooperative research that will benefit all the parties involved.

Enhance Application of Assisted Reproductive Technologies in the Country

To cope with the constant changes in technology, it will be necessary to continually acquire new skills and knowledge. It is essential to apply genetic manipulation technologies for animal species conservation. More globalised agricultural methods such as oestrus synchronization, artificial insemination (AI), somatic cell nuclear transfer (SCNT), and multiple ovulation and embryo transfer (MOET) are used for the improvement and preservation of livestock genetics, as well as the enhancement of reproductive efficiency (Daly *et al*., 2020). The genetic potential of a particular breed can be reactivated through artificial insemination of suitable female animals from existing populations, followed by genetic grading-up in several subsequent generations to increase the gene frequency of the originating breed (Mueller & Van, 2022). Other than that, cloning of different livestock breeds also could provide other substantial benefits, such as improved genetics in terms of fertility and prolificacy as well as increased in milk and meat production (Trzcińska & Samiec, 2021). There will undoubtedly be ups and downs in the application of these current technologies for beginners as it is a slow process and requires perseverance. The development of Mafriwal cattle, on the other hand, demonstrates Malaysia's ability to produce high-quality breeds. This breed was developed as part of the DVS program. It is derived from Sahiwal-Friesian crossbreds to facilitate genetic gain in tropical environments. This breed was developed for both milk and beef production (Panandam & Raymond, 2005).

Strengthen and Integrate Conservation Programmes

The significance of these conservation efforts lies in the supplementing of programmes designed to reactivate animal genetic resources either semen or embryo as well as the prospect of reactivating a few genes that might be required in the near future (Mara *et al*., 2013). This effort requires interdisciplinary collaboration at the local, regional, and national levels. Genetic diversity of each breed should be explored and quantified in order to minimize inbreeding and genetic drift (Vani *et al*., 2022). Through network building and mobilisation of relevant expertise and detailed understanding of optimizing the success of conservation programs including studies on animal morphology and adaptation will be the source of knowledge for action to protect nature.

CONCLUSION

Animal genetic resources play an important role in biodiversity. Through the initiatives made by DVS, some of the economically valuable animal species are being conserved. Future research should focus more on the use of advanced assisted reproductive technology and the reactivation of cryopreserved animal genetic material to improve the genetics of breeding stock that has the potential to be used in conservation programs.

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