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Performance of buffalo sex sorted semen in the southern states of India

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Abstract
The present study was planned with the objective to investigate the performance of buffaloes bred with sex sorted semen in Southern states of India. The data used in the present investigation were collected from 1,037 villages in the jurisdiction of 130 Cattle Development Centres (CDC) being operated in 128 tehsils of 25 districts in Andra Pradesh, Karnataka and Telangana states of Southern India. A total of 7,784 artificial inseminations were performed on 7,248 buffaloes owned by 6,300 farmers during the years October 2019 to September 2023. The effect of different factors such as state, animal owner gender, animal breed, lactation order, heat stage at the time of sex sorted A.I., animal body condition score, sex sorted A.I. season and economic status of animal owner was assessed on the conception rate of buffaloes which were inseminated with the sex sorted semen. Logistic regression analysis was used to compute the odds ratio and probability of conception rate. The results revealed that the overall mean conception rate was 40.54±0.01 percent and it was recorded significantly higher in buffalo animals from Telangana state (41.54%), animals bearing forth lactation (53.56%), animals exhibiting mid heat (43.86%), buffaloes having two ribs exposed (41.78%) and buffaloes inseminated during winter season (42.53%), and conception rate non-significantly recorded highest in animals reared by female owners (42.16%), Pandharpuri buffaloes (46.15%) and animals owned by below poverty line families (40.88%). Statistical analysis of data showed that state, lactation order, heat stage at the time of sex sorted A.I., animal body condition score and sex sorted A.I. season had a significant effect on conception rate of buffaloes from Sothern states of India inseminated with sex sorted semen.

Keywords: Southern states, buffalo, sex sorted semen, field conditions, artificial insemination, conception rate, sex ratio, logistic regression

Introduction
The sex sorted semen is a technology that benefits the farmer community by hastening the production of replacement heifers, reducing dependency on purchase of heifers, reducing the number of male calves, increasing milk production, and better utilizing constrained feed and fodder resources. The use of buffalo sex-sorted semen results in the birth of female up to 90 percent compared to non-sexed semen, which could result in the birth of females' maximum up to 50 percent. The additional heifer calves will help to expand the buffalo dairy herd at a faster pace compared to the use of conventional semen. The fertility of dairy animals is mainly governed by the genetic potential and environmental combinations including nutrition, health, and overall management adopted by farmers and percent conception rate is the accepted indicator for assessing the fertility of farm animals. The available literature on conception rate of buffalos especially bred with sex sorted semen A.I. is inadequate for deciding fertility strategy of such buffaloes at village level. Therefore, an attempt was made in the present investigation to study the performance of sex sorted semen A.I. in buffaloes under field conditions of southern states of India.

Materials and Methods
With financial support from the different agencies in southern states of India the Artificial Insemination (A.I.) program is being operated through BAIF-BISLD performing as a role of service provider. During the period of October 2019 to September 2023, a total of 7,784 buffalo A.I.s' were performed on 7,248 animals owned by 6,300 farmers spread over 1,037

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villages in the jurisdiction of 130 Cattle Development Centres (CDC) in 128 tehsils and 25 districts. All animals were maintained and reared by the individual farmers. The housing ranged from open to permanently constructed sheds. Animals were semi stall-fed with dry and green fodder along with some concentrate feed. The calls for A.I. received through mobile phones and animals were inseminated with sex sorted frozen semen at the doorstep of farmers. Buffaloes not repeated within 90 to 120 days post insemination were examined for conception confirmation by rectal palpation. The information on states (Andhra Pradesh, Karnataka, Telangana), animal owner gender (Male, Female), animal breed (Jaffarabadi, Murrah, N.D. buffalo, Pandharpuri, Surti), lactation order (heifer, 1, 2, 3, 4, 5, 6 & above), heat stage (Late, Mid, No observation), animal body condition score (All ribs expose, No rib expose, One rib expose, Two ribs expose, Three ribs expose), A.I. season (Rainy, Winter, Summer) and economic status of animal owner (APL, BPL) was compiled for studying the performance of buffalo sex sorted semen.

Statistical analysis: Conception is a binary trait having only one of the two possibilities namely, success or failure. The most commonly used multiple analysis techniques pose difficulty when the dependent variable has only two outcomes *viz.* event occurring or not occurring. In such a dataset, the assumption of normal distribution and equality of variances are violated. However, the logistic regression model is found to be a better choice and have used a binary logistic regression model for predicting the probability of conception rate in artificially inseminated buffaloes with sex sorted semen. The data was analyzed using the R project for statistical computing software (version 4.3.2).

