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Field Study on the Performance of Sex-sorted Semen in Buffaloes Maintained under Small Dairy Farming System of Uttar Pradesh

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ABSTRACT

Background: The objective of the study was to evaluate the performance of sex-sorted semen in buffalo population maintained by smallholder dairy farmers and to determine the factors influencing the conception rate.

Methods: The data on 21,422 artificial inseminations on follow-up basis and 1,400 calvings were used to study conception rate and female calf sex percentage. The effects studied were agro-climatic zones, order of lactation and month of insemination. The conception rate, a binomial trait was categorized as "*0*" for not conceived and "*1*" for conceived and the pregnancy diagnosis for all the cows were carried out by per-rectal examination post 60-90 days after insemination. The agro-climatic zones were categorized in seven zones *viz.* Eastern plains, Bundelkhand, Central western plains, Central plains, North-eastern plains, Western plains and south western semi-arid plains. While order of lactation was grouped in six as Heifer, 1-4 and 5 and above. The statistical analysis was performed using the binary logistic regression model to estimate the effect of factors on the conception rate.

Result: The overall mean of conception rate was 43.04±0.34 per cent, while the female calf sex percentage was found to be 91.20%. All of the effects included in the study significantly (P<0.05) influenced the conception rate. The probability of conception rate was higher in central western plains while low probability for conception rate was found in North-eastern Plain. The conception rate increased gradually up to February and later declined till July 39.13. The conception rate was higher in month of February and lowest in July month. The odds of conception were comparatively lower in heifers than multiparous buffaloes. The reason for the low conception rate in heifers was due to preferential treatment given by farmers to milking animals. The performance in terms of conception rate and sex ratio of sex-sorted semen in buffaloes maintained under smallholder dairy system were encouraging and could be replicated with good management practices.

Key words: Buffalo, Sex-sorted semen, Conception rate and Female sex percentage.

INTRODUCTION

Buffaloes have been the integral part of Indian dairy system and major source of livelihood for the many of dairy farmers in India. Such is the contribution of buffaloes towards total milk production in India, nearly accounting for half the milk (97.22 million tonnes of 198.40 million tonnes total produced in India) produced comes from them (GoI Report, 2021). Unlike cows, buffaloes are seasonal breeders and produce a calf on an average 450 days after calving (Shashi *et al*., 2014; NBAGR Report, 2006). The conventional semen or natural service gives the probability of producing a female calf is 50 per cent. With given assumption, it may take around 3 to 4 years to produce female calf with 50 percent uncertainty.

This uncertainty over calf sex can be reduced by adaptation of sex-sorted semen to increase the percentage of female calves. Buffalo sperm can be sorted in X and Y enriched population on the basis of DNA content. The available biotechnological tools like sperm mediated gene transfer (Niemann *et al*., 2011), embryo biopsy *etc*. to select desired sex of calf before birth are expensive and time consuming. While the strategy of using sex sorted semen was proven as an economically feasible through commercial application of new sex sorted semen technology. The use of sex sorted semen not only helps in expansion of the herd size but also with aid of high genetic merit bulls, desired genetic progress and increase in productivity can be achieved in less time. There are few studies, which showed an encouraging results about use of sex sorted semen in different buffalo population (Lu *et al*., 2010; Campanilea *et al*., 2011 and Lu *et al*., 2015).

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The factors affecting conception rate from insemination with the sex sorted semen and, the sex ratio, need to be further addressed to improve further performance in field conditions. In the present study, sex sorted semen was inseminated in different districts of Uttar Pradesh state. The effects of order of parity and season on conception rate were evaluated under field conditions and the outcome of this large field study will be of significant importance for implementing the use of semen sexing technology in buffalo breeding in future.

MATERIALS AND METHODS

Area of study

The present study was conducted in 459 field Artificial Insemination (AI) centres of BAIF Development Research Foundation located in 60 districts of Uttar Pradesh state. BAIF Development Research Foundation (BAIF Development Research Foundation, 2021), a large NGO located close to Uruli Kanchan near Pune, providing door-to-door cattle and buffalo AI service to about 5 million families of small farmers in over 100,000 villages in 13 states.

