Research Article

Seasonality of Reproduction in Buffaloes in Subtropical Regions of Southern Nepal

Gokarna Gautam[1]*, Sajan Rokaya[1], Bhuminand Devkota[1], Saroj Sapkota[2]

[1]Department of Theriogenology, Faculty of Animal Science Veterinary Science and Fisheries, Agriculture and Forestry University, Rampur, Chitwan, Nepal; [2]National Animal Breeding and Genetic Research Center, Nepal Agricultural Research Council, Khumaltar, Lalitpur, Nepal.

Abstract | Reproductive pattern in buffaloes (*Bubalus bubalis*) is affected by photoperiodicity, heat stress and availability of feed supply. The objectives of this study were to understand the seasonality pattern of breeding and calving in buffaloes in subtropical regions of southern Nepal and to characterize the ovarian cyclicity status during the months of long day length. Data regarding seasonality pattern of breeding and calving of Murrah cross bred buffaloes (n=400) from two locations of subtropical region were collected. In a subset of buffaloes (n=104), the ovarian cyclicity status during late spring (May to June) was determined through transrectal ultrasonography and measurement of blood progesterone concentration using ELISA. Seasonal breeding and calving patterns were similar at two locations. The highest proportions of buffaloes were bred during autumn (40.8%), followed by winter (29.5%), summer (19.8%) and spring (10%). While comparing the month-wise patterns, the highest and the lowest proportions of breeding were in November and April, respectively. Likewise, the highest proportions of buffaloes calved in autumn (41.3%), followed by summer (26.8%), winter (20.8%) and spring (12.8%); the highest and lowest proportions of calving were in September and May, respectively. During the low breeding season, 53% buffaloes were non-cyclic (true anestrous) as indicated by inactive ovaries and below basal level (<1ng/ml) plasma progesterone concentration. In conclusion, there was a clear seasonality pattern of reproduction in buffaloes in subtropical regions of southern Nepal with the autumn being the good and the spring being the low breeding as well as calving seasons; and 53% of buffaloes during low breeding season were in true anestrous condition.

Keywords | Buffalo, Reproduction, Seasonality, ELISA, Progesterone, Anestrus

Received | December 05, 2023; **Accepted** | January 31, 2024; **Published** | March 13, 2024
***Correspondence** | Gokarna Gautam, Department of Theriogenology, Faculty of Animal Science Veterinary Science and Fisheries, Agriculture and Forestry University, Rampur, Chitwan, Nepal; **Email:** ggautam@afu.edu.np
Citation | Gautam G, Rokaya S, Devkota B, Sapkota S (2024). Seasonality of reproduction in buffaloes in subtropical regions of southern Nepal. Adv. Anim. Vet. Sci., 12(5):895-901.
DOI | https://dx.doi.org/10.17582/journal.aavs/2024/12.5.895.901
ISSN (Online) | 2307-8316

INTRODUCTION

Buffalo (*Bubalus bubalis*) has been a very important commodity for employment and income generation of rural people for a long time. Due to its important economic role, the buffalo is regarded as the black gold of Asia (Memon and Khushk, 2004). The world buffalo population has been estimated to be approximately 207 million, of which 97% herds are in Asia (FAO, 2020). Buffalo contributes approximately 10-15% of the total world milk production (FAO, 2020) and 50% of the total milk production in Asia (Nasr *et al.*, 2016). Among buffalo rearing countries in the world, Nepal ranks the fourth highest position (Hegde, 2019) with 5,159,931 buffaloes in the year 2021 (MoALD, 2022). Almost all buffaloes in Nepal are riverine type with approximately 65% indigenous breed and 35% Murrah and their crosses (Neopane, 2006). Buffalo is the most important livestock commodity of Nepal contributing through meat, milk, draft power and leather production; buffalo shares 57.2% of the total milk and 36.1% of the total meat produced in the nation (MoALD, 2022). However, the productive efficiency of Nepalese buffalo is quite low due to subfertility, infertility and seasonal breeding pattern (Devkota et al., 2013; Devkota and Bohora, 2009; Gautam et al., 2017).

