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Seasonal Assessment of Semen Quality in Myanmar Indigenous Bull, Shwe Ni

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***Abstract*—** *Successful artificial insemination (AI) is dependent upon libido, semen quality, cryopreservation and post thaw motility in order to accelerate genetic improvement. In Myanmar, the valuable information of semen quality of indigenous bull has not been updated yet. According to disease resistance and environmental adaptability, frozen semen straws of Myanmar indigenous bulls, Shwe Ni (SN) were in high demand. Shwe Ni cattle are native Myanmar cattle characterized by red coat fur on their entire body with strong masculine structure. In the present study, semen quality of 5 SN bulls were evaluated in order to select optimal bull for breeding purpose in nation artificial insemination center. The mean values of semen volume, semen concentration, sperm motility, sperm progressive motility and sperm morphology were assessed. Apart from that, association between semen quality and semen variation between three seasons were also determined. This study revealed that semen volume, sperm motility and sperm progressive motility were not having association within seasons (P>0.05). However, a significant reduction in semen concentration of SN bulls occurred during dry season*.

***Keywords*—** *Artificial insemination, bull, indigenous, season, semen quality*.

I. INTRODUCTION

To obtain better-quality domestic and livestock animals, establishment of animal genetic resources by modern innovation plays crucial. Hence, male reproductive ability is a major factor in which quality of germ cells produced by male animals, artificial insemination technique becomes most accepted biotechnology in livestock sector (Nagata et al., 2019). The procedure of AI includes three main steps-containing semen collection from desired bull, semen processing and deposited semen in female reproductive tract by means of artificial insemination technique (Mohammed, 2018). By improving fertility of bulls, genetic potential of these bulls could increase and may lead to beneficial economic effect for farm owners (Moore & Hasler, 2017). It has been noted that sexual excitement level and bull performance can affect the semen quality (Pound, 2002) as bulls with high libido produce qualified sperm through several ejaculates in a short time (Affandhy et al., 2018).

Nowadays, semen quality has been determined by considering three important parameters of sperm motility, morphology and vitality since these indices had significant correlation with freezability and fertility (Fiaz etal., 2010). Vitality is the proportion of live spermatozoa in a sample in which assessment of membrane integrity has been widely used to determine sperm vitality (Foote et al., 2002). Apart from vitality, the motility of sperm with straight forward direction is also important, hence back flow of poor motile sperm has been observed within 10 minutes of insemination (Dungdung et al., 2016). Differences in sperm morphology could result in differences in fertility rates because a spermatozoon with normal morphology only can reach the site of fertilization in time (Perry, 2021). Therefore, semen evaluation has become critical to consider bull fertility.

Environmental factors play a critical role on semen quality (Fuerst-Waltl et al., 2006). Photoperiod (time interval between sunset and sunrise) has been considered as the most favorable time for semen quality in temperate region (Rizzoto et al., 2020). However, temperature and humidity have been recorded as two significant factors for semen quality variation in tropical region (Llamas-Luceño et al., 2020). High environmental temperature may result in increasing body temperature of bulls, which in turn lead to impair reproduction results and may eventually lead to low conception rates (Morrell, 2020).

Ruminants are known to be susceptible of heat stress than other mammals by means of their habit upon rumination and fermentation process (Bernabucci et al., 2010). In addition to this, heat stress bulls demonstrate increase respiratory rate and rectal temperature (Luo et al., 2021), and it might lead to reduce testes weight, resulting in alteration of testosterone production (Rizzoto et al., 2020). Even Myanmar indigenous bulls are well known for their tolerating ability of environmental conditions (Hlaing et al., 2020), the information on semen quality depending upon seasonal variation is still scarce. Shwe Ni, Myanmar indigenous cattle are well known for their body conformation which aimed to use in agricultural practice as draft power. Apart from that, Myanmar have some regions which tend to rear these type of cattle, Shwe Ni, with red fur as

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their preferences. Therefore, Shwe Ni has strong market in Myanmar especially at the middle part of the country, the most tropical areas in which above 5000 straws per month have been demanded. For that reason, this study was conducted to clarify seasonal effect on semen quality of Myanmar indigenous bull SN between three seasons of dry (from February' 2021 to May' 2021), rainy (from June' 2021 to September' 2021) and winter (from October' 2021 to January' 2022) of their native tropical environment and to determine optimal bull for breeding purpose in artificial insemination center.

II. Materials and Methods

2.1 Study area

This study was conducted at Livestock Upgrading Section, Artificial Insemination Center, Paung Ngu, Yangon Region. The area was located at 16.9969° North latitude and 96.1421° East longitude. The altitude of this area was 9.14 m above sea level.