Results and Discussion
The mean conception rate in artificially inseminated buffaloes with sex sorted semen was recorded as 40.54 ± 0.01 percent which was similar to that obtained (40.00%) by Sawant *et al.* (2022) [10] in buffaloes bred with sex sorted semen, however, much less compared with the finding of Guangsheng *et al.* (2013) [7], Bhave *et al.* (2016) [4], Gokhale and Bhagat (2000) [6] and Syed and Bhagat (2022) [12] who reported an overall conception rate of 52.08, 48.01, 51.84, 45.84 percent, respectively in buffaloes inseminated with the conventional semen. The Central Institute of Research on Buffalo reported 45.75 and 40% conception rates respectively in Murrah and Nilli-Ravi buffalo inseminated with the conventional semen. The other scientists Zicarelli *et al.* (1997) [14] reported the conventional semen conception rate as 42.5–51.1 percent. Thirunavukkarasu and Kathiravan (2009) [13] noticed much less conceptions in buffaloes (25.52%) inseminated with the conventional semen. The sex sorted semen conception rate in present investigation was studied in relation to the following factors;

State: The state had a highly significant effect (*p*<0.01) on the conception rate of buffaloes inseminated with sex sorted semen. The highest percentage of buffaloes covered under sex sorted A.I. (69.36) and conception rate (41.54) was recorded in Telangana state compared with the other two states under study (Table-1). The estimated highest conception rate was recorded from the buffaloes of Andhra Pradesh state (39.44%). The individual farmers' management and agro-climatic conditions of the respective states including feed and fodder availability to the animals might be attributed to significant differences in the conception rate of buffaloes inseminated with the sex sorted semen.

Table 1: States affecting conception rate in sex sorted A.I. bred buffalo

State **	N	Raw mean	SE	% coverage	odds ratio	Relative Probability	Estimated CR%
Andhra Pradesh	1468	39.44%	0.01	18.86	1	0.5	39.44
Karnataka	917	36.42%	0.02	11.78	0.67	0.40	31.76
Telangana	5399	41.54%	0.01	69.36	0.79	0.44	34.91

Animal owner gender: Women are known to be actively involved in handling multi-faceted role in different day to day management practices of dairy animal keeping especially in buffaloes, their involvement in livestock activities range from caring of young calves to grazing of animals, collection of fodder, cleaning of animal shed and milking utensils, milking of animals, making dung cakes etc. Swaminathan (1995) [11] noticed that nature and extent of rural women involvement in dairy farming vary greatly from region to region. In the present study the sex sorted semen conception rate of buffaloes owned and reared by woman folk noticed to be higher (41.26%) compared with the male animal owners (40.52%) although it was non-significant (Table-2). The sex sorted A.I. coverage was highest in animals owned by males (97.14%).

Table 2: Animal owner gender affecting conception rate in sex sorted A.I. bred buffalo

Animal owner gender	N	Raw mean	SE	% coverage	odds ratio	Relative probability	Estimated CR%
Female	223	41.26%	0.03	2.86	1	0.5	41.26
Male	7561	40.52%	0.01	97.14	0.96	0.49	40.50

Animal breed: More than three fourth inseminations (75.48%) were performed from Murrah breed of buffaloes and it was highest coverage among all the buffalo breeds under study however, the probability of conceiving the buffaloes with sex sorted semen was recorded highest in Pandharpuri and Surti buffaloes (each 0.65) and the actual conception rate recorded in these breeds was 46.15 ± 0.08 and 39.45 ± 0.05 percent, respectively (Table-3). Although conception rates within breeds recorded quite different statistically Murrah breed conceptions (40.19 ± 0.19%) differ from N.D. buffaloes (41.82 ± 0.1%), this might be due to the number of observations being relatively less in N. D. buffalo. Gokhale and Bhagat (2000) [6] and Syed and Bhagat (2022) [12] reported comparatively higher conception rate (41.36 ± 4.20 & 44.35 ± 0.29%, resp) in Murrah and N.D. buffalo (50.88 ± 0.85 & 49.22 ± 0.43%, resp) inseminated with conventional semen than that of the present investigation. Anzar *et al.* (2003) [2] and Sarkar *et al.* (2005) [9] reported much lower conception rates (31.30 and 33.19%) in Murrah buffaloes which were inseminated with traditional semen. The animal breed had non-significant effect on sex sorted semen inseminated buffaloes however, results of Gokhale and Bhagat (2000) [6]

and Syed and Bhagat (2022) [12] noticed significant effect of animal breed on conception rate of buffaloes which were inseminated with conventional semen.

Table-3: Animal breed affecting conception rate in sex sorted A.I. bred buffalo.

