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ASSESSMENT OF NON-GENETIC FACTORS AFFECTING THE QUALITY OF BOVINE SEMEN PRODUCTION UNDER BHUTANESE ENVIRONMENT

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ABSTRACT: The study assessed non-genetic factors influencing the quality of bovine semen production under Bhutanese environment. A total of 472 records of semen production from Jersey (*Bos taurus*), Mithun (*Bos frontalis*) and Nublang (*Bos indicus*) were analyzed. The factors studied were age of bull at procurement and semen collection, breed, season and semen collection interval for their effect on semen quality, which was assessed based on volume (Vol.), Mass Activity (MA), Initial Sperm Motility (ISM) and Sperm Concentration (SC) in fresh semen, and Semen Straw Produced (SSP), Semen Straw Discarded (SSD), Semen Straw Stored (SSS) and Post-Thawing Motility at Production (PTM-P) and Certification (PTM-C) in processed semen. Mean semen recovery rate was 78%. A strong correlation was observed between fresh and processed semen qualities. Best output in processed semen in terms of quantity, quality and recovery rate was found for ejaculate with Volume, MA, ISM and SC of ≥3ml, 3, ≥70% and ≥700x10^6/ml, respectively. Age had a significant effect on SSP, indicating lesser production for bulls procured after 18 months of age. Age at first semen collection significantly affected MA, ISM, SSP, SSD, SSS, PTM-P and PTM-C, and quality improved with age of bulls. Breed significantly affected MA, ISM, SSD, SSS, PTM-P and PTM-C, indicating best qualities for Nublang, followed by Mithun and Jersey. Season significantly affected Vol., MA, ISM and SSP, showing better qualities in Autumn, followed by Summer and Spring. The collection interval affected SSP and SSS, revealing best productions for collection interval of 11-15 days than once a week as currently practiced. Hence, greater emphasis be given on more collection in Autumn and Summer, avoiding collection within six days from same bull and processing of ejaculates with volume, MA, ISM and SC of minimum 2ml, 2, 70% and 700x10^6/ml respectively, for optimum semen recovery rate with better quality and minimal waste of resources.

Keywords: Age; artificial insemination; breed; non-genetic; semen quality.

1.INTRODUCTION

Artificial Insemination (AI) has been used worldwide to facilitate animal production effectively and efficiently as one superior bull could produce several thousands of semen straws (doses) to impregnate cows and produce calf with high genetic merit. In Bhutan, AI in cattle was initiated in 1987 using imported progeny tested semen. The in-house production of bovine frozen semen began in 1992 for wider AI coverage in the country. The semen is produced from pedigree selected bulls of different breeds viz; Jersey (*Bos taurus*), Mithun (*Bos frontalis*) and Nublang (*Bos indicus*). The semen produced from these bulls is distributed to all AI centres in the country. As of June 2020, there were 120 AI centres across the country performing around 8,400 AI annually. The overall AI success rate recorded was 32% in 2019-20 (NDRDC 2019).

The semen donor bulls are procured at varying ages ranging from 15- 24 months based on their availability in nucleus herds located in different parts of the country. The bulls are selected based on its pedigree with focus on dam's performance and disease-free condition. The bulls are trained regularly twice a week and some bulls start donating semen as early as 19 months of age and others

at later age depending on type of breed. These bulls are managed under same environment at the National Dairy Research and Development Centre (NDRDC), Yusipang. They are reared as long as they produce standard quality semen.

According to Sethi et al. (1989), bulls donating larger volume of neat semen with higher MA and SC produce more freezable semen. Further, the average age at first semen collection can be reduced, without affecting the semen quality and freezability, by starting training of bulls at an early age. Besides, the availability of semen at the earliest possible age from breeding bulls is not only economical but may increase productive life span and prove the bulls under progeny testing program (Dahiya and Singh 2013).

The semen collection and processing at the centre is done from March to November, when the ambient temperatures is in the range of 5-15 degrees Celsius which is optimal for semen production (Waltl et al. 2006). The production is suspended during the cold months (December to February) owing to very less mounting during the period, which could be affected by low temperatures. However, cold temperatures are harmless unless actual freezing of tissue occurs (Foote 1978).