2.2 Animal and semen collection

A total of 5 SN bulls of 4-6 years old were kept under uniform management practices at Livestock Upgrading Section, Artificial Insemination Center, Paung Ngu, Yangon Region. Animal data of weight, body condition score and evaluation of BSE were also recorded. Semen collection and evaluation was performed during three seasons of dry (from February' 2021 to May' 2021) and rainy (from June' 2021 to September' 2021) and winter (from October' 2021 to January' 2022). Libido was also assessed as described by (Adamczyk et al., 2013) (Table 1), since it is a useful tool to judge the reproductive efficiency (Islam et al., 2018). Fresh semen samples of all experimental bulls (50 ejaculates/ bull in each season) was collected using artificial vagina in the morning. Semen collection was done with identical time interval. The mean ambient temperature of three seasons were recorded. The mean ambient temperature in rainy season is 25.5°C whereas 20°C in winter and the mean ambient temperature becomes high at 32.5°C in dry season during study period.

TABLE 1. Bull libido assessment

Scale	Description
0	Complete lack of self-drive and interest. Bull does not sniff or attempt to mount.
1	Very weak sex-drive. At least 10-minute waiting and bull will only sniff the teaser animal without attempting to mount
2	Weak sex-drive. When taken to the teaser animal, the bull sniffs again and again. Then making a hesitant attempt at mounting, sniffs again, and makes another mount attempt within10 minutes.
3	Moderate sex-drive. When taken to the teaser animal, the bull sniffs it and begins mount attempts after 2-3 minutes.
4	Strong sex drive. When taken to the teaser animal, the bull attempts to mount it at once but remains calm and is manageable for the handler.
5	Very strong sex-drive. When taken to the teaser animal, the bull begins mount attempts at once and becomes unmanageable for the handler.

2.3 Semen evaluation

After semen ejaculation, the semen containing vial was placed in water bath at 37°C. Subsequently, the semen samples were diluted with bull semen extender (OptixCell 2, IMV Technologies, France) at the ratio of 1:50 of ejaculated semen concentration. Then, 4 µl of the diluted sample was placed in a pre-warmed Leija chamber (Leija counting chambers, depth 20 µm; Microptics, Barcelona, Spain) and automatically assessed using Computer Assisted Sperm Analyzer (CASA) system (IVOS-IICASA system, Hamilton Thorne Inc., Beverly, USA). For one semen sample, at least 800 spermatozoa/sample in ten microscopic fields were analyzed. The assessed parameters included bull ID, volume, concentration, sperm motility, progressive sperm motility and sperm normal morphology. Moreover, abnormal morphologies of bent tails, coiled tails, distal and proximal protoplasmic droplets were also assessed by eosin-nigrosin stain (Bred Life Science Technology Inc., Guangdong, China). Briefly, 5% of stain solution was mixed with 10 µl of semen sample followed by incubation at 37°C for 60s. Thereafter, approximately 6 µl of stained sample was smeared on glass slide, and then left air dry for 10 minutes, followed by microscopic examination at a magnification 100x. At least 200 spermatozoa per slide was identified for reliable interpretation.

2.4 Statistical analyses

The statistical procedure was performed using SPSS software version 20.0. Data were expressed as mean ± Standard Error (SE) followed by p-value on each semen quality. The differences with p-values <0.05 were considered as significant. Pearson Chi-square test was used to determine semen quality variation within seasons.

III. Results

The minor changes in mean ambient temperature within different seasons were recorded. Mean values of semen assessment parameters between three seasons are shown in Table 2. As results, semen volume, sperm motility and sperm progressive motility were not significantly affected ($p>0.05$) between seasons. However, semen concentration showed significant association ($p<0.001$) with seasonal variations whereas lower concentration was showed in dry season. According to sperm motility, the average motility of fresh sperm ranged from 94.2% to 98.4% (Table 2). Our results showed that SN 11 had higher motility compared to other 4 SN bulls of SN 9, SN 10, SN 12 and SN 13 (Table 3). The morphology was assessed using eosin-nigrosin staining. Out of 5 SN bulls, SN 11 showed highest percentage of normal spermatozoa (Table 4).

TABLE 2. Mean values of semen assessment parameters between three seasons

Semen parameters	Rainy Season (25.5°C)	Dry Season (32.5°C)	Winter Season (20°C)
Libido (scale)	5 ± 1	5 ± 1	5 ± 1
Volume (ml)	7 ± 2.5	6.5 ± 2.3	6.2 ± 2.1
Sperm concentration ($x10^6$)	4678 ± 400	1527 ± 500**	4521 ± 400
Sperm motility (%)	96.4 ± 1.6	94.2 ± 2.1	98.2 ± 1.2
Sperm progressive motility (%)	63 ± 1.1	62 ± 2.8	63 ± 2.6
Sperm with normal morphology (%)	78 ± 5.4	80 ± 6.1	82 ± 4.3

**p<0.001

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To check out the post-thawed motility of individual SN bulls that freeze and stored in liquid nitrogen of -196°C, post-thawed sperm were analyzed. As results, motility of different ranges between 72 to 80% were investigated whereas SNG 11 showed the highest rate of post-thawed motility with 80% in which 42% of spermatozoa with straight forward progressive motility (Table 5).

