CONCEPTION RATE AND FOETAL SEX DETERMINATION IN CROSSBRED CATTLE ARTIFICIALLY INSEMINATED WITH SEX-SORTED FROZEN BULL SEMEN UNDER FIELD CONDITIONS: A COMPARATIVE STUDY[#]

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ABSTRACT

The present study was performed to test the fertility of commercially procured sex-sorted frozen bull semen straws in crossbred cattle of Thiruvallur district of Tamil Nadu under field conditions. A total of 60 crossbred heifers and 60 crossbred cows were inseminated with sex-sorted semen straws containing 2 x 10^6 X-bearing, frozen spermatozoa from six different bulls. Early pregnancy diagnosis was performed around 28-30 day post insemination using commercially available kits. The pregnancy rates were 31.67 per cent (19/60) and 30.00 per cent (18/60) in heifers and cows, respectively and were 40.9 (9/22), 30.77 (4/13), 27.27 (3/14) and 18.18 (2/11) between cows in first, second, third and fourth calving, respectively. The mean pregnancy rates between animals inseminated with Jersey crossbred and Red Sindhi sex-sorted frozen bull semen were 30.00±5.0 and 31.25±6.9, respectively. The conception rate was not statistically significant (P>0.05) between the groups. Foetal sex determination at 65-80 days post insemination using ultrasonography confirmed female foetus in all the pregnancies. Hence, the present field trial concludes similar pregnancy rates with the commercially procured X-bearing sex-sorted frozen semen straws in crossbred cattle along with foetal sex determination revealing female foetus in all pregnant animals.

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INTRODUCTION

Sexed semen technology being a latest breakthrough in the field of bovine reproduction is being mainly focused nowadays to increase the efficiency of breeding programs in dairy herds. Some of the upgrading factors like reduction in the number of cows needed for progeny testing,

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production of replacement dairy heifers from a handful population of genetically superior cows and crossbreeding of dairy cows with both dairy or beef bulls has made the use of sex-sorted semen more advantageous in the field of bovine breeding. Flow cytometric sperm sorting is being widely adapted nowadays in the large-scale commercial production of sex-sorted frozen bull semen, which is considered more cost-effective, reliable and fatest method of sperm sexing compared other traditional methods with unique separation accuracy of about 90 per cent.

The important steps in flow cytometric sorting reported to cause sperm damage are the optimization of sperm dilution rate, laser power, rotational change in the use of dye types and sorting pressure. The alterations in sperm membrane protein framework, integrity of sperm plasmalemma, acrosome and DNA and the capacitation-like effects produced by flow cytometric sorting serve to be the main causes of reduced conception rate in cattle. Other factors contributing to the success rate of sex-sorted semen at field level are sperm concentration used for insemination dose, site of semen deposition, temperature-humidity index, timing of insemination and bull fertility (Cerchiaro *et al.,* 2007; Bhalakiya *et al.,* 2018; Xie *et al.,* 2020).

In consideration to the above factors, the present field trial was conducted to assess or validate the fertilizing ability and purity of commercially procured sex-sorted frozen bull semen straws in oestrus synchronized heifers and cows of crossbred cattle population, under prevailing tropical conditions.

MATERIALS AND METHODS

Animals

A total of 60 crossbred heifers and 60 pluriparous crossbred cows at various parities in Thiruvallur district of Tamil Nadu, aged between 4-8 years, weighing approximately 300 kg, apparently healthy with normal reproductive status were utilized. The selected heifers and cows were screened for infectious diseases like Brucellosis, Johne's disease and Tuberculosis through TANUVAS developed diagnostic kit and intradermal testing. Deworming and mineral mixture supplementation to selected disease free animals was carried out and additional feeding was advised for one month.