Depending on the geographical location, the buffalo can be considered as seasonal breeder. In the geographical regions of the world away from the equator such as India, Pakistan and Italy, the breeding activities in buffalo are seasonally dependent being favored in decreasing day light length whereas countries near the equator like in Brazil it depends upon the availability of green pasture and thus the calving is regulated by pasture availability (Zicarelli, 1997; Campanile et al., 2010; Phogat et al., 2016). However, there were no consistent findings regarding seasonal pattern of anestrus, breeding and pregnancy in water buffalo even in the regions away from equator (Singh et al., 2000; Bughio et al., 2000; El-Wishy, 2007; Khan et al., 2009; Kushwaha et al., 2011; El-Tarabany, 2018; Gunwant et al., 2018). In a previous study consisting of a small number of buffaloes from a single farm in subtropical area of mid southern Nepal, there was a seasonal breeding pattern with July to December as active breeding months and January to June as poor breeding months (Devkota and Bohora, 2009). However, it was not known whether the similar seasonal breeding pattern prevails in other parts of the country. Understanding the seasonality pattern of breeding and calving in the buffalo can provide useful guidelines to manage their reproductive performance throughout the year. Therefore, the objectives of the present study were to understand the seasonality pattern of breeding and calving in buffaloes in subtropical regions of southern Nepal and to characterize their ovarian cyclicity status during the months of long day length.

MATERIALS AND METHODS

The use of animals in this study complied with the ARRIVE (Animal research: Reporting of *in vivo* experiments) guidelines and all the experiments were carried out in accordance with the U.K. Animals (Scientific procedures) Act, 1986 and associated guidelines.

Study site

This study was conducted in buffaloes of two different locations in southern Nepal namely: Plain areas of Chitwan and Surkhet districts (Figure 1). Both locations lie in the inner terai region of Nepal under subtropical climatic condition having seasonal climatic variations with cold and semi-dry to dry winter (December to February), rapidly increasing hot and dry spring (March to May), very hot and rainy monsoon summer (June to August) and moderate autumn (September to December) (Devkota and Bohora, 2009). However, Surkhet is slightly cooler than Chitwan throughout the year (https://tcktcktck.org).

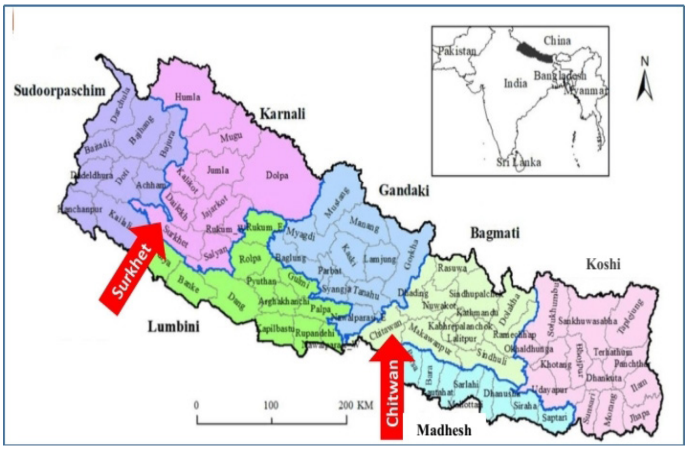

Figure 1: Map of Nepal showing study areas (Chitwan and Surkhet) in subtropical region of Nepal.

Chitwan, located in southern central part of Nepal, has annual average temperature 29.3°C that ranged from minimum 11.36°C in January to maximum 40.31°C in May (https://tcktcktck.org/nepal/narayani/bharatpur). Surkhet, located in southern western part of Nepal, has annual average temperature 24.1°C that ranged from minimum 5.47°C in January to maximum 36.65°C in May (https://tcktcktck.org/nepal/bheri/birendranagar). Most of the buffaloes at plain areas of both districts were Murrah and their crossbred, although the exact blood level was not known.