Studies elsewhere have revealed that the environmental factors influence the fertility of bulls, which is determined by its semen quality, beside genetic and management factors. Season affected the fresh semen characteristics in *Bos taurus* breed (Snoj et al. 2013). Similarly, the performance in terms of semen quality production was observed better in winter than in summer **(**Mathevona et al. 1998). Conditions imposed at the time of semen collection such as frequency of collection and degree of sexual preparation may influence the quality of semen harvested (Foote 1978).

The bovine frozen semen production in Bhutan has been established for almost three decades but no scientific studies have been conducted so far on the factors influencing semen quality under Bhutanese environment. Therefore, this study was undertaken to assess the effect of non-genetic factors on quality of bovine semen production. Besides, the outcomes from this study will guide in production of higher semen recovery rate with better semen quality for higher conception rate and control wastage of resources during semen processing. Further, the study was also aimed to determine an ideal age of young bulls for procurement and use for frozen semen production.

1. MATERIALS AND METHODS

1.1 Study area

The study was conducted at NDRDC Yusipang under the Department of Livestock from September to Dec. 2020. The centre is located at cool-temperate zone in Bhutan at 2,738 masl at latitude 27º 27’52” N and longitude 89º42’22" E. The area experiences four seasons in a year; Spring, Summer, Autumn and Winter.

1.2 Animals, semen collection and evaluation

The study included information from 19 pedigree selected bulls of different breeds; Jersey (11nos), Mithun (5nos) and Nublang (3nos), procured for frozen semen production at the centre. All bulls were managed under the same environment and the young bulls were trained regularly (twice a week) for mounting and semen collection.

Semen was collected once a week per bull but in the event if the scheduled bull for collection did not donate semen, then other bull in queue was used. Semen was collected using artificial vagina between 8.00-8.30 am in the morning. Immediately after collection, semen was assessed for appearance, color and volume using graduated collection tube. The semen was also assessed for MA and ISM. The semen with MA and ISM of acceptable quality was used for further processing and freezing. A light microscope equipped with phase contrast optics was used at 40x magnification to determine MA (0 = no MA, 1 = slow waves, 2 = quick waves, 3 = very quick waves) and ISM at 400x magnification on the percentage of individual spermatozoa depicting a pattern of progressive/ rectilinear movement. Sperm concentration was evaluated using a Spectrophotometer. The total sperm count per ejaculate was calculated by multiplying spermatozoa per ml by volume of ejaculate and expressed in millions. Sperm motility of processed semen before-cooling/freezing and post-thawing was assessed under a light microscope at 400x magnification. Following equations were adopted to calculate:

*Total motile sperm count = Vol. **x** SC **x** sperm motility*

*Frozen semen dose = (Vol. **x** SC)/ 20x10^6*

All semen production procedures followed were as per Bhutan standards for bovine frozen semen production (BAFRA 2020), which requires minimum MA of 2 (scale of 0-3) and ISM of 60% to be considered as fit for processing. Ax et al. (2000) noted that the minimum standard for bull semen should have the sperm concentration of at least 500x10^6/ml. The ejaculates (fresh semen) with Vol., MA, ISM and SC of 1 ml, 1, <60% and <500 x10^6/ml respectively were considered as "low level' quality and discarded in any collection, and other ejaculates of higher levels were processed. The processed (frozen) semen which had sperm PTM below 40% was discarded following the Bhutan standards for bovine frozen semen production.

1.3 Data collection

The study included data maintained by the centre from Mar. 2015 – Nov. 2019 on semen collection and production (Table 1). The information included date of production, bull no., age, breed, volume donated per collection, MA, ISM, SC, SSP, SSS, SSD, PTM-P and PTM-C.

For analysis, semen parameters were grouped as <2ml, 2-2.9ml and ≥3ml for Vol., 0, 1, 2 and 3 for MA (scale of 0-3), <60%, 60-69% and ≥70% for ISM and <500, 500-699 and ≥700 x 10^6/ml for SC. Further, the ejaculates with Vol., MA, ISM and SC of <2ml, 1, <60% and <500x10^6/ml respectively were categorized as "low level" quality, 2-2.9ml, 2, 60-69% and 500-699x10^6/ml respectively were categorized as "medium level" quality, and ≥3ml, 3, ≥70% and ≥700x10^6/ml respectively were categorized as "high level" quality. Similarly, for uniform distribution of bulls, they were grouped into four categories as <12 months, 13-15 months, 16-18 months and >18 months for age at procurement, ≤48 months, 49-72 months, 73-96 months and >96 months for age at semen collection, and <5 days, 6-10 days, 11-15 days and >15 days for frequency of collection to assess various effects. Thus, semen collected and processed was evaluated on Vol., MA, ISM and SC as fresh semen quality and SSP, PTM-P, PTM-C, SSS and SSD as processed semen quality.

