Assess the quality of Murrah Buffalo (Bubalus bubalis) Bull Semen regarding seasonal and geographical variability in Nepal

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ABSTRACT

We carried out this study to assess the quality of semen regarding the seasonal and geographical variability of Murrah buffalo breeding bull in Nepal. We aimed to access the quality of seme n of murrah buffalo bull considering the seasonal and geographical variability to help standardize quality semen producing protocol and concern. A total of eight Murrah buffalo bulls were used. Semen collection was done at once week and two ejaculations were considered at an interval of 30 minutes during the Summer (June to August), Autumn (September to November), Winter (December to February), and Spring (March to May) from 2017 to 2020 AD. Immediately after collection, initial motility, sperm concentration, pre-filling motility, post-thaw motility, live sperm, and abnormality of sperms were evaluated. Findings revealed that the season had affect ($p<0.005$) to the semen volume, individual motility, and post-thawing motility except for semen volume and sperm abnormality($p>0.05$). However, initial motility, sperm concentration, and pre-filling motility did not vary in months ($p>0.05$). It is thus well reflected that several traits related to quality and quantity of semen Murrah buffalo bulls may vary depending on the season of collection, location and age ($p<0.05$) with the semen traits of initial motility, sperm concentration, and pre-filling motility. Accordingly the semen collected during the autumn and spring season is revealed the percentage of average post thaw motility above the 52% that considered the success of artificial insemination is good for buffaloes.

Keywords: *Murrah buffalo, location, Season, Semen quality, months*

INTRODUCTION

Buffalo is called the 'Black Gold', and farmers rearing all parts of Nepal with varying population densities, but the majority (70%) of the milking buffaloes are found in Terai

and Mid-hill. The higher progress rate of buffalo in the Terai and mid-hill because of the increasing demand for buffalo milk, meat, and superior buffalo germplasm. It offers a daily source of income and is well recognized as a means for poverty reduction and food security. Milk and milk product demand is increasing day by day in Nepal. There is a need for high-yielding breeds of dairy buffaloes to meet the increased demand for milk. Genetic improvement is one of the prioritized activities of the Nepal government to improve the productivity of the buffalo herd (S.et al.,2018). Therefore, an enormous scope is to upgrade the nondescript buffaloes using superior germplasm to improve productivity. Artificial insemination has played an important role in the improvement in productivity through the use of cryopreserving semen of superior bulls. The importance of artificial insemination in buffaloes increasing at a faster rate, as a result, the demand for buffalo bull semen by the rural inseminator is as well high. So far the maximum number of females been served with the quality of frozen semen of superior genetic make-up (Singh and Pant, 2000).

Bull could produce superior quality semen with a high breeding value for the milk-producing ability. Quality semen production is vital for assessing the bull's fertility to enhance the artificial insemination programs (Lemma & Shemsu, 2015). However, semen traits are often influenced by environmental effects than genetic effects. There are very limited studies for the scientific research carried out so far including appropriate records of the semen on different buffalo bull breeds (Bhakat et al. 2015, Ramajayan 2016, Pathak et al. 2018) in Nepal.

Buffaloes are the main source of milk production in Nepal producing about 65.3% of the total annual milk. Daily milk production is more in Murrah buffaloes. On an average Murrah Buffaloes give about 8-16 liters per day. The other big advantages these animals are, they are more disease resistant when compared to cross-bred cows. They can thrive on any crop residue in absence of concentrates during drought. The demand for milk and meat is large, and these animals are also important for religious purposes.

Semen quality parameters are important indicators of bull fertility, whereas, poor quality semen results in female impregnation failure, affecting the production and reproductive life of buffaloes. Due to natural limitations, the breeding potential may not be reached at the maximum level also due to the fact that the area behind the size of the testes is relatively smaller, the daily rate of sperm production is low, and the epididymal sperm reserve is smaller in the buffalo bull (Suryaprakasa,1993; Sudheer, Xavier 2000).

Semen quality in bulls reflects the normality of the function of their testicles, epididymal ducts, and genital tract. The reproductive system depends on the hormonal balance of the father, which is sensitive to changes in health, nutrition, and management. Variations in these situations affect sperm yield, accessory gonadal secretion, and epididymal function, all of which are reflected in ejaculate volume and sperm characteristics (Seri et al.,2007). Semen volume, sperm concentration, sperm motility, mass activity, sperm morphology, and semen pH are common criteria for evaluating sperm quality (Den Daas, 1992). Breed of the reproductive health status of bulls, technical, skills, and age (Mandal et al. 2010)

influence the quality of semen and genetic constitution of bulls (Koivisto et al. 2009) for a long time not to be reliable because it is not under one hand of management. (Seri et al., 2007).