Oestrus synchronization protocol

Both heifers and cows received Eazi-Breed™ CIDR® (Zoetis, US) containing 1.38 g of progesterone followed by 500 µg of Inj. PG Estro® (Cloprostenol, Hester Biosciences Ltd.) intramuscular on day 8. On day 9,Eazi-Breed™ CIDR® was removed and AI was performed with commercially procured sex-sorted frozen bull semen on days 11 and 12 with 2.5mL of Inj. Gynarich® (Buserelin, Intas Pharmaceuticals Ltd.) intramuscular on day 11. At the time of AI, both heifers and cows were randomly divided in to six groups of 10 each under each category. Each group of 10 animals in both heifers and cows categories received inseminations with commercially procured sex-sorted frozen semen straws from six different bulls containing approximately 2 million sperm cells per straw with 95 per cent of these sperm cells expected to be X sperm.

Early pregnancy diagnosis

Pregnancy was confirmed around 28-30 days from the day of second AI using commercially available readymade ELISA kits (IDEXX, US) (Fig. 1).

Fig. 1. Early pregnancy diagnosis with ELISA kit

Foetal sex determination

Foetal sex determination was carried out using ultrasound at 65 - 80 days from the day of second AI using an ultrasound device and a trans-rectal linear transducer 6 MHz, as outlined by Stroud (2005). Briefly, during examination of the foetus, the transducer was moved distally to where the umbilicus was attached to the abdomen. At this time, the transducer was moved slowly back and forth to diagnose the presence or absence of a male genital tubercle. If a male genital tubercle was not observed, the transducer was moved distally to the perineal area to detect the presence of a female genital tubercle. The female genital tubercle was generally bilobed, whereas the tail was a monolobed structure. Frequently, the tail and female genital tubercle were seen simultaneously (Fig. 2).

Ethical committee approval

The present field trial was funded by and carried out under NADP (2019-20) project on "Augmenting reproductive and production potential through adoption of assisted reproductive techniques using sexed semen in dairy cattle-SC No. 22298", where foetal sex determination is the vital part of the project in ascertaining the purity of sex-sorted semen. Hence, ethical committee approval was not considered mandatory for this trial.

Statistical Analysis

The statistical analysis was carried then out as per Snedecor and Cochran (1994) using SPSS computer program (Version 20.0; SPSS Co., Chicago, IL, USA). The mean value of $P<0.05$ was considered statistically significant and $P<0.01$ was considered highly significant and $P>0.05$ was considered non-significant.

RESULTS

Conception rate in oestrus synchronized cattle artificially inseminated (AI) with sex-sorted frozen thawed bull semen

Out of 120 crossbred heifers and cows inseminated with sex-sorted frozen thawed bull semen, 37 heifers and cows were pregnant with a conception rate of 30.83 per cent.

In the present study, the ELISA kit test for early pregnancy diagnosis provided 100 per cent accuracy with blood samples collected from sex-sorted frozen bull semen inseminated crossbred heifers and cows at 28-30 days post insemination. The development of blue colour in the sample wells similar to that of control wells were confirmed as pregnancy positive and the sample wells that remained colourless were confirmed as pregnancy negative samples (Fig. 2).

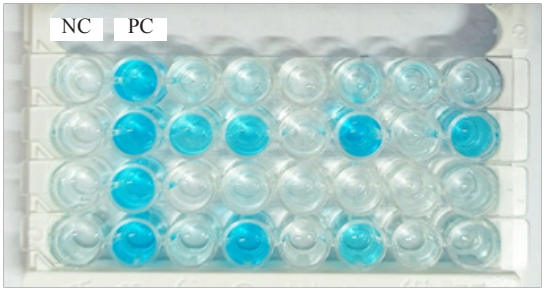

Fig. 2: Blue colour indicates pregnancy positive samples and colourless wells describe negative samples
NC: Negative control
PC: Positive control

The mean percentage of conception rate between heifers and cows was 31.67±7.96 and 30.00±5.16 per cent, respectively and between inseminations with sex-sorted bull semen of different breeds was 30.00±5.0 and 31.25±6.9 per cent, respectively. Chi-square test analysis revealed non-significant difference (P>0.05) in the conception rate between heifers and cows and between inseminations with sex-sorted frozen bull semen of different breeds (P>0.05).