1.4 Statistical analysis

Data were compiled and computed in Microsoft Excel spread sheet. The data on 472 semen ejaculates collected, processed and discarded were analyzed descriptively. Statistical analysis was performed using Statistical Package for Social Science (SPSS version-21). Person's Chi Square test was applied to assess association of age at procurement with mounting and semen production. The relation between fresh and processed semen qualities was assessed using Pearson's co-relation. The effect of factors on semen quality was evaluated using analysis of variance (ANOVA) and data were expressed as a mean ± standard error (SE). The significance level for effect of factors was set at $p<0.05$.

2. RESULTS AND DISCUSSIONS

The overall mean semen Vol., MA, ISM, SC, SSP, SSD, SSS and PTM-P and PTM-C were 6.8ml, 2.8, 75%, 1268x10^6/ml, 360 straws, 84 straws, 280 straws, 39% and 42% respectively. In general, the semen produced had fulfilled the Bhutan standards for bovine frozen semen production (BAFRA 2020), which requires minimum PTM of 40% to consider as fit for storage and distribution for AI. According to the Indonesian National Standard [INS] (2017), the bovine semen used for AI should have ISM and PTM of at least 70% and 40%, respectively. The semen recovery rate based on semen certified fit for storage and distribution for AI was 78%.

3.1 Fresh and Processed semen quality

Table 2 presents the number of ejaculates collected, processed and discarded based on

Table 1: Semen collection and production during the study period

Parameters		Breed			Total
		Jersey	Mithun	Nublang	
Collection attempts (no)		373	35	64	472
Mounting & collection (no)	Yes	302	16	52	370
	No	71	19	12	102
SSP		98,508	4,866	19,766	123,140
SSD (<40% PTM)		26,432	890	1,130	28,452
SSS (>40% PTM)		72,076	3,976	18,636	94,688

its quality. Overall, the ejaculates discarded of "low level" in Vol., MA, ISM and SC comprised of 2.2%, 2.7%, 4.3% and 3.8% respectively in total ejaculates collected (n=370). The ejaculates of "medium level" were comparatively more in numbers than those of low level, but negligible when compared to ejaculates in high level. Similarly, the ejaculates of "high level" were much more in numbers that qualified for processing than those of medium level. Any ejaculate collected with one or more parameters of low level was discarded based on Bhutan standards for bovine frozen semen production (BAFRA 2020). Overall, bulls donated more semen of high quality. The ejaculates of medium level in Vol., MA, ISM, and SC after processing resulted in very less numbers of ejaculates worth cryopreservation with 17% (n=6), 42% (n=65), 12% (n=16) and 13% (n=15) respectively. The ejaculates of high level in Vol., MA, ISM, and SC after processing resulted in more numbers of ejaculates worth cryopreservation with 75% (n=337), 82% (n=273), 77% (n=322) and 73 % (312) respectively (Table 2).

Similarly, the combinations of different levels; among medium level, high level and combined medium and high levels in different combinations, resulted in wide differences in number of ejaculates worth cryopreservation than at individual level (Table 2). The combination of all quality traits in medium level resulted in very few numbers of ejaculates worth cryopreservation similar to findings of individual medium level, whereas the combination all quality traits in high level resulted in maximum number of ejaculates worth cryopreservation, and slightly better than the findings of individual high level. The mixed medium and high levels in different combinations resulted in slightly higher number of ejaculates worth cryopreservation than the combination of medium levels but markedly lower than the combination of high levels.

Therefore, in terms of minimizing the waste of resources while processing semen, it is worth processing the ejaculates of high-level semen qualities to ensure more better-quality straws for cryopreservation. However, considering the combination of mixed model (Table 2), not much differences were observed in number of ejaculates cryo-preserved and discarded after processing for MA in medium level with ISM and SC in high level (A1xM2,

