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KVK, GUNTUR IMPACTFUL INTERVENTION WITH SEX-SORTED SEMEN: A FIELD STUDY ON CONCEPTION PERFORMANCE AND FEMALE CALF YIELD IN BUFFALOES (SEX SORTED SEMEN IN GRADES MURRAH BUFFALOES)

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ABSTRACT

A field study was conducted under the impactful interventions of Keishi Vigyan Kendra (KVK), to popularise and evaluate the performance of Sex Sorted Semen (SSS) in Graded Murrah (GM) buffaloes under farmer managed field conditions of Guntur District, Andhra Pradesh. A total 600 doses of SSS straws derived from three breeding bulls (Mahabali, GM401, GM404) were used between November 2023 to June 2024 across two adopted villages of KVK. The inseminations were carried out as per the guidelines given by Andhra Pradesh Livestock Development Agency (APLDA) in enrolled GM buffaloes receiving either single or double dose of SSS straws. Pregnancy diagnosis was done between 80 to 100 days post insemination and animals were monitored for possibility of dystocia, still births and calf sex. Out of 393 inseminated buffaloes, 139 animals were found pregnancy positive (PD +ve) resulting in birth of 120 female and 13 male calves while six pregnant animals were sold before calving. Conception rates ranged from 25 to 38.71% across months, with a tendency of higher success (PD +ve) in double insemination as compared to single inseminations. Bull wise conception rate varied from 25 to 38.9% reflecting interactive effect of bull efficiency, seasonal variations, time of inseminations and insemination protocol. Overall, this trial achieved a conception rate of 35.4% with 90.2% female and 9.77% male calf births. Minimum reproductive complications were recorded (1.44% dystocia, 2.16% abortion cases and no still births or premature calves). These findings confirm the practical utility of SSS in reliably skewing the sex rate towards female calves, there by supporting herd replacement and sustainable dairy productivity under field conditions.

Keywords: *Bubalus bubalis*, buffaloes, sex sorted semen, graded Murrah buffalo, conception rate, female calf, insemination

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INTRODUCTION

Livestock production systems have been revolutionized globally with the introduction of assisted reproductive technologies (ASTs). In India, artificial insemination (AI) has significantly transformed dairy sector by improving the genetic potential through rapid dissemination of superior germplasm. AI technique provided easy access to high quality semen from progeny tested sires without the burden of maintaining the high potential breeding males at farms and aided in accelerate the herds 'genetic potential and reproductive efficiency. Despite the remarkable progress, Indian dairy farmers who typically maintain small and medium scale dairy farms, continue to face persistent challenges such as the frequent birth of male calves with limited utility in milk-oriented production and marketing systems affecting farm profitability in terms of feeding, labour and maintenance cost of male calves (Sharma *et al*., 2024).

To address these challenges Sex Sorted Semen (SSS) emerged as a transformative innovation in the field of ASTs with assured birth of female calves to sustain herd replacement and enhance milk productivity (Weigel, 2004). Sexed semen/sex sorted semen refers to semen in which natural proportions of X- and Y- bearing chromosomes have been altered based on sorting and selection processes (Johnson and Welch, 1999). Several techniques have been developed for sperm sexing such as albumen gradient separation, binding with sex specific antibodies, multitube swim-ups, gradient fractionation and free flow electrophoresis, FACS- florescence activated cell sorting (Cerchiaro *et al*., 2007). Among these techniques (FACS), a specialized flow cytometry is regarded as most reliable, and potentially cost-effective technique (Welch and Jhonson, 1999). Combining the SSS technology with the popular AI technique farmers can get benefitted with desired offsprings with higher productivity that can act as a replacement stock reducing the burden of male calves (De Vries *et al*., 2008).

Globally research with SSS application have focused on dairy cattle with relatively scanty research attention to buffaloes (Campanile *et al*., 2011). This is particularly significant in Indian Scenario where buffaloes contribute nearly 49% of national milk production and forms the cornerstone of dairy economy. despite their critical role adaptation of AST in buffaloes has been slower as compared to cattle due to physiological differences and farmers reluctance. There is pressing need to generate field-based evidence on the application of SSS technology to support sustainable dairy herd development In India.