Artificial insemination is an important tool to improve production and productivity in buffalo by breeding animals with high genetic potential (Baruselli and Carvalho 2005). Different seasons significantly affect the quality of sperm production (Snoj et al. 2013, Bhakat et al. 2015). Research findings revealed the fact that post-thaw plasma membrane integrity, stability, and DNA fragmentation index (DFI) could significantly better in ejaculates processed during winter than in other seasons (Koonjaenak et al. 2007). In bulls, differences in sperm quality have been attributed to seasonal variation in the expression of low-density lipid receptors on the sperm surface that influence the utilization of seminal plasma components between seasons (Argov et al. 2007).

However, little information is available about the scientific facts on the study of sperm quality of Murrah buffalo bull in the Nepalese context with sperm production performance. Under such contxt quality of sperm is largely very important to consider as it could be well influenced by several environmental factors. The main objective of the research was to evaluate semen quality traits and parameters in terms of seasonal and geographic variability in frozen semen of Murrah buffalo breeding bulls.

MATERIALS AND METHODS

Study area

This research was done at the National Livestock Breeding Office Pokhara, which was located at 28°18′19.08″ North and 84°04′37.20″ East. According to the rainfall data, we divided the climatic condition into the summer (June to August), autumn (September to November), Winter (December to February) and Spring (March to May).

Animals

8 Murrah buffalo bulls were used included in this study in the fiscal year 2017/2020. Five to seven years old buffalo bulls having an average body weight of 450 to 550 kg were used to collect semen.

Semen collection

Buffalo bulls were trained for semen collection through an Articial Vagina (AV) set before starting the experiment. Semen was collected twice/week regularly in the early morning (6-6.30 AM) with an AV set (Walton, 1945) and consedering two ejaculations at an interval of 30 minutes at each collection.

The AV comprises of outer rubber cylinder, inner rubber line, rubber band, cone and collecting tube. Semen was collected with the help of a teaser animal. All the apparatus

used for semen collection was sterilized in autoclave machine before collection of semen. The inner liner temperature of AV was conserved at 42-43^{0}C water packing at 52-54^{0}C two thirds of its volume.The rest of one-third area of water jacket was filled with air. Some non spermicidal gel was applied into the inner side of artificial vagina before semen collection. The semn collected tube was labeled with aluminium foil and kept in water bath at 37^{0}C for five minutes. Collected semen from each bull was transferred to the laboratory for evaluation and further processing.

Motility, morphology and concentration were measured using a Computer Assisted Sperm Analyzer (CASA) (Miraz et al. 2022). The CASA settings for analyzing sperm motility were set as; Frame rate 60Hz, Frames acquired 30, Minimum contrast 35, Minimum cell size, 5 pixels, Cell size, 9 pixels, Cell intensity 110 pixels, Path velocity (VAP) 50 μm/s, Straightness (STR) 70%, VAP cut-off, 30 μ/s and VSL cut-off 15 μ/s. Prepared semen (1 μl) sample was loaded on a prewarmed Leja® slide and analyzed for sperm motility.

Seasonally available green fodder (maize, teosinte in the summer rainy season and oats during the winter) were fed to each bull at the rate of 25-30 kg green herbage (8 to 10 kg DM on an average per day bull along with adlibitum feeding of paddy straw. Besides this, a concentrate of 3 to 4 kg/bull/day (CP 16%, energy 4000 kilo calorie fortified with mineral and vitamin mixture) planned in Pokhara Livestock farm was given along with an ad-libitum supply of fresh water.