Table 2: No. of ejaculates collected, discarded, processed and cryo-preserved

Semen parameters		Ejaculates Collected	Discarded as fresh	Processed	Discarded after freezing	Cryo-Preserved
Low level	Vol. <2 ml	8	8	0	0	0
	MA = 1 degree	10	10	0	0	0
	ISM <60%	16	16	0	0	0
	SC <500x10^6/ml	14	14	0	0	0
Medium level	Vol. = 2-9 ml	11	5	6	5	1
	MA = 2 (A1)	87	22	65	38	27
	ISM =60-69% (M1)	26	10	16	14	2
	SC = 500-699x10^6/ml (C1)	23	8	15	13	2
High level	Vol. ≥3 ml	351	14	337	83	254
	MA = 3 (A2)	273	0	273	50	223
	ISM ≥70% (M2)	328	6	322	74	248
	SC ≥700x10^6/ml (C2)	333	21	312	84	228
Combination of medium levels*	A1xM1	10	0	10	8	2
	A1xC1	17	8	9	1	8
	A1xM1xC1	10	4	6	6	0
Combination of high levels	A2xM2	271	0	271	48	223
	A2xC2, M2xC2 & A2xM2xC2	266	0	266	45	221
Mixed medium & high levels**	A1xM2	51	6	45	26	19
	A1xC2	63	3	60	37	23
	M1xC2	10	0	10	8	2
	A1xM2xC2	52	3	49	25	24
	A2xM1 / A2xC1 & A2xM1xC1	2 / 1	0 / 0	2 / 1	2 / 1	0 / 0
	A2xM2xC1 / A1xM2xC1	5 / 3	0 / 0	5 / 3	3 / 2	2 / 1

**M1xC1 and **M2 x C1 had zero frequency*

A1xC2, A1xM2xC2). Hence, ejaculate with MA, ISM and SC of 2, ≥70% and ≥700x10^6/ml respectively can be considered for processing but any ejaculate with ISM and SC of <70% and <700x10^6/ml should be discarded.

Figure 1 presents the quality of frozen semen produced with medium and high-level semen qualities. The ejaculates of high level in Vol., MA, ISM and SC yielded higher semen straws worth cryo-preservation of 77%, 83% 79% and 78% respectively with PTM of 42-46%, whereas the ejaculates of medium level in Vol., MA, ISM and SC yielded semen straws worth cryo-preservation of 73%, 45%, 16% and 34% respectively with PTM of 40-42% only. Thus, the frozen semen straw production both in quantity and quality was comparatively much better for high level than medium level semen qualities, particularly in terms of ISM and SC. The semen with higher PTM is directly proportional to higher fertility rates on AI. Therefore, processing of frozen semen is worth focusing on ejaculates with high level for higher semen recovery rate with better semen quality and conception rates on AI.

Further, the study found strong correlation between the fresh and processed semen qualities (Table 3). All fresh semen parameters such as Vol., MA, ISM and SC had direct influence on the SSP and SSS,

Table 3: Pearson Correlations between fresh and processed semen qualities

Parameters	Test level	SSP	SSS	SSD	PTM-P	PTM-C
Vol.	Pearson Correlation	0.563**	0.429**	-0.014	0.183**	0.007
	Sig. (2-tailed)	0.000	0.000	0.797	0.001	0.912
	N	342	338	337	338	275
MA	Pearson Correlation	0.291**	0.350**	-0.224**	0.352**	0.092
	Sig. (2-tailed)	0.000	0.000	0.000	0.000	0.126
	N	342	338	337	338	275
ISM	Pearson Correlation	0.276**	0.326**	-0.201**	0.357**	0.103
	Sig. (2-tailed)	0.000	0.000	0.000	0.000	0.089
	N	342	338	337	338	275
SC	Pearson Correlation	0.176**	0.159**	-0.050	0.066	0.006
	Sig. (2-tailed)	0.001	0.003	0.362	0.227	0.922
	N	342	338	337	338	275