In India, National Dairy Development Board (NDDB) pioneered the application of SSS technology in high yielding dairy cattle alongside private firms such as ABS India (Sexcel) and sexing technologies (ST) (Sharma *et al*., 2024). To promote adoption several state governments subsidized the cost through Rashtriya Gokul Mission (RGM), A Government of India mission of promoting animal husbandry reducing the cost of each straw to Rs. 100 to 300/- as compared to the market price of Rs 1000-2000/-. As per the Ministry of Fisheries, Animal Husbandry and Dairying (Year End Review, 2022) approximately 6 million doses of SSS were produced in India (2.78 million from government units and 3.11 million from private and cooperative stations).

In Andhra Pradesh the Andhra Pradesh Livestock Development Agency APLDA introduced this technology was introduced in the year 2022 at a subsidised price of Rs. 500/- per

straws (50% subsidy). despite this, farmers initially expressed reluctance citing concerns regarding the possibility of male calf births, 10 times higher price of the SSS straws compared to conventional AI (Rs. 40/- vs.Rs.500/-) and fears reproductive complications as revealed in a field survey conducted by KVK, Guntur. Furthermore, there was limited field-based evidence on conception rates, sex ratio and calving outcomes in Graded Murrah (GM) buffaloes at practical farming situations. To address these gaps and generate practical data a trial was undertaken in Guntur district to evaluate the effectiveness of SSS in GM buffaloes under real farming conditions, while simultaneously creating awareness and addressing farmers apprehensions.

MATERIALS AND METHODS

Location selection

The study was conducted in Vallabhapuram and Munnangi villages of Kollipara mandal, Guntur district, Andhra Pradesh. Kollipara mandal is recognised as source of high producing Graded Murrah (GM) buffaloes with average body weigh exceeding 420 kg and an average milk production of 8.4±1 lt/day. The dairy farmers in this area were having vast experience in dairy farming with an ideal attitude of adopting innovative techniques. In addition, the area has abundant resources of feed and fodder owing to its location along the fertile Krishna riverine belt (Lakshmi *et al*., 2024). Considering these factors the current study was executed in this location of Guntur district.

Procurement and maintenance of SSS straws

For the study 600 SSS straws were procured by KVK, Guntur from District Livestock Development Authority (DLDA), Chebroule and transported in liquid nitrogen to Veterinary Dispensary (VD), Vallabhpuram, where liquid nitrogen storage facilities were available. Flow cytometrically segregated sperms carrying X chromosomes in SSS straws were supplied from DLDA in 4 different batches and the labels on the straws belong to 3 bulls i.e. Mahabali, GMB401, GMB404.

Record maintenance

A detailed record of SSS inseminations was maintained at Veterinary Dispensary, Vallabhapuram consisting of the information of farmers name, buffalo tag no, age, stage of lactation, no. of parturitions, bull number of SSS straws, no. of inseminations, Pregnancy status, and birth of calves, sex of calves. A separate repeat animal data was also maintained for the animals that exhibited oestrous symptoms after first and second SSS insemination.

SSS Insemination

The SSS straws were inseminated to GM buffaloes in accordance with the APLDA guidelines to increase the conception. The following criteria were considered before selecting the animals for insemination.

1. The selected animal should not have the history of abortions, endometritis.

2. The buffalo should be in 2^{nd}, 3^{rd} or 4^{th} lactation.

3. Insemination was done in GM buffaloes between 3 to 5 months after parturition.

4. Inseminations were done when the animals were in mid to late heat.

5. The body condition score (BCS) should be 2.5 to 3.

The selected animals were initially

inseminated with single dose of SSS straw. Those that did not conceive with first insemination were and exhibited heat symptoms were re inseminated with second dose of SSS straw during the late heat and classified under double inseminations. If the animals returned to oestrus even after second insemination, the possibility of endometritis was assessed by examining the uterine discharge collected using the AI gun. Only healthy and infection free animals were inseminated with third dose. Animal failed to conceive even after 3rd dose were excluded from the current study. In few cases, double inseminations (12 to 24 h interval) were carried out based on practical field requirements to accommodate farmer preferences, and these animals were also listed under double insemination category.