A total of 21,422 inseminations with semen produced from 14 Murrah bulls were carried out in 20,146 graded Murrah buffaloes belonging to 18,771 farmers. The inseminations were carried out over a period of 2 years, between January 2019 and September 2021. A total of 1,015 calving were followed which included abortion, still birth, dystocia and normal calving. All the cows inseminated with sex sorted semen were tagged with 12-digit unique identity number and the data pertaining to inseminations were collected through an online digital platform, stored and analysed using BAIF' data storage and data management pipeline.

Processing of sex sorted semen

The BAIF frozen semen station and the semen sex sorting facility is located in Maharashtra state, India, in the outskirts of Pune city (18.5°N latitude 73.8°E) and at an altitude of 559 m above sea level. Sex sorted semen is being produced at BAIF frozen semen station using Sex ULTRA™ sperm sex sorting technology, a patented technology of Sexing Technologies (ST), USA. Sexing Technologies (ST) is the pioneer in the field of sex sorting technology in the world. The bovine semen sex sorting techniques were originally invented by ST's subsidiary XY, LLC and licensed to ST. The semen sorting and the processing was done as per the method explained by Gonzalez-Marín *et al.* (2018). Every single batch of sex sorted semen was subjected for quality control using standard protocols. The quality standards of the sex sorted frozen semen used in the study as per the prescribed norms were gender purity, as assessed during the laboratory process was greater than 90 per cent; Post thaw incubation of progressive motility at 0 and 3 hours were above 50 and 30 per cent, respectively; bacterial count was below 100 CFU; The final concentration of spermatozoa was minimum 2.1 million sperms per frozen semen straw.

Parameters studied and influencing factors

The parameters studied were conception rate (coded as 0: not conceived and 1: conceived) and sex ratio of sex sorted semen. Conception rate (CR) was estimated as the proportion of pregnancies confirmed by the rectal palpation of the genital tract at 90 to 120 days of post-insemination, against the total number of inseminations followed for pregnancy diagnosis, over a period of time. Different factors included in the analysis were agro-climatic zone, month of insemination, body condition score and order of parity in order to determine their effect on the conception rates. Eight agro-climatic zones *viz*. Eastern plains, Bundelkhand, Central western plains, Central plains, North eastern plains, Western plains, Vindhyan and South western semi-arid plains as described by Jha and McKinley (2014) were included as a separate class. Seasonal variation was captured by month of inseminations as a separate class. Order of lactation was group in heifer, first to forth lactations and five and more than five lactations. Body condition score were categorized as 1 to 5 on the basis of visible ribs 1): All ribs exposed: 2): Three ribs exposed: 3): Two ribs exposed: 4): One rib exposed: 5): No rib exposed:

Data processing and statistical analysis

The artificial insemination data was trimmed in order to remove outliers. Agro climatic zones with less than 100 inseminations records were removed. Vindhyan agro climatic zone was removed (Due to 19 inseminations). Conception rate follows the binary data structure having only one of the two possibilities namely, success or failure. To handle such binary data structure, logistic regression model is found to be a better choice (Dyke and Patterson, 1952; Ron *et al*., 1984 and Hosmer and Lemeshow, 1989) and hence adopted in the present studies. To investigate the differences in conception rate between different sub classes of independent variables, a binary logistic regression model was constructed with conception rate as the dependent variable and different independent variables of interest along with an interaction effect was included. It was observed that body condition score was found non-significant, hence removed from the analysis. All the procedure of data cleaning and statistical analysis was performed using R statistical software (R Core Team, 2021).

The statistical model used in the study is given below:

$$Y_{ijkl} = \mu + A_i + P_j + M_k + e_{ijkl}$$

Where,

Y_{ijk} = Conception rate (a binary trait with value 0= not conceived, 1= conceived).

μ = Overall mean.