Semen dilution and equilibration

The egg yolk-tris-fructose-citric acid-glycerol was used as an extender. Briefly, a stock solution was prepared by dissolving tris (24.20 gms), fructose (10.00 gms), citric acid (13.60 gms), penicillin G-sodium (1000 I.U/ml), and streptomycin sulfate (1 mg/ml) in glass distilled water. The fresh yolk was added with the buffer at a concentration of 20% (v/v). We divided the volume the of extender into two equal parts. Thereafter, glycerol was added to one part of the extender at times of the desired concentration (12.8%). The other part of the diluents was used to make the initial dilution of semen that contained two time the desired concentration of spermatozoa. The initial dilution of semen was made at +37^{0}C. Three equal parts of initially diluted semen and double concentration glycerol containing extender were mixed at four steps during a 2 to 3 hours cooling process, the dilution steps were at +18, +12, +8, and +4^{0}AC Finally, the diluted semen contained 6.4% (v/v) glycerol and 20x10 motile spermatozoa per individual insemination doses were sucked in 0.25 ml French straws and the open ends of the straws were sealed by using an automatic filling, sealing and printing of straw by IS-4 (IMV Company, France), and quattro automatic filling, sealing and printing machine (Minitube Company, Germany) . After loading, the straws were left for equilibration at +40c for 4 hours. We did the cooling and equilibration operation in a cold handling cabinet. After equilibration, the freezing operation was conducted in a deep semen freezer to cool the semen from +4 to -1400c, and then the straws were directly plunged into liquid nitrogen (-1960C). Only semen that showed equal to or 50% post-thaw motility was preserved for artificial insemination.

Sealing, filling and printing of straw

Filling, sealing and printing of semen straws was done with the help of IS-4 (IMV Company, France), minitube automated machine (Minitube Company, Germany. This machine were used for this purpose. 0.25 ml of semen was fillied in each straw. Bull number , breed, date of semen collection, batch number, address and date of prepared straw.

Cooling of straw

After printing, straws are arranged on freezing racks using ramp meant for Mini straws. These racks are kept in Cold Handling Cabinet at 4-6º C for 4 hours for equilibration. The cabinet should be maintained at the prescribed temperature during this period so that sperms are not exposed to temperature fluctuation.

Freezing of straw

Then racks are transferred into chamber of freezing unit (Bio-freezer for bovine semen) controlled by a computerized programmer. Pre-decided freezing protocol on the programmer takes care of freezing of semen doses. Freezing completes at -140 ºC. Frozen semen doses are now taken from freezing unit and plunged into liquid nitrogen (LN) for cryo-preservation. It is uses the integration of SMILE software for data recording and the CASA system for semen evaluation. We used different quality parameters for the evaluation of the semen as mentioned in (Table 1).

Table 1: Quality parameters for the evaluation of bull semen

Parameters	Descriptions
Semen volume	Volume of Semen in ml ejaculated by bull/ejaculation
Initial motility of sperms	Initial motility % of individual sperms of fresh semen
Concentration of sperms	Billion (10^9/ml) of sperms/ml of semen
Pre-filling motility %	Motility % of sperms after mixing with diluters and before filling into the straws.
Post thaw motility %	Motility % of sperms after freezing of semen by post thawing process of it.

Thawing of Semen

Post-thaw evaluation of cryopreserved semen was conducted after 24 hours of storage at $+37^0$ of warm water for 20 sec. Frozen semen samples of these genotypes were evaluated by CASA for motility and morphology of fresh semen. Articial insemination (AI) was conducted with frozen semen in naturally estrous crossbred murrah buffalo at their different parity. After 60 days post-AI, inseminated animals were checked for pregnancy with rectal palpation and the non-return rate was calculated. Articial insemination and pregnancy diagnosis was conducted by the same technician to avoid the variation in

conception rate

Data Analysis Techniques

All the concerned data were tabulated, and it carried statistical analysis out using Harvey's (1990) software. We presented data as the mean followed by the standard error (SE). We considered the data to be statistically significant at $P<0.05$.

The following fixed effect model was used to express each observation of semen quality produced from breeding buffalo bulls.

Yijkno = μ + ai + bj + ck + gn + ho + eijkno

Where, μ is the overall mean

ai is the effect of ith year (i =1, 2, 3, 4)

bj is the effect of jth bull (j =1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11)

ck is the effect of kth interval of semen collection (k =1, 2)

gn is the effect of nth density of fresh semen (n =1, 2, 3)

eijkno is the random element (error mean) assumed to be normally and independently distributed among the sampled population.

RESULTS AND DISCUSSION

Quality Parameters of Buffalo Bull Semen Production

Physical quality parameters of bull semen include semen volume. In this study, microscopic quality of bull semen were semen volume, prefilling motility, the concentration of sperm, and post-thaw motility (Table 2 and 3).