**. Correlation is significant at the 0.01 level.

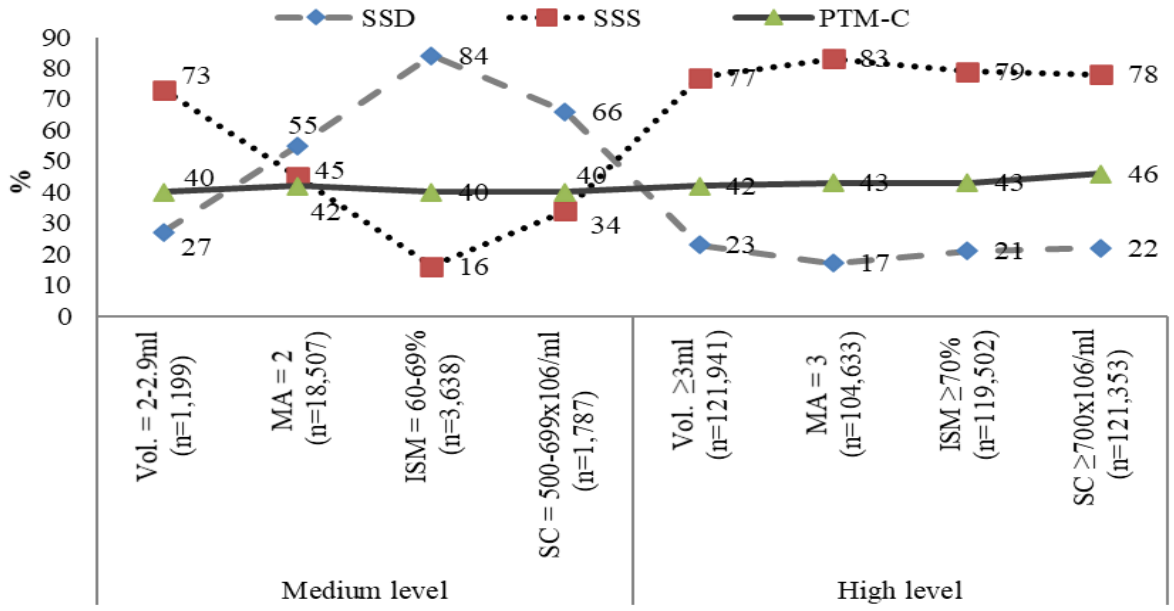

Figure 1: Percent of semen stored, discarded and quality with different levels (n=straw produced)

whereas inverse influence on SSD. The findings in this study are in complete agreement with the observation of Sethi et al. (1989) who reported that bulls donating larger Vol. of neat semen with higher MA are supposed to produce more freezable semen. Hence, it is highly recommended to consider the high level of fresh semen qualities at the time of processing for higher semen recovery rate with better quality as well as minimize resources.

3.2 Age at procurement and semen production

The overall mean age at procurement was 15 months, and by breed it was 14 months for Jersey, 13 months for Mithun and 25 months for Nublang (Table 4).

The bulls procured after 18 months of age (n=4) had posed with lot of difficulties in training them to mount on dummy, and bulls procured after 27 months of age (n=2) mounted rarely and did not donate semen that qualified for processing and freezing. In the study, no significant association (Chi-Square test) was observed between the age at procurement and mounting (X^2 (3) =2.8; p=0.434), but age at procurement and semen production were strongly related (X^2 (348) =393.9; p=0.045). Similarly, no significant effect of age at procurement was observed on mounting (p=0.436), but SSP was significantly lower (p<0.046) for bulls procured after 18 months of age. Therefore, appropriate age for young bull procurement would be before 15 months of age for ease of training and more semen straw production in lifetime. This finding is in concurrence with the recommendation of Sethi et al. (1989) that the average age at first semen collection can be reduced, without affecting the semen quality and freezability, by starting training of bulls at an early age. Further, the availability of semen at the earliest possible age from breeding bulls is not only economical but may increase productive life span and prove the bulls under progeny testing program (Dahiya and Singh 2013).

3.3 Age at collection and semen quality

The result indicated that mean age duration of semen collection in bull irrespective of breeds was found to be 68 months; 58 months for Jersey, 72 months for Mithun and 123 months for Nublang respectively (Table 4). At the same time, the mean age at first semen collection in bull irrespective of breeds was accounted at 33 months; 25 for Jersey, 47 for Mithun and 36 for Nublang respectively. Table 5 shows the

Table 4: Age at procurement and semen production

Breed	Age at proc. (month)			Age duration of collection* (month)			Age at 1st collection (month)		
	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.
Jersey	14	10	36	58	29	113	25**	19	42
Mithun	13	9	16	72	31	104	47***	31	63
Nublang	25	17	36	123	22	150	36***	22	50

** Inclusive of all bulls in the study, **4 bulls, *** 2 bulls*

Table 5: Effect of age at collection (month) on semen quality (Mean ± SE)