Animal breed	N	Raw mean	SE	% coverage	odds ratio	Relative probability	Estimated CR%
Jaffarabadi	20	30.00%	0.11	0.26	1	0.5	30.00
Murrah	5875	40.19%	0.01	75.48	1.60	0.62	36.93
N.D. Buffalo	1741	41.82%	0.01	22.37	1.56	0.61	36.58
Pandharpuri	39	46.15%	0.08	0.50	1.84	0.65	38.86
Surti	109	39.45%	0.05	1.40	1.82	0.65	38.75

Order of lactation: Animal order of lactation had very highly significant ($p<0.001$) effect on the conception rate of buffaloes bred with sex sorted semen (Table-4). The observations of Gokhale and Bhagat (2000) [6], Bhave *et al*. (2016) [4] and Syed and Bhagat (2022) [12] supported the present findings for the animals inseminated with conventional semen. Compared with multiparous animals, the lowest conception rate was observed in buffaloes having six and more lactations (31.59±0.03%). The findings of Gokhale and Bhagat (2000) [6] and Syed and Bhagat (2022) [12] supported the present results as they noticed fewer conceptions in old-age buffaloes inseminated with traditional semen. In heifers, the overall conception rate was recorded as 28.74±0.01 percent which was noticed to be much less in comparison with that of buffaloes having first to fifth lactations. The lower conception rate in heifers noticed in the present investigation agreed with findings of Gokhale and Bhagat (2000) [6] and Syed and Bhagat (2022) [12]. The lower conceptions in heifers might be due to farmers paying more attention to the animals which were under production. In multiparous buffaloes, the likelihood of getting more pregnancies with sex sorted semen was noticed in forth lactation animals (0.69), followed by fifth (0.66), first (0.62), third (0.60), and second (0.50). The author Syed and Bhagat (2022) [12] noticed likelihood of more pregnancies in first lactation animals (0.54), followed by second (0.50), fourth (0.48), while third and fifth lactation was at par (0.46). The percentage coverage of sex sorted A.I. was highest in buffaloes having second lactation (25.86), followed by third lactation (24.91), heifer (15.65), first (14.02), and forth (10.82). The remaining buffaloes having fifth and six and more lactation recorded less than 5% coverage under sex sorted A.I.

Table-4: Lactation order affecting conception rate in sex sorted A.I. bred buffalo.

Lactation order ***	N	Raw mean	SE	% coverage	odds ratio	Relative probability	Estimated CR%
6 & more	320	31.56%	0.03	4.11	1	0.5	31.56
Fifth	361	47.65%	0.03	4.64	1.91	0.66	41.44
First	1091	46.01%	0.02	14.02	1.63	0.62	39.11
Forth	842	53.56%	0.02	10.82	2.25	0.69	43.72
Heifer	1218	28.74%	0.01	15.65	0.76	0.43	27.30
Second	2013	36.91%	0.01	25.86	1.00	0.50	31.56
Third	1939	43.17%	0.01	24.91	1.48	0.60	37.63

Heat stage at the time of Sex sorted A.I.: The deficiencies of various trace minerals, inadequate vitamin intakes, energy/protein imbalances, and excessive protein intake could lead to infertility and poor reproductive performance reflected through the poor expression of estrus symptoms (Balakrishnan, 2003) [3]. The nature and variation in expression of estrus behavior had a significant ($p<0.05$) effect on the conception rate in buffaloes bred with sex sorted semen (Table-5). The probability of getting more animals pregnant was noticed in mid heat animals (0.64) but coverage of animals was highest (84.90%) in animals in which no observations were recorded. The late heat animals' coverage was less than 2 percent and conception rate was also recorded lowest as 31.52±0.05 percent. Syed and Bhagat (2022) [12] noticed higher conception rate (46.64±0.43%) in Telangana state buffaloes bred with conventional semen in which no observations were recorded. The conceptions of mid and late heat buffaloes recorded by Syed and Bhagat (2022) [12] were 28.99±1.37 and 45.72±0.47 percent, respectively.

Table-5: Heat stage affecting conception rate in sex sorted A.I. bred buffalo.

Heat stage at the time of sex sorted A.I. *	N	Raw mean	SE	% coverage	odds ratio	Relative probability	Estimated CR%
Late heat	92	31.52%	0.05	1.18	1	0.5	31.52
Mid heat	1083	43.86%	0.02	13.91	1.75	0.64	40.11
No observation	6609	40.13%	0.01	84.90	1.64	0.62	39.18

Animal body condition score: The body condition score of animals provides an objective evaluation of the amount of fat covered on the body disregarding animals body frame size and indicates the status of overall animal management adopted by farmers but having involved more technicalities, it requires skill and experience (Anitha *et al*., 2005) [1]. To overcome this problem a simple method of visualization of exposure of ribs has been applied for studying its effect on conception rate of buffaloes bred with sex sorted semen. All animals under study were divided into five subgroups *viz*. no ribs exposed, exposure of one rib, exposure of two ribs, exposure of three ribs, and exposure of all ribs. The study results indicated that a significantly ($p<0.01$) higher conception rate (41.78±0.01%) was recorded in buffaloes having two ribs exposed but the percentage of animals covered under sex sorted semen A.I. was highest (43.09) in buffaloes having three ribs exposed (Table-6). It was further noticed that the lowest conception rate (26.54±0.03%) was recorded in buffaloes having no ribs were exposed.