The conception rate in crossbred cows of different parity inseminated with sex-sorted frozen bull semen were 40.90, 30.77, 27.27 and 18.18 per cent with first, second, third and fourth calving, respectively which was found to decrease with increase in the number of calvings. However, Chi-square test analysis revealed a non-significant difference (P>0.05) in conception rate among parity in crossbred cows inseminated with sex-sorted frozen bull semen.

Foetal sex determination by ultrasound examination at 65 - 80 days of pregnancy

Ultrasound based foetal sex determination was accurate and easy to perform at 65-80 days post insemination in crossbred heifers and cows inseminated with sex-sorted frozen bull semen. Foetal sex determination with ultrasonography was possible only in 17 heifers (54.84 per cent) and 14 cows (45.16 per cent) out of 37 pregnant heifers and cows. Out of 31 foetal sex confirmed pregnancies, the gender determined was found to be female in all animals based on the location of the genital tubercle near the tail head (Fig. 3).

DISCUSSION

The mean percentage of conception rate with sex-sorted frozen bull semen in crossbred heifers and cows in the present study was 31.67±7.96 and 30.00±5.16 per cent, respectively. The results of the present study were in accordance with the reports of Sales *et al.* (2011) and Karakaya-Bilen *et al.* (2019) who reported 31.40 per cent in heifers and 33.90 per cent in cows, respectively. But Hutchinson *et al.* (2013) reported a higher conception rate of 53.00 per cent in heifers

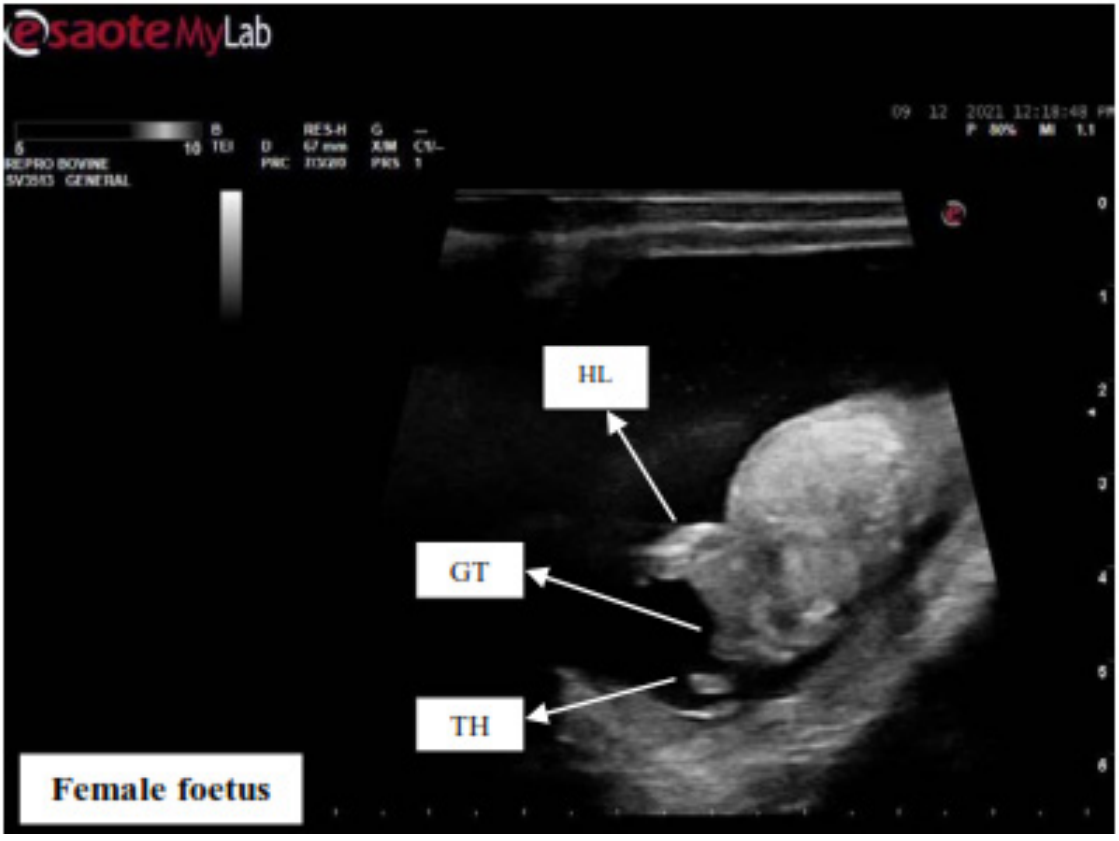

Fig. 3: Identification and location of genital tubercle (GT) in foetus
HL: Hind limb; TH: Tail head