Table 2:The mean values (±S.E.) for traits of semen of Murrah buffaloes bull

Factors		N	Initial Volume of Semen (%)	Premotility (%)	Sperm Concentration (10^9/ml)
			SSM±SEM	SSM±SEM	SSM±SEM
Overall Mean		532	6.15±0.13	74.99±0.26	1599.33±27.95
Location			P<0.001	P<0.001	P<0.001
	Hill	394	5.42±0.10	75.94±0.21	1489.86±22.61
	Terai	138	6.87±0.20	74.04±0.42	1708.80±44.06
Year			P<0.002	P<0.001	P<0.001
	2017	44	5.93±0.27	75.19±0.57	1663.76±60.61
	2018	66	6.09±0.23	76.93±0.49	1565.86±51.40
	2019	348	6.56±0.10	75.52±0.20	1399.73±21.14
	2020	74	6.00±0.22	72.32±0.46	1767.97±49.03

Season			P<0.033	P<0.001	P<0.001
	Summer	133	5.53±0.33	74.00±0.70	1655.34±73.61
	Autumn	126	6.44±0.31	76.01±0.66	1681.94±69.23
	Winter	143	5.97±0.32	75.06±0.67	1612.92±70.44
	Spring	130	6.65±0.34	74.88±0.71	1447.12±74.63
Months			NS	NS	NS
	January	54	6.02±0.38	75.90±0.79	1530.93±83.47
	February	44	6.34±0.39	75.48±0.82	1501.43±86.91
	March	51	5.88±0.34	75.32±0.71	1631.91±75.29
	April	29	5.73±0.46	76.01±0.97	1797.06±102.39
	May	56	5.97±0.39	75.21±0.83	1830.92±87.18
	June	39	6.40±0.37	75.42±0.77	1727.79±81.41
	July	44	6.89±0.42	75.41±0.88	1578.53±92.89
	August	43	6.89±0.43	75.89±0.89	1606.87±94.23
	September	44	5.93±0.34	73.74±0.71	1598.99±75.04
	October	30	5.90±0.43	73.40±0.91	1511.98±95.61
	November	48	5.72±0.39	73.28±0.81	1491.31±86.00
	December	50	6.07±0.32	74.80±0.66	1384.25±69.76
CV			28.20	4.69	26.07
R squared			0.19	0.17	0.16

Initial volume of semen

The overall least squares mean of the initial ejaculate volume was 6.15±0.13 ml (Table 2) at two times of the same day at an interval of one hour. It was differ significantly ($p<0.05$) between location, year, season, and month.

Tomar et al. (1966) reported ejaculate volume ranging 2-12 ml (5.11±0.17 ml), Shukla and Mishra (2005) in Murrah bulls and Javed et al. (2000) also reported similar findings in Nili-Ravi bulls. Several authers (Bhakat et al., 2011; Pant et al., 2003; Koonjaenak et al., 2007; Rehman et al., 2012) also reported lower ejaculate volume compared to Jaffrabadi in Murrah, Swamp, and Kundhi breeds, respectively. Differences in semen volume among different buffalo breeds may be due to differences in genetics, the reproductive health status of bulls, age of bulls, collection frequency, pooled volume, nutrition, season, and management (Nazir, 1988). Differences may also be due to the skill of the semen collector and the temperature of the artificial vagina. Andrabi (2014) reported that the sperm volume

and sperm concentration of Murrah bulls ranged from 2.0 to 8.0 mL and 320 million to 5.9 billion, respectively. Mahmoud et al. (2013) observed that the concentration of buffalo bull sperm varied from 1.02 to 1.17 billion/mL, while their motility varied from 65.00 to 68.30% (fresh semen) and from 39.33 to 45, 87% (thawed sperm). Therefore, variation in frozen sperm production between two bulls could not prevent both bulls from being used for artificial insemination. In addition, Bull had a similar result in post-thaw sperm motility, which was previously categorized as the best single predictor for buffalo field fertility (Ahmed et al. (2016) & Ahmed et al. (2017).

Sperm motility

During the study, the initial progressive sperm motility ranged from 73.28±0.81 to 76.01±0.97 and the overall average sperm motility was 74.99±0.26 (Table 2). It reports a similar finding from 65 to 95%, with an average of 79.41±0.60 (Ghodasara et al.2016). Sahu and Pandit (1997) and Shukla and Mishra (2005) reported higher initial progressive motility in Murrah bulls. Bhakat et al. (2011) also reported a lower percentage of initial motility than the present findings in Murrah bulls.