Animals and data collection

Murrah and their crossbred buffaloes that were calved at least once were used for this study. Total 200 buffaloes from 84 households of Chitwan and 200 buffaloes from 78 households of Surkhet were selected. Data were collected during a period from January to July 2021. Data regarding date of breeding and last calving and other various information were collected by the authors through face-to-face interview with the farm owners or attendants. All the buffaloes were reared in almost similar management conditions with 24 hours tie stall barn, hand milking twice daily and feeding seasonally available roughage and locally available concentrate feed consisting of maize, rice bran, wheat bran, mustard oil-cake etc. The number (Mean±SE) of buffaloes at each farm was 2.59±0.48 (range: 1 to 16) in Chitwan and 2.34±0.43 (range: 1-13) in Surkhet. Parity of buffaloes included in the study was 4.09±0.12 (range: 1-9) in Chitwan and 3.4±0.12 (range: 1-8) in Surkhet. Body condition score (BCS) was 3.11±0.23 (range: 1.75-4.5) in Chitwan and 2.40±0.27 (range: 1.75-4.0) in Surkhet. There was no proper recording of milk yield. Among the data available, the milk yield ranged from 1 to 9 liters per day.

Reproductive examination

In a subgroup of anestrous buffaloes (n=104) randomly selected from 12 farms in Chitwan during May-June 2021 (the buffalo owners/attendants had not noticed any estrous signs in those buffaloes for over last three months), transrectal ultrasonography (USG) (Ebit 30VET, CHISON Medical Technologies Co., Ltd, China) was performed using 7.5 to 10 MHz transducer to determine the ovarian cyclicity status. Presence of corpus luteum (CL) and/or follicle (F) if any, was recorded.

Determination of plasma progesterone concentration

Blood samples were collected from the jugular veins of same 104 buffaloes using EDTA containing vacutainers. Collected samples were immediately kept in the cool box containing ice packs and transported to the laboratory within 2 hours. Then, the plasma was harvested by centrifuging blood samples at 1,000 x g for 10 minutes, and stored at -70 °C until ELISA was done. Plasma progesterone concentration was determined using a progesterone ELISA kit (Abnova, Taiwan). Sensitivity of assay was 8.57 pg/ml. Intra-assay and iter-assay CVs (%) were 4.9-7.6 and 2.7-6.8, respectively. Cross reactivity with related steroid compounds was <1%.

Categorization of buffalo based on ovarian cyclicity status

Ovarian cyclicity status was determined based on two criteria. Based on transrectal ultrasonography and plasma progesterone concentration, the buffaloes were categorized into two groups as follow. Buffaloes having CL in the ovary and plasma progesterone concentration ≥ 1 ng/ml were considered to be in 'silent estrus' (or to have persistent CL) and those having plasma progesterone concentration < 1 ng/ml and no evidence of regressing/regressed CL were considered be to non-cyclic (true anestrous).

Statistical analyses

Data were entered into MS Excel 2010 and the statistical analyses were done using SPSS (version 25.0). Proportion of buffaloes bred and calved during different seasons between two locations were compared using Chi-square test. Month-wise breeding and calving patterns of buffaloes at two different locations were expressed in the form of bar diagram using MS Excel. Plasma progesterone concentrations between true anestrous and silent estrous buffaloes were compared using z-test assuming equal variance.

RESULTS

Seasonality pattern of breeding in buffaloes at two different locations

Table 1 shows the seasonality pattern of breeding in buffaloes at two different locations. Seasonality pattern of breeding in buffaloes was similar at both locations (P=0.98). Overall, the highest breeding proportion was in autumn (40.75%) and the lowest breeding proportion was in spring (10%).

Table 1: Seasonality pattern of breeding in buffaloes at two different locations.