Parameters	≤ 48[a] (n)	49-72[b] (n)	73-96 [c](n)	> 96[d] (n)	*p* value
Vol. (ml)	6.6 ± 0.4 (80)	6.6 ± 0.2 (188)	7.4 ± 0.4 (51)	7.1 ± 0.4 (51)	0.231
MA	2.7 ± 0.1[d] (77)	2.7 ± 0.0[d] (183)	2.8 ± 0.1 (49)	2.9 ±0.0[a,b] (51)	0.003
ISM (%)	74 ± 0.9[d] (77)	74 ± 0.6[d] (183)	76 ± 0.8 (49)	79 ± 0.9[a,b] (51)	0.001
SC (..x10^6/ml)	1122 ± 32 (73)	1313 ± 92 (173)	1162 ± 55 (49)	1422 ± 68 (50)	0.213
SSP (no)	322 ± 12[c, d] (73)	348 ± 12[d] (170)	398 ± 30[a] (49)	417 ± 24[a,b] (50)	0.002
SSD (<40% PTM)	64 ± 15 (73)	103 ± 13[d] (167)	100 ± 28 (48)	23 ± 14[b] (49)	0.009
SSS (>40% PTM)	258 ± 20[d] (73)	252 ± 17[d] (167)	306 ± 38 (48)	394 ± 27[a,b] (50)	0.000
PTM-P (%)	39 ± 1[d] (73)	39 ± 0.5[d] (167)	40 ± 0.9 (47)	43 ± 0.9[a,b] (50)	0.005
PTM-C (%)	43 ± 0.7 (57)	40 ± 0.6[d] (131)	41 ± 0.9[d] (39)	44 ± 0.8[b,c] (47)	0.002

[a,b,c,d] *Different superscript within a row show a difference (p<0.05)*

effect of age at semen collection on semen quality. The age at collection had significant effect on MA (p=0.003), ISM (p=0.001), SSP (p=0.002), SSD (p=0.009), SSS (p=0.000), PTM-P (p=0.005) and PTM-C (0.002) but no effect was observed on Vol. and SC. Among the significant effect of age at collection on the semen quality parameters, the aged bulls had better semen quality than the younger bulls. The bulls above 96 months in production produced better quality semen than those below 72 months of age, and in addition bulls above 72 months produced significantly higher doses of semen straws than those up to 72 months. No significant difference in semen quality was observed between the higher age group bulls of 73-96 months and >96 months in production.

Therefore, if estimated doses of semen are not produced at younger age, the bulls can be reared for long years until it donates estimated doses of quality semen. Generally, aged group of bulls had produced better quality semen than the younger ones. Nevertheless, according to Dahiya and Singh (2013), the availability of semen at the earliest possible age from breeding bulls is not only economical but increase productive life span and allow proving the bulls under progeny testing program**.** Overall, the study found that semen quality was much better with the bulls of higher age groups, indicating improvement of fertility with age. The findings are coherent with the observations of Mathevona et al. (1998a and 1988b) who reported that semen characteristics generally improved significantly with age of bulls. Further, the findings are in complete agreement the with the observation of Mathevona et al. (1988b) who reported that SC remained relatively constant with age, but in partial agreement with Vilela & Smith (2018) who reported that age has moderate effect on semen quality parameters. However, some findings in this study deviated from observation in other studies wherein the highest fertility of bull has been observed at around 2-4 years of age and started declining once bull attained more than 4 years of age (Thomas 2009). Age of bull did not affect ISM, and ejaculate volume increased with age of bull while sperm concentration was lower in higher age classes (Waltl et al. 2006). All semen traits like Vol., SC were significantly affected by age groups that increased with the increasing age of bull up to 5 years and then decreased (Bhakat et al. 2011).

3.4 Breed and Semen quality

The study found significant effect of breed on semen quality; MA (p=0.032), ISM (p=0.013), SSD (p=0.013), SSS (p=0.002), PTM-P (p=0.006) and PTM-C (p=0.002), but no effect was observed on Vol., SC, and SSP (Table 6). With the significant effect of breed in the above parameters between Jersey and Nublang and not with Mithun, the study concluded that Jersey, Mithun and Nublang demonstrated semen of lower, moderate and higher quality respectively. The significant effect of breed on majority of semen quality parameters observed in this study is in agreement with Vilela & Smith (2018) who reported that the effect on semen quality parameters was highly significant for breed.

The better semen quality of Nublang could be attributed to semen donation at higher age than Jersey and Mithun, which is in agreement with Mukhopadhyay et al. (2010) who reported that the age of starting semen donation varies according to breed and age at first semen collection is lower in crossbred than indigenous cattle breed.