A_i = Effect of the i^{th} agro-climatic zones (i= 1 to 7).

P_j = Effect of the j^{th} parity (j= 1 to 6).

M_k = Effect of the k^{th} month of insemination (j= 1 to 12).

e_{ijkl} = Random error associated with the Y^{ijkl}.

The regression coefficients were derived to odds ratio. The probability was calculated as odds ratio divided by (1 +

odds ratio). The figure of 0.5 under relative probability indicates the reference figure for comparison with others as chosen by the Logit Regression Analysis method. The figures are odd ratio of Success (or Failure)/ Number of events, *viz*. Conceived (or Not Conceived)/ Number of AI.

RESULTS AND DISCUSSION

The overall conception rate of sex sorted semen was 43.04±0.34 per cent. The sex ratio of female to male was found to be 91.20: 8.80. Lu *et al*. (2015) studied conception rate in 4,521 river-type buffaloes inseminated by sex sorted semen of Murrah and Nili-Ravi buffalo bulls and found conception rate of 48.5 percent with 86.6 percent of sex ratio. The results are presented in Table 1.

Factors influencing conception rate

Agro climatic zones

The conception rate of sex sorted semen was highly affected ($p<0.01$) by agro climatic zones. The Bundelkhand zone was considered as a reference and other classes were compared with it (Table 1). The probability of high conception rate was found in agro-climatic zones of Central plain, Central western plain and Eastern plain while low conception rate is observed in remaining agro-climatic zones. The lowest probability was obtained in North eastern plain and highest in Central western plain zone. The difference in the conception rate in different agro climatic zones could be due to differences in the management practices carried out by farmers, skill of AI technicians, veterinary services available in the given areas *etc*. A study conducted by Singh *et al*. (2018) elaborated the difference in dairy management the practices followed in different agro-climatic zones in Uttar Pradesh. It was observed that lack of information on scientific breeding understanding, preventive and control measures for various diseases, deficient in knowledge of improved farming practices in western plain, north eastern plain and Bundelkhand region. The lack of knowledge on scientific dairy practices and breeding in Bundelkhand and North eastern plain can be understood from the fact that most of the districts coms from low agriculture and farming region in comparison to the other agro-climatic zones. Apart from these, difference between the experienced and less

Table 1: Class of variables, number of inseminations, raw conception rate, odds ratio, probability. .and estimated conception rate in cows conceived by sex sorted semen.

Levels of variable	No of observations	Raw means	Odds ratio	Probability
		Agro-climatic zones**		
Bundelkhand	17803	42.51±0.01^{ab}	1.00	0.50
Central plain	1916	46.92±0.01^{a}	1.15	0.53
Central western plain	664	47.13±0.02^{a}	1.20	0.55
Eastern plain	492	47.56±0.02^{a}	1.13	0.53
North-eastern plain	190	38.50±0.03^{b}	0.91	0.47
South-western semi-arid plain	170	44.11±0.04^{ab}	0.97	0.49
Western plain	189	40.79±0.04^{ab}	0.80	0.44
		Order of parity*		
Heifer	5729	35.80±0.01^{b}	0.67	0.40
Parity_1	3653	45.80±0.01^{a}	1.00	0.50
Parity_2	4714	46.01±0.01^{a}	1.02	0.50
Parity_3	4299	46.12±0.01^{a}	1.02	0.50
Parity_4	2151	44.95±0.02^{a}	0.98	0.49
Parity_5 and above	878	46.22±0.02^{a}	1.04	0.51
		Month of insemination**		
January	3023	45.74±0.01^{ab}	1.00	0.50
February	1642	49.26±0.02^{a}	1.14	0.53
March	402	46.51±0.03^{ab}	1.05	0.50
April	85	41.17±0.05^{b}	0.78	0.44
May	86	41.86±0.05^{b}	0.76	0.43
June	84	45.23±0.03^{ab}	0.93	0.48
July	253	39.13±0.02^{b}	0.75	0.42
August	726	39.53±0.01^{b}	0.79	0.44
September	2205	39.77±0.01^{b}	0.82	0.45
October	3777	42.46±0.01^{b}	0.91	0.48
November	4854	42.23±0.01^{b}	0.90	0.47
December	4287	42.96±0.01^{b}	0.91	0.48

Significance level- **: $p<0.01$; *: $p<0.05$; NS: $p>0.05$. Classes with different superscripts within a variable differ significantly.

experienced AI technicians' skill could make difference in conception rate.