Season of breeding	Proportions of breeding (%)			P value
	Overall (n=400)	Chitwan (n=200)	Surkhet (n=200)	
Summer (Jun-Aug)	19.75	19.50	20.00	0.98
Autumn (Sep-Nov)	40.75	41.00	40.50	
Winter (Dec-Feb)	29.50	30.00	29.00	
Spring (Mar-May)	10.00	9.50	10.50	

When the breeding pattern was analyzed on month-wise basis, similar pattern of breeding was seen in both locations (Figure 2). Buffaloes were bred round the year. However, the highest proportion of breeding at both locations (Chitwan and Surkhet) was in November (15% and 17%, respectively) and the lowest was in April (2% and 3%, respectively). There was a trend that the breeding proportion started to increase gradually from June until November, and then decreased gradually from November to April and May.

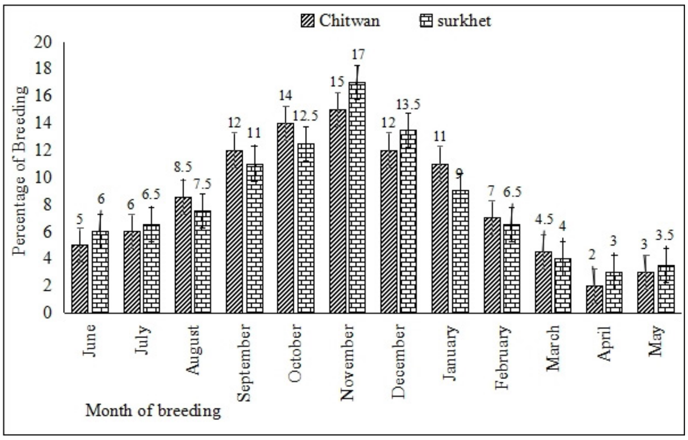

Figure 2: Month wise breeding pattern of Nepalese buffaloes at two different locations.

Seasonality pattern of calving in buffaloes at two different locations

Table 2 shows the seasonality pattern of calving in buffaloes at two different locations. Seasonality pattern of calving in buffaloes was similar at both locations. Overall, the highest proportion of calving was in autumn (39.50%) and the lowest calving proportion was in spring (12.25%).

When the calving pattern was analyzed on month-wise basis, similar pattern of calving was seen at two locations (Figure 3). Although the buffaloes calved round the year, the highest proportion of calving in both locations (Chitwan and Surkhet) was in September (16% and 16.5%) and the lowest proportion of calving was in May (3% and 3.5%), respectively. There was a trend that the proportion of calving started to increase gradually from June until September and then started to decrease from September until May.

Table 2: Seasonality pattern of calving in buffaloes at two different locations.

Season of calving	Proportions of calving (%)			P value
	Overall (n=400)	Chitwan (n=200)	Surkhet (n=200)	
Summer (Jun-Aug)	26.75	26.00	27.50	0.92
Autumn (Sep-Nov)	39.50	40.00	39.00	
Winter (Dec-Feb)	21.50	22.50	20.50	
Spring (Mar-May)	12.25	11.50	13.00	

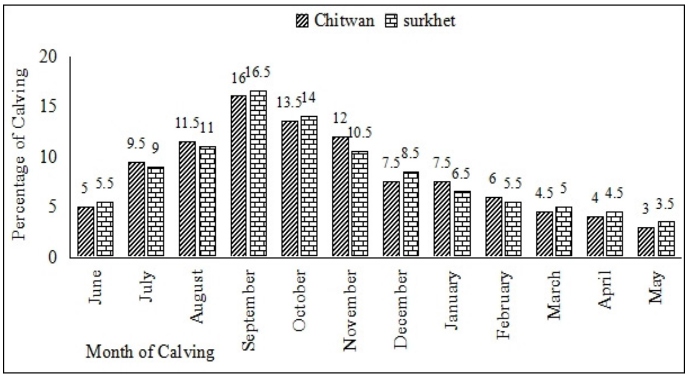

Figure 3: Month wise calving pattern of Nepalese buffaloes at two different locations.