TABLE 3. Comparison of semen parameters within 5 SNG bulls

Semen parameters	SN 9	SN 10	SN 11	SN 12	SN 13
Libido (scale)	5 ± 1	5 ± 1	5 ± 1	5 ± 1	5 ± 1
Volume (ml)	6.5 ± 0.5	6.7 ± 1.1	7 ± 0.4	6.6 ± 0.5	6.5 ± 0.8
Concentration (x10^6)	2527 ± 500	3823 ± 400	4678 ± 400	2113 ± 650	1527 ± 740
Motility (%)	94.2 ± 2.1	93.4 ± 1.8	98.4 ± 1.6	95.3 ± 2.0	96 ± 1.5
Progressive motility (%)	62 ± 2.8	62 ± 3.4	63 ± 1.1	61.5 ± 2.3	61 ± 3.1

TABLE 4. Morphology assessment of 5 SN bulls

Bull ID	Normal(%)	Head abnormality(%)	Midpiece abnormality(%)	Tail abnormality(%)
SN 9	74 ± 6.1	10.1 ± 2.6	13.5 ± 4.4	2.4 ± 0.3
SN 10	75 ± 2.4	9.8 ± 1.6	7.8 ± 2.3	7.4 ± 2.5
SN 11	76 ± 5.4	9.2 ± 1.3	10.7 ± 0.7	4.1 ± 1.2
SN 12	75 ± 1.6	11.3 ± 1.5	7.8 ± 2.1	5.9 ± 2.3
SN 13	74 ± 5.4	5.6 ± 1.2	12.1 ± 2.3	8.3 ± 2.1

TABLE 5. Motility of fresh and frozen-thawed sperm from individual SN bull assessed by CASA system

Bull ID	Fresh, %		Frozen-thawed, %	
	Motility	Progressive motility	Motility	Progressive motility
SN 9	94.2 ± 2.1	55 ± 3.1	72.2 ± 2.2	24.2 ± 1.8
SN 10	93.4 ± 1.8	54 ± 2.8	74.2 ± 3.1	33.2 ± 2.1
SN 11	98.4 ± 1.6	62 ± 2.3	80.2 ± 2.4	40.2 ± 2.2
SN 12	95.3 ± 2.0	40 ± 2.1	76.2 ± 3.8	34.2 ± 4.1
SN 13	96 ± 1.5	38 ± 3.2	73.2 ± 2.1	32.2 ± 2.7

IV. Discussion

Currently, the information on semen quality of Shwe Ni bulls in Myanmar is still scarced. To improve genetic traits and to preserve Myanmar indigenous bulls, collection of elite bulls for breeding purpose is important. For this, evaluation of semen quality plays crucial to provide sufficient motile sperm for successful fertilization. Since SN bulls are known as Myanmar indigenous breed, the information on their tolerance of tropical environment still need to be updated. Therefore, the present study was conducted to update variation in semen quality within different seasons and to evaluate semen quality of SN bulls which selected for frozen straw production at Yangon artificial insemination center.

According to the results, semen volume, sperm motility and sperm progressive motility were not affected ($p>0.05$) between seasons. However, semen concentration showed significant association ($p<0.001$) with seasonal variations whereas lower concentration was found in dry season. Therefore, SN bulls might not be heat tolerant by means of concentration whereas endocrine system could alter and resulted in reduction of sperm number (Krishnan et al., 2017). However, the resilient motility of SN bulls has been shown in this study within dry season, therefore it was approved the capability of heat stress in indigenous cattle without affecting sperm motility (Madhusoodan et al., 2020). It was in lined with (Prastowo et al., 2019) that reported season plays critical role in alteration of bull reproduction, since the sperm quality might have been changed in different seasons (Landaeta-Hernández et al., 2020). Therefore, it could be stated that even though the motility of SN bulls remained the same, remarkable reduction of semen concentration might be due to high environmental temperature that affect spermatogenesis process in any way.

The determination on normal morphology is critical while the pubertal bull need to have >70% normal sperm (Menon et al., 2011). In present study, all of 5 SN bulls showed >75% normal morphology, whereas SN 11 showed highest percentage compared to others. It has been noted that the sperm normality is critical not only for successful fertilization but also to diagnose male infertility clinically (Kastelic, 2013). Since simply result about abnormalities percentage could not make strong prognosis, interpretation on type of abnormalities correlating with breeding soundness examination should be further performed along with history of environmental stress factors (Perry, 2021).

Nowadays, selection of elite bulls for breeding purposes is critical to preserve and improve their genetic traits (Yoon et al., 2022). To meet the targeted conception rate, semen quality assessment for Myanmar indigenous bulls becomes important. The present study, therefore evaluated semen quality of Shwe Ni bulls which bred in artificial insemination center for frozen semen straw production. This study provided valuable information about characteristics of sperm from Shwe Ni bulls that could further be used in advanced reproductive technologies.

V. Conclusion

It can be concluded that there were not significant variations on the semen quality of Myanmar indigenous 5 SN bulls between three seasons of their native tropical environment. However, the semen concentration is notably reduced during the dry season, which is considerably associated with the higher sunshine duration of dry season. Further development of in vivo studies should be performed in order to better understanding nation traits of Shwe Ni.

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Authors contribution

BKS designed the work, analyzing the data and drafting the manuscript. All of the authors equally contributed to analyze the data and revised the manuscript. All authors read and approved the final manuscript.

Conflict of interest

The authors declared that they have no competing interests.

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