3.5 Season and Semen quality

The effect of season on semen quality is illustrated in Table 7. The season had significant effect on Vol. (p=0.000), MA

Table 6: Effect of breed on semen quality (Mean ± SE)

Parameters	JP[a] (n)	Mithun[b] (n)	Nublang[c] (n)	*P* value
Vol. (ml)	6.8 ± 0.2 (302)	5.5 ± 0.5 (16)	6.8 ± 0.4 (52)	0.185
MA	2.7 ± 0.0[c] (293)	2.9 ± 0.1 (15)	2.9 ± 0.1[a] (52)	0.032
ISM (%)	74 ± 0.4[c] (293)	77 ± 1.9 (15)	78 ± 1.1[a] (52)	0.013
SC (..x10^6/ml)	1239 ± 58.2 (281)	1218 ± 51.2(15)	1441 ± 69.7 (49)	0.347
SSP (no)	354 ± 9.5 (278)	324 ± 28.5 (15)	403 ± 24.3 (49)	0.093
SSD (<40% PTM)	94 ± 10.0[c] (274)	59 ± 32.6 (15)	24 ± 14.1[a] (48)	0.013
SSS (>40% PTM)	266 ± 13.0[c] (274)	265 ± 44.7 (15)	380 ± 27.6[a] (49)	0.002
PTM-P (%)	39 ± 0.4[c] (273)	40 ± 1.9 (15)	42 ± 0.9[a] (49)	0.006
PTM-C (%)	41 ± 0.5[b, c] (216)	45 ± 2.0[a] (12)	44 ± 0.8[a] (46)	0.002

[a,b,c] *Different superscript within a row show a difference ($p<0.05$)*

Table 7: Effect of season on semen quality (Mean ± SE)

Parameters	Spring[a] (n)	Summer[b] (n)	Autumn[c] (n)	*p* value
Vol. (ml)	5.5 ± 0.2 [b,c] (113)	7.4 ± 0.2[a] (191)	7.3 ± 0.3[a] (66)	0.000
MA	2.7 ± 0.1[b] (106)	2.8 ± 0.0[a] (188)	2.7 ± 0.1 (66)	0.033
ISM (%)	73.5 ± 0.8[b] (106)	76 ± 0.5[a] (188)	74.7 ± 0.8 (66)	0.028
SC (..x10^6/ml)	1260 ± 85 (103)	1251 ± 77 (119)	1325 ± 58 (63)	0.853
SSP (no)	330 ± 16[c] (102)	361 ± 12 (177)	406 ± 20[a] (63)	0.011
SSD (<40% PTM)	81 ± 14 (101)	71 ± 11 (174)	116 ± 26 (62)	0.164
SSS (>40% PTM)	252 ± 22 (101)	294 ± 16 (175)	297 ± 29 (62)	0.235
PTM-P (%)	38 ± 0.8 (101)	40 ± 0.5 (174)	40 ± 0.8 (62)	0.198
PTM-C (%)	42 ± 0.7 (76)	41 ± 0.6 (145)	42 ± 1.0 (53)	0.992

[a,b,c] Different superscript within a row show a difference (p<0.05)

Table 8: Effect of semen collection frequency on semen quality (Mean ± SE)

Parameters	< 6 days[a] (n)	6-10 days[b] (n)	11-15 days[c] (n)	> 15 days[d] (n)	*p* value
Vol. (ml)	6.4 ± 0.3 (61)	6.9 ± 0.2 (170)	7.3 ± 0.4 (50)	6.6 ± 0.3 (89)	0.240
MA	2.7 ± 0.1 (61)	2.8 ± 0 (166)	2.8 ± 0.1 (49)	2.7 ± 0.1 (84)	0.116
ISM (%)	74.8 ± 0.8 (61)	76 ± 0.6 (166)	74.3 ± 1.1 (49)	73.7 ± 0.9 (84)	0.136
SC (..x10^6/ml)	1127 ± 39 (59)	1305 ± 86 (160)	1500 ± 190 (45)	1166 ± 39 (81)	0.125
SSP (no)	317 ± 19[c] (57)	356 ± 11 (160)	402 ± 26[a] (45)	375 ± 26 (80)	0.043
SSD (<40% PTM)	73 ± 18 (56)	87 ± 13 (158)	50 ± 19 (43)	106 ± 20 (80)	0.282
SSS (>40% PTM)	251 ± 25[c] (56)	273 ± 16 (158)	362 ± 33[a] (44)	269 ± 8 (80)	0.046
PTM-P (%)	39 ± 1 (56)	39 ± 1 (158)	40 ± 1 (44)	39 ± 1 (80)	0.777
PTM-C (%)	43 ± 1 (43)	41 ± 1 (134)	42 ± 1 (39)	42 ± 1 (59)	0.208