Pregnancy diagnosis and birth of calves

Animals inseminated with SSS straws were examined for the pregnancy status between 80 to 100 days post insemination by rectal palpation. The pregnancy diagnosis information was entered in the register and the animals with confirmed pregnancy were further monitored until calving. The buffalo owners were advised to monitor the animals closely for signs of abortion, parturition and any signs of dystocia 20 to 30 days prior to the expected date so that immediate veterinary aid could be provided. The health status and sex of calves born were entered in the register to determine the % calving and male calves born through SSS straws.

Data collection and presentation

As the current study was undertaken at real farming situations of farmer managed conditions, we found difficulties in maintaining uniformity in several variables like age, parity of animal, milking status, feeding conditions etc. influencing the outcome. In addition to these facts sex sorted semen (SSS) straws used in the current study were sourced from three different bulls causing unavoidable heterogeneity in semen quality and fertility potential. Considering these practical limitations, the recorded data was unable to meet the robust statistical modelling and comparative analysis. Instead, the collected information was consolidated top reflect the field level performance trends of SSS inseminations. The mean conception % following single and double inseminations overall conception %. Bull wise conception, month wise information and sex ratio of calves born was consolidated and tabulated.

RESULTS AND DISCUSSIONS

A total of 600 SSS straws from three different bulls were used in this study to inseminate 393 GM buffaloes (Table 1). Of these 139 animals were confirmed pregnant, corresponding to an overall conception rate of 35.4%. The remaining animals fell in the category such as non-pregnant, repeat breeder even after triple insemination, those inseminated subsequently with conventional semen insemination, abortions cases, sold by the farmers before pregnancy diagnosis.

Inseminations included both single (n=65) and double/triple (n=325) attempts with double insemination being the predominant method practiced with 12 h interval in a single oestrous cycle. Among total conceptions (n=139) 20.4% occurred with single inseminations in first dose of SSS while higher proportion 38.9% was achieved through double inseminations administered at 12-hour intervals in a single oestrous cycle. First time repeat breeders contributed 37.4% of total

conceptions whereas triple inseminations (second time repeaters) contributed only 3.6%. These results indicate that double insemination protocols are more efficient than single insemination for improving the conception % with SSS inseminations. Given the inherent ability in reproductive biology/estrous behaviour of cattle and buffaloes, refinement of insemination strategies from single insemination to double inseminations at 12 h interval appears beneficial for improving the conception rate at small holder field conditions. The relatively modest conception rate of current study (35.4%) is consistent with global experiences using SSS in Italian buffalo heifers (37.7%; Campanile *et al*., 2011) and in Indian crossbred cattle (40%; Sharma *et al*., 2018).

Fertility with sexed semen is typically lower than that of conventional semen due to reduced sperm concentration (2 million per dose Vs 20 million in conventional semen straws; Seidel and Schenk, 2007) and physical stress during sorting which lowers sperm viability (Amann, 1999). A direct comparison between sexed and unsexed semen was precluded in this study as field data was used and unsexed semen from the same bulls was unavailable. Earlier research in mediterranean Italian buffaloes (Gaviraghi *et al*., 2013) indicated that a dose of 4 million sperms represents optimal compromise while using SSS with conventional insemination technologies unlike dairy cattle where 3 million sperms were considered adequate (Biswas *et al*., 2016). The conception percentage in the current study was relatively lower than the 46 to 52% reported in Murrah and Niliravi buffaloes (Lu *et al*., 2015) possibly reflecting the variations in breed, field level conditions and insemination protocols. Nevertheless, the improved observation with double inseminations in present study corroborates with earlier findings (Gaviraghi *et al*., 2013) that emphasised the importance of adjusting sperm dose and insemination strategy to maximise the fertility outcome.

Monthly inseminations in the current study ranged from 21 animals (June 2024) to 65 animals (December 2025) (Table 1). These variations were largely contributed from the season, and oestrous cycles of the animals. Climatic factors particularly temperature and seasonal variations are known to influence the conception rates (Kunavongkrit *et al*., 2005) and semen quality (Hirabhai *et al*., 2022). Despite such variability, conception rates remained consistent across months ranging from 25% (November 2023) to 38.7% (February 2024). The lower conception % in the month of November associated with exclusive use of single dose of SSS whereas higher conception in February coincided with double inseminations at 12 h interval suggesting the superiority of latter protocol. Minor variations across months might have been resulted from managemental factors such as time and site of insemination.