Table-6: Animal body condition score affecting conception rate in sex sorted A.I. bred buffalo.

Body condition score **	N	Raw mean	SE	% coverage	odds ratio	Relative probability	Estimated CR%
All ribs expose	793	36.82%	0.02	10.19	1	0.5	36.82
No rib expose	260	26.54%	0.03	3.34	0.52	0.34	25.34
One rib expose	91	34.07%	0.05	1.17	1.01	0.50	36.93
Three ribs expose	3354	41.47%	0.01	43.09	1.25	0.56	40.90
Two ribs expose	3286	41.78%	0.01	42.21	1.28	0.56	41.41

Sex sorted A.I. season: The study results indicated that maximum (50.41%) sex sorted semen inseminations were performed in the Winter season (November to February), followed by 32.86 percent in the summer season (March to June), and lowest 16.73 percent in the rainy season (July to October). The sex sorted A.I. season had very high significant ($p<0.001$) effect on the conception rate in buffaloes bred with sex sorted semen. These results agreed with the findings of Gokhale and Bhagat (2000) [6]. The animals inseminated in the winter season recorded the highest conception rate ($42.53\pm0.01\%$) compared with animals inseminated in the other two seasons. The study results of Gokhale and Bhagat (2000) [6] supported the present investigation findings of higher conception rate in winter season for buffaloes bred with conventional semen.

Table-7: Sex sorted A.I season affecting conception rate in sex sorted A.I. bred buffalo.

Sex sorted A.I. Season ***	N	Raw mean	SE	% coverage	odds ratio	Relative probability	Estimated CR%
Rainy	1302	36.79%	0.01	16.73	1	0.5	36.79
Summer	2558	39.41%	0.01	32.86	1.11	0.53	38.66
Winter	3924	42.53%	0.01	50.41	1.27	0.56	41.22

Economic status of animal owner: The economic status of the animal owner did not affect the conception rate in buffaloes bred with sex sorted semen (Table-8). More than half (61.00%) inseminations were performed in above-poverty line (APL) farmer animals, but buffalo animals owned by below-poverty line (BPL) category groups of farmers recorded higher conception rate ($40.88\pm0.01\%$). The higher conception rate in BPL section of society revealed their major livelihood dependence on animals resulting in caring for animals. Anzar *et al*. (2003) [2] reported from Punjab province of Pakistan higher reproductive performance which apparently was attributed to the availability of green fodder, supply of adequate ration, and condition of farmers resulting in overall better management of dairy animals with commercial views.

Table-8: Economic status of animal owner affecting conception rate in sex sorted A.I. bred buffalo.

Economic status of animal owner	N	Raw mean	SE	% coverage	odds ratio	Relative probability	Estimated CR%
APL	4748	40.33%	0.01	61.00	1	0.5	40.33
BPL	3036	40.88%	0.01	39.00	1.02	0.50	40.72

Cavling status: During the study period a total of 1,986 calvings have occurred of which 86.86 percent were normal calving, 0.15 percent dystocia, 9.21 percent animals were sold before calving, 0.55 percent animals were transferred to other places, 0.45 percent animals died, and 2.77 percent animals were aborted (Table-9). The overall female-to-male sex ratio was noticed to be as 92:8. The sex ratio observed in the present investigation is differed to the findings of Ingawale *et al*. (2022) [8] who recorded a female-to-male sex ratio of 88:12 for buffalo sex-sorted semen, however Sawant *et al*. (2022) [10] noticed 100 female birth with the sex sorted semen in buffaloes with small number of data set of only four pregnancies.

with sex sorted semen was significantly affected within states, due to lactation order, heat stage at the time of sex sorted A.I. animal body condition score, and sex sorted A.I. season these factors need to be emphasized for having better conception rate in sex sorted A.I. bred buffaloes under field conditions of Southern states of India. The overall female to male sex ratio was noticed as 92:8.

Table-9: Calving status

Particulars	Numbers	Percent to total calving
Normal calving	1725	86.86
Dystocia	3	0.15
Sold	183	9.21
Transfer	11	0.55
Died	9	0.45
Abortion	55	2.77
Total calving	1986	Percent to normal calving
Male calves born	135	8
Female calves born	1590	92

Conclusion
The study indicated that the conception rate of buffaloes bred

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