and 45.00 per cent in cows inseminated with sex-sorted frozen bull semen. The lower conception rate in heifers and cows of the present study might be due to the variations in the time of insemination and temperature-humidity index, since a part of the study was carried out during summer months (Healy *et al.,* 2013; Thomas *et al.,* 2014; Correa-Calderón *et al.,* 2020). Further, in the present study, a non-significant difference reported between the conception rate in heifers and cows inseminated with sex-sorted frozen bull semen was in contrast to the reports of Yamaguchi *et al.* (2020) who reported a higher conception rate of 68.50 per cent in heifers than that of 56.30 per cent conception in cows. In the present study, similar conception rate obtained in cows as that of heifers might be due to the use of superior quality sex-sorted semen samples (SEXEDULTRA™) resulting in promising conception rate in cows inseminated with sex-sorted semen (Maicas *et al.,* 2019; Kasimanickam *et al.,* 2021).

In the present study, although the conception rate among cows in different parities did not differ significantly, it was found to decrease with increase in the number of calvings. These results were in accordance with the results of DeJarnette *et al*. (2008) who reported the conception rate with sex-sorted semen in cows of first parity, second parity and cows of third or greater parity as 31.10, 30.40 and 25.60 per cent, respectively. Similarly, Karakaya *et al.* (2014) reported a higher conception rate of 41.7 per cent in primiparous cows compared to that of 25.0 per cent in multiparous cows. Decrease in conception rate with increase in number of calvings might be due to differences in milk yield, energy balance and metabolic status between primiparous and pluriparous cows, where greater energy demands were needed for milk synthesis might increase the risk of reproductive disorders due to higher susceptibility of high yielders to endocrine and metabolic disorders (Chebel *et al.,* 2004).

The conception rate in crossbred heifers and cows inseminated with sex-sorted frozen bull semen of different breeds (crossbred Jersey and Red Sindhi) reported a non-significant difference, which was in contradiction to the results of Joshi *et al.* (2021) who reported the conception rate as 45.00 and 33.00 per cent in cattle inseminated with sex-sorted frozen semen from crossbred Jersey and Red Sindhi bulls. The results of the present study reporting a non-significant difference in conception rate between inseminations from different bull breeds might be due to smaller sample size of bulls under each breed in the present study, which was insufficient for a complete statistical analysis.

Further in the present study, foetal sex determined by ultrasound examination was more feasible at 65 to 80 days post insemination and the foetal sex confirmed using ultrasonography was found to be female in almost all pregnant heifers and cows inseminated with sex-sorted frozen bull semen samples utilized for the study. During this period of 65-80 days post insemination, the genital tubercle was visualized below the tail head enabled genuine examination of the whole foetus followed by gender identification as female.

Out of the 37 confirmed pregnancies in the present study, foetal sex was determined in 83.78 per cent pregnancies and was questionable in 16.22 per cent pregnancies. The difficulty faced during gender determination in the present study was due to non-cooperative behaviour and highly distended abdominal viscera imparing the movement of the ultrasound probe coupled with abdominal straining gravid uterus into the abdominal cavity in case of heifers and pluriparous cows. The foetal sex determination per cent was higher as 54.84 per cent in heifers when compared to 45.16 per cent cows. These results of the present study were in accordance with the reports of Curran *et al.* (1989) and Quintela *et al.* (2011) who reported 100 and 86.25 per cent of foetal sex determination in heifers and cows, respectively.

CONCLUSION

The commercially procured sex-sorted frozen bull semen straws used for field trial in the present study possessed assured purity and hence can be recommended for desired calf production with comparably equal

pregnancy rates in both crossbred heifers and cows.

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