Bull wise conception efficiency further highlighted the variability (Table 1). The SSS straws of bull Mahabali was reported with lowest conception rate of 25% (Single inseminations done in November 2023), whereas GMB401 achieved the highest conception of 38.9% followed by GMB404 at 34.4%. These variations were largely attributed to seasonal influences (Hirabhai *et al*., 2022) and inseminator related skills rather than intrinsic bull fertility where all the three bulls were pre-screened, standardised for sperm concentration in the straws maintained under uniform storage and transportation conditions. Similar variations in conception rate due to non-genetic factors has been reported earlier (Lu *et al*., 2015). Overall, the pooled bull wise conception rate averaged at 32.8%. Notably, Cerchiaro *et al*., 2007 also

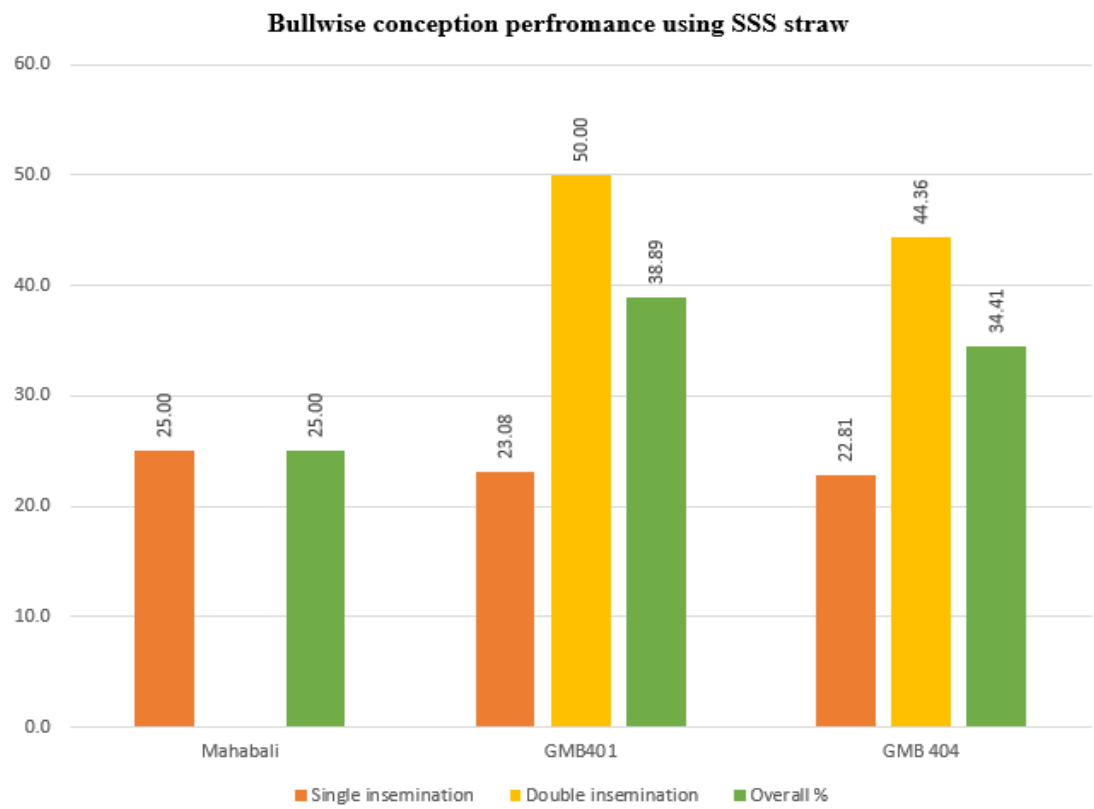

Figure 1. Bull wise conception performance using sex sorted semen straws straws.

Table 1. Insemination and conception % in GM buffaloes using sex sorted semen straws.