Ovarian cyclicity status of buffaloes during low breeding season

In in a subset of buffaloes randomly selected from 12 farms in Chitwan during low breeding season (n=105), 47% buffaloes had the luteal activity (either silent estrus or persistent CL) as indicated by plasma progesterone concentration above the basal level (i.e. ≥1ng/ml) and the presence of CL in the ovary, while 53% buffaloes were non-cyclic (true anestrus) as indicated by plasma progesterone concentration below the basal level plasma (i.e. <1ng/ml) and absence of CL in the ovary (Table 3). Among those 53% true anestrous buffaloes, 16.7% had DF (i.e. follicle size ≥ 8mm) and 36.3% had small ovaries without DF. Plasma progesterone concentration (mean±SE) was significantly higher (P<0.001) in buffaloes having silent estrous or persistent CL (3.54±0.32 ng/ml) than in true anestrous buffaloes (0.61±0.06 ng/ml) (Table 4).

DISCUSSION

This study tried to understand the seasonal pattern of reproduction in buffaloes at two different locations in subtropical area of Nepal. Although both locations were the southern plains located in the inner terai region having hot humid summer and dry cool winter, Chitwan was hotter than Surkhet throughout the year (https://tcktcktck.org). However, the seasonal pattern of reproduction in buffaloes was almost similar at both locations.

Table 3: Ovarian cyclicity status of buffaloes during low breeding season.

Ovarian cyclicity status		Proportion of buffaloes (%)
Luteal activity (Silent estrus or persistent CL)		47.0
No luteal activity	Follicle size ≥8 mm	16.7
	Follicle size <8 mm	36.3

Table 4: Plasma progesterone (P4) levels in true anestrous and silent estrous buffaloes during low breeding season.

Plasma P4 level (ng/ml)	Type of anestrus		P value
	True anestrus (n=55)	Silent estrus (n=49)	
Mean±SE	0.61±0.06	3.54±0.32	<0.001
95% Confidence intervals for estimated mean of population	0.6082±0.0646	3.5414±0.6418	
Range	0-0.96	1.1-10.4	

The findings of the present study revealed that the breeding activities of buffaloes in subtropical region of southern Nepal were the highest during autumn (40.8%) followed by winter (29.5%), summer (19.8%) and the lowest during spring (10.5%). Such seasonal breeding pattern was similar to that reported in India (Chaudhari et al., 2015) in which the breeding proportion was the highest (51.65%) during November to February and the lowest (16.5%) during March to June. Seasonal pattern of calving in buffaloes in the present study was similar to that reported by previous study in Chitwan that consisted of a small number of buffaloes only from the single farm (i.e. Livestock farm of the Institute of Agriculture and Animal Science, Chitwan) (Devkota and Bohora, 2009); in the same study the authors, based on calving season, retrospectively concluded that the period from August to February was the active breeding season and that from April to May was the low breeding season for the buffalo in Chitwan. The lowest breeding activities were during spring (March to May) that corresponded with the increasing day length, dry and hot climatic conditions and the scarcity of green roughage. On the contrary, the highest breeding activities were during autumn (September to November) that corresponded with the decreasing day length, cool climatic conditions and the availability of enough green roughage. It has been stated that that breeding season of buffaloes in equatorial region (where day length fluctuates very less) is mainly affected by availability of sufficient feed resources and that of subtropical and temperate region is affected by photoperiod, heat stress and availability of green forages (Zicarelli, 1997; El-Wishy, 2007; Zicarelli, 2007; Campanile et al., 2010). It has been suggested that the buffalo during increasing day length (i.e., late spring and summer seasons) have changes in the pineal metabolism that lead to hyperprolactinemia (Paraneswaran et al., 1983), and hyperprolactinemia has been suggested to be a possible reason of summer anestrus in buffaloes (Singh and Madan, 1989).

Thus, it can be speculated from the present study that the breeding activities of buffaloes in subtropical region of Nepal are also controlled by photo period, heat stress and availability of green forages. However, even in spring and early summer a few buffaloes had breeding activities. This finding was in consistent with a previous study in subtropical area of Egypt in which the conception and pregnancy loss rates were not affected by the day length (El-Tarabany, 2018). That means there is potentiality that we can improve the reproductive efficiency of buffaloes during low breeding season through nutritional and hormonal management because various health, nutritional and hormonal interventions have been able to induce estrus, ovulation and pregnancy during low breeding season. However, the outcome of such intervention was largely affected by the ovarian cyclicity status and body condition score of animals (Devkota et al., 2012).