Order of parity

The order of parity had a significant ($p<0.05$) effect on the conception rate of sex sorted semen. The first parity was considered as a reference class (Table 1). It was observed that the heifers showed very low odds ratio and subsequently low probability conceiving than the other parities. While other parities showed more or less similar probabilities of conception rate. This result could be stated with an explanation on preferential treatment of lactating animals over non-lactating animals (Bhave *et al*., 2022). For a farmer in rural India, a lactating animal is a source of their livelihood. The lactating animals are preferentially fed with green and dry fodders supplemented with concentrate and mineral mixture unlike that of non-lactating animals which are non-productive. An important fact behind lower fertility in heifers observed in this study could be due to insemination before reaching the optimum weight at maturity. In rural field conditions data on weight of animals is not available and farmers are in a haste to impregnate their animals. It was observed that farmers often insisted on insemination of heifer irrespective of their body weight. Moreover, similar pregnancy rates were seen in multi-parous buffaloes regardless of the number of their parities (Lu *et al*., 2015).

Apart from these reason, low Body condition scores (BCS) due to poor nutrition management in heifer could be reason for the low conception rate (López-Gatius *et al*., 2003). Also, a study conducted by López *et al*. (2018) reported that the heifers with lightest weaning weight were found to have low conception rate (approximately 13%) than the heifers with heaviest weaning weight. Unfortunately, data related to body weight and BCS are rarely recorded but could be consider for recording in future studies.

Month of insemination

The month of insemination had significant ($p<0.01$) effect on the conception rate of sex sorted semen. The April month was considered as a reference class (Table 1). A peculiar trend of conception rate could be observed over the months. There was gradual increase in the probability of conception rate from August onwards until February, while later the conception rate started reducing until July except for the June when it was slightly high. It was also observed that number of inseminations during March to July ranged from 84 to 402. The decrease of conception rates of in buffaloes likely was due to inability to maintain normal body temperature under heat stress conditions due to higher internal heat production associated with lactation. Aliston *et al.* (1965) reported a low fertility in cows following exposure to high environmental temperatures having detrimental effects on developing embryos.

Calving difficulties

A total of 1,400 calvings were followed out of which, 35 abortions (2.50 per cent), 25 still births (1.78 per cent), 3 dystocia (0.21 per cent) and 1337 (95.50 per cent) buffaloes had normal calving. The incidence of normal calving was in close accordance with findings of Lu *et al*. (2015). The higher percentage of female calves led to reduced calving difficulties.

CONCLUSION

With the conception rate of 43 per cent and female to male sex ratio of 91:09, the application of sex sorted semen looked promising. The buffaloes with higher parities should be preferred over heifers while inseminating with sex-sorted semen. The inseminations during cooler months seemed reasonable, as the conception rate was better than summer months. The strategy of using sex-sorted semen could reduce the burden of unwanted male calves by increasing the proportion of female calves, which will be used as replacement stock.

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REFERENCES

Alliston, C.W., Howarth, B., Ulberg L.C. (1965). Embryonic mortality following culture *in vitro* of one- and two-cell rabbit eggs at elevated temperatures. Journal of Reproduction and Fertility. 9: 337340.

BAIF Development Research Foundation, (2021). Available at http://www.baif.org.in/.

Bhave, K., Koilpillai, T.P.J., Gaundare, Y., Shirsath, T., Potdar, V., Swaminathan, M., Khadse, J., Ragothaman, V. (2022). Genetic and non-genetic factors affecting conception rate of frozen semen in small holder dairy farmer system of rural India. Tropical Animal Health and Production. 54(1): 9-13.