Sperm motility of fresh semen of buffalo bull varied significantly ($p<0.001$) concerning location, season, and, months, while the year is not significantly ($p>0.001$) affected. The results of this study indicate that the total average of the thawed sperm was 52.17±0.28. And this study may cause higher field fertility as noted by Mahmoud et al. (2013) that buffalo bull semen with post-thaw sperm motility between 39.33 and 43.84% had a similar result in pregnancy rate, which has the potential to be used as a male sire for artificial insemination. The difference in motility in different reports could be due to changes in motility assessment, the few bulls studied, or differences in seasons. However, Mohan et al. (1977) and Gill et al. (1974) reported higher motility in winter (75 and 65%) in buffaloes, while Saeed et al. (1990) reported no effect of seasons on sperm motility.

Sperm concentration

The total average sperm concentration in the semen of the Murrah bull was 1599.33±27.95x10^9/ml (Table 2). Sperm concentration per unit of sperm volume is perhaps one of the most studied seminal attributes of seasonal changes in sperm quality. The results showed that sperm concentration per ml (SPC) varied significantly ($P<0.01$) between site, year, and season, with a maximum in autumn followed by summer winter, and spring (1681.94±69. 3 ,1655.34±73.61, 1612.92±70.44 and 1447.12±74.63 × 10^9/ ml), which is support with the statement of Mandal et al. (2000) and Ravimurugan et al. (2003) at Murrah Bulls.

Bhosrekar (1980) and Manik & Mudgal (1984) reported the highest SPC during summer at Murrah, while Prajapati (1995) and Dhami et al. (1998) obtained the highest value in the rainy season; however, in Egyptian buffalo bulls, Oloufa et al. (1959) recorded the

highest value in spring. On the contrary, Zafar et al. (1988) in Nili Ravi buffalo bulls and Koonjaenak et al. (2007) found no significant seasonal differences in sperm concentration in swamp buffalo.

Table 3: The mean values (±S.E.) for various characteristics of semen of Murrah buffaloes bull of National Livestock Breeding Office(NLBO) Pokhara and Lahan

Quality parameters	Location	N	Final Volume (ml)	Post thaw mortality (%)	Live sperm (%)	Sperm abnormality (%)
			SSM±SEM	SSM±SEM	SSM±SEM	SSM±SEM
Overall Mean		532	84.54±1.78	52.17±0.28	66.98±0.08	4.21±0.05
Location			P<0.001	P<0.001	P<0.001	NS
	Hill	394	78.04±1.44	50.58±0.22	68.42±0.06	4.26±0.04
	Terai	138	91.05±2.81	53.77±0.44	65.54±0.12	4.16±0.08
Year			P<0.0326	NS	P<0.001	P<0.001
	2017	44	86.84±3.86	53.02±0.60	68.56±0.17	4.37±0.11
	2018	66	84.98±3.27	52.11±0.51	68.56±0.14	5.30±0.09
	2019	348	79.19±1.35	52.23±0.21	65.66±0.06	3.55±0.04
	2020	74	87.16±3.12	51.34±0.48	65.14±0.14	3.62±0.09
Seasons			NS	P<0.011	NS	P<0.001
	Summer	133	87.47±4.69	51.23±0.73	67.14±0.20	4.85±0.14
	Autumn	126	91.04±4.41	51.25±0.68	67.10±0.19	4.21±0.13
	Winter	143	82.09±4.49	51.82±0.70	66.96±0.19	4.03±0.13
	Spring	130	77.57±4.75	54.41±0.74	66.72±0.21	3.75±0.14
Months			NS	P<0.0002	NS	P<0.0002
	January	54	83.45±5.32	54.20±0.82	66.82±0.23	4.59±0.15
	February	44	80.88±5.54	54.25±0.86	67.11±0.24	4.53±0.16
	March	51	90.69±4.80	50.94±0.74	67.09±0.21	4.74±0.14
	April	29	95.02±6.52	49.55±1.01	67.30±0.28	4.48±0.19
	May	56	102.57±5.55	48.85±0.86	67.46±0.24	4.30±0.16
	June	39	90.77±5.19	50.25±0.80	67.02±0.23	3.77±0.15
	July	44	84.58±5.92	51.38±0.92	67.19±0.26	3.53±0.17
	August	43	83.43±6.00	52.41±0.93	66.91±0.26	3.67±0.17
	September	44	78.92±4.78	52.84±0.74	66.85±0.21	3.99±0.14

	October	30	76.14±6.09	52.42±0.94	66.72±0.26	4.28±0.18
	November	48	75.66±5.48	54.91±0.85	66.60±0.24	4.40±0.16
	December	50	72.40±4.44	54.10±0.69	66.68±0.19	4.27±0.13
CV			30.39	7.19	1.55	17.75
R squared			0.11	0.24	0.79	0.48

Final Volume and Post thaw mortality

Results of this study revealed that the overall mean final volume of semen was 84.54±1.78 ml in the present study (Table 3). In line with the findings of the present study, According to NLBO, Poudel et al. (2021), we reported this finding lower than the present study 57.9.2±1.79.