The proportions of buffaloes that calved during different seasons did not differ between two locations. The calving pattern of buffaloes in the present study was similar to that of buffaloes in a government farm of eastern terai of Nepal where the highest proportion of calving was in autumn (46.6%) followed by summer (41.3%), winter (9.3%) and in spring (2.8%) (Sah et al., 2013). Seasonal calving pattern of buffaloes in subtropical regions of southern Nepal in the present study was also similar to that reported by previous studies in India where the calving proportion was maximum in autumn and minimum in spring season (Chaudhari et al., 2015; Gunwant et al., 2019). Month-wise calving pattern in the present study was also similar at both locations; proportion of calving gradually increased from June, reached to peak in September and gradually declined until reached to nadir during March to May. Considering the gestation period of approximately 310 days, the highest calving in September corresponded with the highest breeding activities in November. All of those buffaloes in this study were bred with bull and most of the buffaloes became pregnant with single service; the number of services per conception was 1.25±0.20 (unpublished data). Calving in late summer (monsoon) and autumn can ensure the availability of enough green roughage to the buffalo when they require good nutrition during their early lactation period.

In a subgroup of anestrous buffaloes in Chitwan during low breeding season (May-June), the ovarian cyclicity status was examined using transrectal ultrasonography of ovaries and progesterone determination of blood samples. It was found that 47% buffaloes had luteal activity as indicated by plasma progesterone concentrations above basal level. However, estrous signs were not noticed in those buffaloes for over last three months. Thus, it can be presumed that those buffaloes had either silent estrus or persistent of CL. Further detailed study is recommended to differentiate between silent estrus and persistent CL cases by determining the ovarian cyclicity status of anestrous buffalo more frequently at one to two weeks interval. Remaining 53% buffaloes in the present study were in true anestrous condition (characterized by small ovaries and sub-basal level of plasma progesterone concentrations in peripheral blood circulation). These results were almost similar to the findings of a previous study in Nepal that demonstrated that during low breeding season among the buffaloes having good BCS, 53.8% had silent estrus and 46.2% had true anestrus as diagnosed by transrectal palpation and ultrasonography (Devkota et al., 2012). Among 53% true anestrous buffaloes, 16.7% had DF (i.e., follicle size ≥ 8mm) and 36.3% had small ovaries without DF. Although 16.7% buffaloes had follicle size ≥ 8mm, those buffaloes might not have been able to express estrus and ovulate because it has been stated that the anestrous buffaloes during low breeding season have insufficient pulsatile of LH to support the final stages of follicular development, so that estrus behavior and ovulation would not occur (Carvalho et al., 2016).

In the present study, the plasma progesterone concentration in true anestrous buffaloes was significantly lower than in silent estrous buffaloes. Progesterone concentrations in true anestrous and silent estrous buffaloes in the present study were almost similar to the findings of a previous study that demonstrated that the progesterone concentrations were 3.29 ng/ml in buffaloes having developed CL and <0.1 ng/ml in true anestrous buffaloes (Quereshi et al., 2000). Results of the present study suggest that the low breeding activities in Nepalese buffaloes during late spring and early summer season were primarily due to inadequate functioning of the ovaries in secreting optimum concentrations of progesterone. Thus, interventions to improve the seasonal anestrus in Nepalese buffaloes during low breeding season may require frequent monitoring of ovarian cyclicity status, proper heat detection as well as the application of progesterone based estrous synchronization protocols.

CONCLUSIONS

There was a clear seasonality pattern of breeding and calving in buffaloes in subtropical regions of southern

Nepal with autumn being the best and the spring being the lowest breeding as well as the calving seasons. Nearly 53% buffaloes during low breeding season had true anestrus with no luteal activity while 47% had luteal activity without overt signs of estrus.