Campanilea G., Gasparrinia, B., Vecchioa, D., Negliaa, G., Senatoreb, E.M., Bellac, A., Presicceb, G.A., Zicarellia, L. (2011). Pregnancy rates following AI with sex sorted semen in Mediterranean Italian buffalo heifers (*Bubalus bubalis*). Theriogenology. 76: 500-506.

Cerchiaro, I., Cassandro, M., Dal Zotto, R., Carnier, P., Gallo, L. (2007). A field study on fertility and purity of sex-sorted cattle sperm. Journal of Dairy Science. 90(5): 2538-2542.

Dyke, G.V. and Patterson, H.D. (1952). Analysis of factorial arrangements when the data are proportional. Biometrics. 8: 1-12.

GoI Report, (2021). Department of animal husbandry and dairying. Livestock census and integrated sample survey. Documents. Annual report 2020-21. pp. 5. Available at https://dahd.nic.in/.

González-Marín, C., Góngora, C.E., Gilligan, T.B., Evans, K.M., Moreno, J.F. and Vishwanath, R. (2018). *In vitro* sperm quality and DNA integrity of SexULTRA™ sex-sorted sperm compared to non-sorted bovine sperm. Theriogenology. 114: 40-45.

Hosmer, D.W. and Lemeshow, S. (1989). Applied Logistic Regression. John Willey and Sons, New York.

Jha, K.K. and McKinley, C.R. (2014). Demography and ecology of Indian sarus crane Grus antigone antigone in Uttar Pradesh, northern India. Asian Journal of Conservation Biology. 3(1): 8-18.

López, E., Véliz, F.G., Carrillo, E., De Santiago, Á., García, J.E. and Mellado, M. (2018). Effect of birth weight, weaning weight and pre-weaning weight gain on fertility of Holstein heifers under hot Mexican conditions. Slovenian Veterinary Research. 55(1): 35-42.

López-Gatius, F., Yániz, J., Madriles-Helm, D. (2003). Effects of body condition score and score change on the reproductive performance of dairy cows: a meta-analysis. Theriogenology. 59(3-4): 801-812.

Lu, Y., Ming, Z., Shengsheng, L., Dianxin, X., Weihong, H., Bing, M., Huiyan, X.,Kehuan, L. (2010). Sex-preselected buffalo (*Bubalus bubalis*) calves derived from artificial insemination with sex sperm. Animal Reproduction Science. 119: 169-171.

Lu, Y., Yanqiong, L., Ming, Z., Bingzhuang, Y., Xianwei, L., Xiaogan, Y., Shengsheng, L., Zhuyue, W., Huiyan, X., Yunbin, L., Kehuan, L. (2015). A field study on artificial insemination of swamp and crossbred buffaloes with sex-sorted semen from river buffaloes. Theriogenology. 84(6): 1-6.

NBAGR Monograph Report, (2006). Buffalo genetic resources of India: Murrah. pp.15. https://nbagr.icar.gov.in/wp-content/uploads/2020/02/Murrah-Buffalo.pdf.

Niemann, H., Kuhla, B., Flachowsky, G. (2011). Perspectives for feed efficient animal production. Journal of Animal Science. 89: 4344-4363.

R Core Team, (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. (Version: 4.1.0.). https://www.R-project.org/.

Ron, M., Bar-Anan, R., Wiggans, G.R. (1984). Factors affecting conception rates of Israeli Holstein cattle. Journal of Dairy Science. 67: 854-860.

Shashi, S., Dhirendra, K., Mandal, K.G., Taggar, R.K., Das, A.K. (2014). Factors influencing the dry period and calving interval in different grades of buffaloes. Buffalo Bulletin. 33(1): 120-126.

Singh, P.K., Tiwari, R., Dutt, T. (2018). Dairy husbandry information needs of farmers in different agro-climatic zones of Uttar Pradesh. Indian Journal of Animal Sciences. 88(8): 972-976.