Post-thaw sperm motility: Results of this study showed that the overall mean of post-thaw motility was 52.17±0.28.The post-thaw motility ranged from 48.85% to 54% with an average mean of 52.17±0.28. during the entire study period. According to NLBO, Poudel et al. (2022), reported this finding was lower than 48.2±0.25 which is similar to the present study. Maurya et al. (2003) emphasized that the success of AI depends upon the effective prolongation of fertile life of spermatozoa under in-vitro conditions and he reported that the acceptable percentage (40%) of post-thaw sperm motility considered fit for artificial insemination in buffaloes was maintained up to 60 minutes of incubation in semen, which was frozen in winter and rainy seasons, whereas semen frozen in spring and summer seasons could maintain it only up to less than 30 min of post-thaw incubation. No significant difference between progressive and rapid sperm motility was observed at different time intervals of incubation across the seasons. Argaman et al. (2007) also reported a significant reduction in sperm motility during summer. Majic-Balic et al. (2012) stated that it may be due to more oxidative stress during summer to seminal plasma and spermatozoa that lead to a decrease in sperm progressive motility, which further leads to semen quality deterioration.

Live and sperm abnormalities

The overall least square means of live and abnormal spermatozoa percentages were 66.98±0.08 and 4.21±0.05 respectively (Table 3). Effect of location and year was significant ($p<0.05$)in live spermatozoa but effect of seasons and months were nonsignificant ($p>0.05$) in the case of status of live sperm. The lowest live sperm percentages were observed during the spring season (Table 3), followed by the summer, autumn, and winter seasons. Salem et al. (1973) documented lowest live sperm percentage and greatest sperm abnormality during the spring season which is partly in agreement with the present results. Values of live sperm were lowest in November and then increased up to April (Table 3). According to the semen quality control at the NLBO lab, the standard of average live sperms has been recorded 55% and lowest at 45%, and best at 75% (Annual Technical Report, 2021). Percentages of abnormal sperm were significantly ($p<0.05$) in different seasons (Table 3). The sperm abnormalities of 4.85±0.14, 4.21±0.13, 4.03±0.13 and 3.75±0.14 percent

observed in summer, autumn, winter and spring, respectively.The highest percent of abnormal spermatozoa were noted in March, January, February, April, October, November, and December, and the lowest in June-September (Table 3), respectively. Singh et al. (1992) and Igboeli et al. (1987) also reported higher sperm abnormalities in winter. Heuer et al. (1987), Bhavsar et al. (1990) and Younis (1996) observed higher percentage of sperm abnormalities in buffalo bulls during summer season, while Ahmad et al. (1984) reported it autumn in Nili-Ravi buffalo bulls. The higher sperm abnormalities during the winter have been related to effect of cold or improper handling during semen collection (Javed, 2019). Ahmed et al. (1984) observed total abnormalities of 12.68±7.06, 9.83±3.64, 11.48±6.57 and 15.04±6.59 percent in winter, spring, summer and autumn, respectively (lowest in spring and highest in autumn). Koonjaenak et al. (2007) reported overall total mean percentages of sperm abnormalities of buffalo bull spermatozoa (13.7 ± 0.5%) in the rainy season, (12.4 ±0.5%) in winter and (10.7 ± 0.5%) in summer. The rate and pattern of sperm removal seemed to depend particularly on the quality of spermatozoa (Rao et al., 1980). The maximum allowed limit for bulls is 10 percent for young and 20 percent for older bulls with 18 percent upper limit in all bulls (Lagerlof 1934).

It was also observed that abnormalities of the spermatozoa could occur due to disorder of the seminiferous tubules or germinal epithelium (Hossain et al., 1990). However, the sperm concentration did not differ significantly between the groups.

CONCLUSION

The present study provides evidence that the seasons of a collection influenced the semen volume of Murrah buffalo bulls. During the autumn and spring semen collection is more profitable than in the other two seasons. The quality of product semen used in Nepal is good and meets international standards and requirements. The existing breed of buffaloes bulls for semen production traits would help to scale up the genetic make-up in the hill and terai buffaloes to produce good progeny.

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