ACKNOWLEDGMENTS

This study was financially supported by the University Grants Commission (UGC) Nepal [Collaborative Research Grant (CRG-75/76-Ag and F-1)]; the authors would like to acknowledge the UGC Nepal for its financial support. The authors are also thankful to the buffalo owners for their cooperation and support.

NOVELTY STATEMENT

To our knowledge, this is the first detailed study that reported the seasonality pattern of Nepalese buffalo, especially in subtropical region of Nepal.

AUTHOR'S CONTRIBUTION

GG: Conceptualization, funding acquisition, project administration, resources, investigation, methodology, formal analysis, supervision, writing original draft, writing review and editing. SR: Methodology, data curation, formal analysis, writing original draft. BD: Methodology, investigation, supervision, writing review and editing. SS: Methodology, investigation, supervision.

Conflict of interest

The authors have declared no conflict of interests.

REFERENCES

Bharatpur Climate Summary (2023). Available at: https://tcktcktck.org/nepal/narayani/bharatpur (accessed 7 Nov 2023).

Birendranagar Climate Summary (2023). Available at: https://tcktcktck.org/nepal/bheri/birendranagar (accessed 7 Nov 2023).

Bughio S, Shahani AK, Mirani AH, Moriani AA, Oad FC, Bughio BA (2000). Seasonal trend of calving and subsequent service period in buffaloes. Pak. J. Biol. Sci., 3: 2169-2170. https://scialert.net/abstract/?doi=pjbs.2000.2169.2170.

Campanile G, Baruselli PS, Neglia G, Vecchio D, Gasparrini B, Gimenes LU, Zicarelli L, D'Occhio MJ (2010). Ovarian function in the buffalo and implications for embryo development and assisted reproduction. Anim. Reprod. Sci., 121(1–2): 1–11. https://doi.org/10.1016/j.anireprosci.2010.03.012

Carvalho NAT, Soares JG, Pietro SB (2016). Strategies to overcome seasonal anestrus in water buffalo. Theriogenology, 86(1): 200-206. https://doi.org/10.1016/j.theriogenology.2016.04.032

Chaudhari R, Mehrotra S, Krishnaswamy N, Ajevar G, Chaudhary J, Kumar A (2015). Effect of season on the incidence of estrus and calving in cattle and buffaloes. Indian Vet. J., 92: 24–26.

Devkota B, Bohara TP (2009). Effects of season on pregnancy rates in water buffaloes of Southern Nepal evaluated by using different estrus synchronization protocols during active season and low breeding season. Pak. J. Zool. Suppl., 9: 763-770.

Devkota B, Bohara TP, Yamagishi N (2012). Seasonal variation of anestrus conditions in Buffaloes (*Bubalus bubalis*) in Southern Nepal. Asian J. Anim. Vet. Adv., 7(9): 910-914. https://doi.org/10.3923/ajava.2012.910.914

Devkota B, Nakao T, Kobayashi K, Sato H, Sah SK, Singh DK, Dhakal IP, Yamagishi N (2013). Effects of treatment for anestrus in water buffaloes with PGF2 alpha and GnRH in comparison with vitamin-mineral supplement, and some factors influencing treatment effects. J. Vet. Med. Sci., 75: 1623-1627. https://doi.org/10.1292/jvms.12-0515

El-Tarabany MS (2018). Survival analysis and seasonal patterns of pregnancy outcomes in Egyptian buffaloes. Livest. Sci., 213: 61–66. https://doi.org/10.1016/j.livsci.2018.05.008

El-Wishy AB (2007). The postpartum buffalo. II. Acyclicity and anestrus. Anim. Reprod. Sci., 97(3–4): 216–236. https://doi.org/10.1016/j.anireprosci.2006.03.003

FAO (2020). Gateway to dairy production and products. FAO. www.fao.org/dairyproduction-products/production/dairy-animals/buffaloes/en/

Gautam G, Gyawali RR, Nath B, Pant S (2017). Characterization and treatment of infertility in buffaloes of hills and Terai regions of mid Nepal. In: The proceedings of the international buffalo symposium, 15-18 Nov 2017, Chitwan, Nepal, pp. 98-102.