S. No.	Month	Straws used	Animals inseminated	Singles inseminations	Double inseminations	Pregnancy diagnosis		Conception %	Sex of calves		
						Positive	Negative		Female	Male	Sold
1	November-2023	20	20	20	0	5	15	25.00	4	1	0
2	December -2023	82	65	48	17	23	42	35.38	17	4	2
3	January-2024	71	49	27	22	17	32	34.69	16	1	0
4	February -2024	57	31	5	26	12	19	38.71	12	0	0
5	March-2024	80	48	16	32	18	30	37.50	12	3	3
6	April-2024	79	50	21	29	19	31	38.00	18	1	0
7	May-2024	72	55	38	17	18	37	32.73	17	1	0
8	June-2024	39	21	3	18	8	13	38.10	8	0	0
9	In field	100	54	8	46	19	35	35.19	16	2	1
	Total	600	393	186	207	139	254	35.36	120	13	6

Table 2. Bull wise conception and calving data using sex sorted semen straws.

Bull name	Straws used	Single inseminations	Double inseminations	Total no. of animals inseminated	Pregnancy diagnosis		Conception %	No. of calves	Sex of calves		
					Positive	Negative			Female	Male	Sold
Mahabali	20	20	0	20	5	15	25.00	5	4	1	0
GMB401	200	52	148	126	49	77	38.89	49	38	5	4
GMB 404	380	114	266	247	85	162	34.41	85	78	7	2
	600	186	414	393	139	254	32.77	139	120	13	6

Table 3. Reproductive outcomes of GM buffaloes inseminated with Sex Sorted Semen straws under field conditions.

S. No.	Component	No. of animals reported	% of pregnancy +ve animals (n=139)	% of Enrolled animals (n=393)	% of straws used
1	Conception with single dose	28	20.4	7.12	4.67
2	Conception with double dose (12 hrs interval)	54	38.9	13.7	9.00
3	Conception with double dose (First time repeater)	51	37.4	12.98	8.50
4	Conception with triple dose (2nd time repeater)	5	3.60	1.27	0.83
5	Still births	Nil	0.00	0.00	0.00
6	Abortions (First trimester)	3	2.16	0.76	0.50
7	Dystocia	2	1.44	0.51	0.33
8	Premature calves	Nil	0.00	0.00	0.00
9	Male calf births	13	9.77	3.31	2.17
10	Female calf births	120	90.2	32.1	21.0
11	Calves born to sold animals	6	4.31	1.52	1.00
12	Total calvings	139	100	35.37	23.2

reported interactive effect of bull type and season of insemination on pregnancy outcomes (Figure 1.).

The inseminated animals were examined for pregnancy status between 80 to 100 days post insemination in animals that did not return to oestrous. Of 393 inseminations, 139 animals were confirmed pregnancy positive. Pregnancy outcomes further confirmed the safety of SSS in GM buffaloes with no still births reported three abortions (2.16%) and two cases of dystocia (1.44%). No premature births were recorded in current study. These findings were consistent with Chebel *et al*., 2010 who reported negligible cases of calving difficulties, assisted parturitions and retention of placenta in heifers that received sex sorted semen though they reported 8.8% of dead male calves. Collectively the present results indicate that SSS use in GM buffaloes does not predispose to significant reproductive complications under field conditions (Table 3).

Among of 139 reported pregnancies positive buffaloes, six animals were sold (4.31%) before calving. Excluding these 133 calvings were considered to analyse the sex ratio of calves yielding only 13 male calves (9.77%) and 120 were female calves (90.23%) corresponding to a sex ratio of 9:1. This outcome aligns with the earlier reports (Tuman *et al*., 2004; Cerchiaro *et al*., 2007; Chebel *et al*., 2010; Dejarnette *et al*., 2008) and exceeds the values reported in Indian cattle (82.14%) by Sharma *et al*., 2018. Such skewed ratio strongly validates the ability of SSS to reliably increase female calf production in GM buffaloes under practical field conditions. Crucially this directly addresses the farmers concern of availability of heifers as replacement stock (Weigel, 2004).

The field level strategy of SSS straw distribution by KVK Guntur coupled with farmers awareness programme, ensured participation reduced the financial burden and promoted equitable access to this technology. Collectively these demonstrate that SSS as a transformative tool in buffalo based dairy farming. In present study use of 600 doses of SSS, resulting in 35.4% conception rate and 90.2% female calf birth with minimum reproductive complications addressing the concerns of dairy farmers in adopting new technology. These findings confirms that when coupled with appropriate insemination protocols with good management practices, SSS can substantially enhance replacement stock, contribute to long-term productivity, profitability, and genetic advancement in dairy systems.

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