[a,b,c,d] Different superscript within a row show a difference (p<0.05)

(*p*=0.033), ISM (*p*=0.028) and SSP (*p*=0.011). The volume of semen produced in Summer and Autumn was significantly higher than Spring. The MA and ISM of semen produced in Summer was significantly higher than Spring but did not differ with Autumn. The semen straw produced in Autumn was significantly higher than Spring but did not differ significantly with Summer. Therefore, semen produced in Summer and Autumn was better than Spring season. Other parameters of semen quality such as SC, PTM, SSD and SSS were not affected by season. The null effect of season on SC in this study deviates from the findings of Rehatin et al. (2016) and Koivisito et al. (2009) who reported that SC was highly influenced by seasons.

Overall, the effect of season on semen quality parameters was inconsistent in this study as only volume, MA, ISM and SSP were affected, which is in partial agreement with other findings (Vilela & Smith 2018; Bhakat et al. 2011; Koivisito et al. 2009; Mathevona et al. 1998a), who observed that the effect on semen quality parameters is highly dependent on season.

3.6 Semen collection interval and semen quality

The effect of collection interval on semen quality was observed only on SSP and SSD (Table 8). The SSP was significantly higher (*p*=0.043) for collection interval of 11-15 days than weekly collection interval, but not with other collection intervals. Correspondingly, the SSS was significantly higher (0.046) for collection interval of 11-15 days than weekly collection interval. So, weekly semen collection interval for a particular bull should be avoided. However, the findings in this study deviates away from the observations in other studies. Mathevona et al. (1998a and 1998b) reported that the highest numbers of motile spermatozoa per ejaculate were obtained with collection intervals of atleast 4 to 5 days and 5 to 9 days between collections respectively. Similarly, Waltl et al. (2006) reported that with increasing collection interval, ejaculate Vol. and SC increased significantly, and SC and ISM were superior for collection intervals between 4-9 days and 1-6 days respectively, and Rehatin et al. (2016) reported that the ejaculation frequency affected to all semen variables.

3. CONCLUSION & RECOMMENDATION

Overall, the frozen semen production under Bhutanese environment had fulfilled our standards required for bovine from semen production. The semen recovery rate was 78%. The best output in semen production in terms of quantity produced, recovery rate and quality were found for ejaculates having Vol., MA, ISM and SC of ≥3ml, 3, ≥70% and ≥700x10^6/ml respectively. The ejaculates processed with MA, ISM and SC of 2, 60-69% and 600-699 x10^6/ml respectively has resulted in discard of 55%, 84% and 66% of semen processed respectively, which was evident from the strong correlation observed between fresh and processed semen qualities. Therefore, only ejaculates having Vol., MA, ISM and SC of minimum 2ml, 2, ≥70% and ≥700x10^6/ml respectively should be considered for processing for optimum semen recovery rate with better semen quality that ensures higher conception rate on AI. Any ejaculate with lesser than the indicated level should be discarded in view of minimizing the waste of resources through discard of processed semen based on low PTM. All factors studied have significant effect on one or other semen quality parameters except on sperm concentration. The age at procurement showed significant effect on SSP, with lesser production for bulls procured after 18 months of age coupled by difficulties in training them. Therefore, young bull procurement intended for semen production should be done before the age of 15 months. The semen quality parameters such as MA, ISM, SSP, SSD, SSS, PTM-C and PTM-C were significantly affected by the age at collection, which improved with the age of bulls. Hence, bulls that continue to donate quality semen can be reared for longer duration. Similarly, breed had significant effect on majority of semen quality parameters; MA, ISM, SSD, SSS, PTM-P and PTM-C, indicating best qualities for Nublang, followed by Mithun and then Jersey, with significant difference between Nublang and Jersey. Season had inconsistent effect on semen quality. However, with significantly better results obtained in Autumn and Summer when compared to Spring, greater emphasis may be given on more semen collection in Autumn and Summer. The semen collection interval also affected the semen quality. Based on significantly higher results in terms of SSP and SSS for collection interval of 11-15 days, the weekly collection of semen from same bull should be avoided.

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