Gunwant P, Pandey AK, Ramkaran, Kumar P (2019). Effect of season of calving on reproductive characteristics of Murrah buffalo at organized dairy farm. Pharma Innov. J., 8(2): 435–436.

Gunwant P, Pandey AK, Singh I, Phogat JB, Kumar S, Kumar S (2018). Seasonal variation of calving in Murrah buffalo at organized dairy farm. Int. J. Pure Appl. Biosci., 6(1): 1283–1287. https://doi.org/10.18782/2320-7051.5814

Hegde NG (2019). Livestock development for sustainable livelihood of small farmers. Asian J. Res. Anim. Vet. Sci., 3: 1–17.

Khan MS, Hassan F, Rehman MS, Ahmad S, Rehman Z (2009). Days open and seasonality of calving in buffaloes and cattle in Pakistan. Pak. J. Zool., 9: 195–199.

Kushwaha BP, Singh S, Maity SB (2011). Seasonality of calving in Bhadawari and Murrah buffaloes in Bundelkhand, India. Buffalo Bull., 30(4): 256-261.

Memon A, Khushk AM (2004). Economic analysis of buffalo dairy farms in Sindh. Indus J. Biol. Sci., 1(2): 231-240.

MoALD (2022). Statistical information on Nepalese agriculture 2020/21. Ministry of Agriculture and Livestock Development, Government of Nepal, Kathmandu, Nepal.

Nasr M, Awad A, El-Araby IE (2016). Associations of leptin and pituitary- specific transcription factor genes' polymorphisms with reproduction and production traits in dairy buffalo. Reprod. Domestic Anim., 51: 597-603. https://doi.org/10.1111/rda.12726

Neopane SP (2006). Characterization of indigenous animal genetic resources of Nepal. In: The proceedings of the 6th national workshop on livestock and fisheries research, 1-2 July 2004, NARC, Khumaltar, Lalitpur, Nepal, pp. 1-11.

Paraneswaran M, Thakkar TP, Janakiraman K (1983). Pineal activity in relation to ovarian structures and breeding seasons in the water buffaloes. Indian J. Anim. Reprod., 4: 5-8.

Phogat JB, Pandey AK, Singh I (2016). Seasonality in buffaloes reproduction. Int. J. Plant Anim. Environ. Sci., 6(2): 46-54.

Qureshi MS, Habib G, Nawab G, Siddiwqui MM, Ahmad N, Samad HA (2000). Milk progesterone profiles in various reproductive states in dairy buffaloes under field conditions. Proc. Natl. Sci. Counc. Republ. China. B, Life Sci., 24(2): 70-75.

Sah AK, Jha PK, Ali MS, Pathak LR (2013). Seasonal effect on calving pattern of Murrah crossbred buffaloes at National Buffalo Research Programme, Tarahara, Sunsari, Nepal. In: 9th National Proceedings of Livestock and Fisheries Research, Nepal. Nepal Agric. Res. Counc.,

Singh J, Madan ML (1989). Hyperprolactinaemia: A possible cause of summer anoestrus in buffaloes. Proceedings of National Symposium of Applied Reproduction in Farm Animals and 8thNational Convention of ISSAR, Anand, 1989, November 10–12.

Singh J, Nanda AS, Adams GP (2000). The reproductive pattern and efficiency of female buffaloes. Anim. Reprod. Sci., 60–61: 593-604. https://doi.org/10.1016/S0378-4320(00)00109-3

Zicarelli L (2007). Can we consider buffalo a non precocious and hypofertile species? Ital. J. Anim. Sci., 6: 143-154. https://doi.org/10.4081/ijas.2007.s2.143

Zicarelli L (1997). Reproductive seasonality in buffalo. Bubalus Bubalis, 4(Suppl. 1): 29-52.