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Relative Merits of Homo and Heterospermic Bull Semen in Respect of Fertility

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Abstract: The experiment was conducted to compare the relative efficiency of homo and heterospermic insemination in respect of fertility judged by conception and calving rate. Spermatozoa from three different breeds of bull namely Holstein Friesian (HF), Red Chittagong (RC) and Sahiwal (SL) were mixed in equal number and preserved for 3 days and inseminated at different days. In this study 168 cows were inseminated and 42 of them were inseminated with heterospermic semen containing equal numbers of spermatozoa from a HF, a RC and a SL bull. After calving the paternity of calves was established by comparing coat colour and conformation. Difference between homo and heterospermic insemination on conception and calving rate was not significant ($p>0.05$). The heterospermic conception and calving rates were 80.95±0.07 and 64.29±0.08%, with the homospermic rates being 66.67±0.04 and 50.79±0.04%, respectively. The conception and calving rate from heterospermic insemination was found insignificantly ($p>0.05$) different from that of homospermic insemination separately at first, second and third day of preservation. Conception rates of homo and heterospermic insemination at first day were 76.19±0.07 and 85.71±0.12%, respectively. The corresponding values at third day were 59.52±0.07 and 78.57±13%. Calving rate of homo and heterospermic insemination at first day were 57.14±0.08 and 64.29±0.13%, respectively. The corresponding values at third day were 45.24±0.08 and 64.29±0.13%. The numbers of offspring (12:8:7) sired by the three bulls (HF: RC:SL) after using heterospermic semen did not differ significantly from an assumed 1:1:1 ratio ($\chi^2 = 1.55$: $p>0.05$). The sex ratios among calves from heterospermic insemination (15♂: 12♀) also did not differ significantly ($\chi^2 = 0.34$: $p>0.05$) from 1:1. From the above results, it was concluded that heterospermic insemination did not however, show any significant superiority in conception and calving rate in cows compared to homospermic insemination. However, heterospermic insemination offers no apparent risk to co-operating farmers, since overall conception and calving rate were either numerically higher or at least equal to that obtainable from homospermic insemination.

Key words: Homospermic, heterospermic, conception rate, calving rate, paternity of calves, sex ratio

INTRODUCTION

Artificial Insemination (AI) in farm animals has been evolved to be a key technique devised for genetic improvement, control of venereal disease, improvement of herd fertility by better record keeping and to provide economic service. It also facilitates in providing aids to many reproductive manipulations. Low reproductive efficiency of cattle is considered to be a one of the major problem to the livestock producers. Since fertility is obtained from the results of mating between male and females, a part of low breeding efficiency in cattle may probably arise from bull. Attempts to maximize fertility of semen to be used in AI have been made in various ways. Improving conception rate is a continuous effort in every artificial breeding programme. In this connection mixing up of spermatozoa from different males (heterospermic semen) showed to exhibit many advantages in different species of animals[1-7]. In usual practice the female is inseminated in one heat with semen from one male (homospermic insemination). In contrast heterospermic insemination of a female in estrus with semen from more than one male can be carried out by multiple natural mating, or by artificial insemination with semen from more than one male. Upon heterospermic insemination offspring fathered by different sires are sometimes born in the same litter. The paternity of offspring after heterospermic insemination can be traced/scored by using

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visual appearance like coat colour and conformation[6], by means of prearranged genetic markers[8] and by blood-typing[7]. The predominance in the proportion of resulting progeny of one male over another is reported to be consistent and reproducible[9,3,10]. Kushner[11] reviewed the works so far been published in Russia and claimed four distinct merits can be obtained from heterospermic insemination. These are increased conception rate, better offspring in terms of heavier birth weight and faster growth, larger litter in polytocous animals and in some species offspring inherits characteristics from both fathers. On the contrary, many investigators[3,10,12] failed to reproduce the advantages obtainable from heterospermic insemination. Heterospermic insemination as viewed by Lopyrin and Loginova[13] not to be always encouraging. They argued that mixed semen could be utilized only in special circumstances. It is postulated that if two or more males are considered equal in other ways, if differences in fertility have not been established and if pedigree of offspring is not of importance (or can be established by characteristics of the offspring), heterospermic insemination may be utilized to improve overall fertility of semen samples[14]. The conflicting results obtained by the various authors mentioned above necessitate undertaking further research to examine the effects of heterospermic insemination. On the above context present research with AI service in cattle was, therefore, designed to examine the relative merits of homo and heterospermic bull semen in respect of fertility.

MATERIALS AND METHODS

Site of experimentation: Maintenance of bulls, collection of semen, its evaluation following processing and insemination of cows were accomplished at the Bangladesh Agricultural University Artificial Insemination Center (BAU AI Center). Diagnosis of pregnancy in the previously inseminated cows and tracing of calves for identification of parentage were done at the farmers premises in the villages located around the BAU AI center.

Breeding bulls used: The semen was obtained from bull each of three different breeds namely Holstein Friesian, Red Chittagong and Sahiwal maintained at BAU AI Center.

Semen collection, evaluation, processing, mixing and preservation: Semen was collected using artificial vagina from each of three bulls twice a week. As soon as the collection was made ejaculate was brought into the laboratory. Each sample was subjected to estimate initial motility and sperm concentration. Ejaculate to be used in the experiment must had to have at least 60% initial motility and $800x10^6$ spermatozoa per mL. Fresh semen in part from each ejaculate was mixed to give heterospermic semen. Dilution was accomplished for both homo and heterospermic semen. Concentration of spermatozoa per unit volume of heterospermic semen was also found out before dilution. Volume of inseminate (dose) was determined in such a way so that each inseminate contain 20 million motile sperm cell. The formula used to find out the volume of diluted semen (V) to be inseminated;

$$V = \frac{20}{C} \times motility\,\%$$

when C stands for concentration of sperm cell/ml diluted semen in million. Each dose was kept in separate vial and preserved in the refrigerator at 4°C for a period not exceeding 3 days.

Fertility of breeding bulls: Bull fertility was assessed by conception rate and calving rate calculated from the number of cows inseminated by each bull.

Conception rate: Conception rate is defined as the proportion of cows conceived out of total cows given service. Conception was ascertained by per rectal examination in cows at 90 days post service. Conception rate of cows was calculated by following formula:

$$\text{Conception Rate (CR)} = \frac{\text{No. of cows pregnant}}{\text{No. of cows given service}} \times 100$$

Calving rate: Calving rate of cows was calculated by using the following formula:

$$\text{Conception Rate (CR)} = \frac{\text{No. of cows calved}}{\text{No. of cows given service}} \times 100$$

Paternity of calves: As the sires used were from three distinctly different breeds, calves were likely to be of distinctly and correspondingly different. After freshening of cows the paternity of each calf was established by recognizing phenotype of each calf (coat colour and conformation) for further statistical interpretation.

Sex of calves: Sexes of the calves were recorded against each type of semen used for further statistical interpretation.

Insemination of cows and record keeping: Cows from private farms, mostly of subsistence level, when brought to BAU AI center were given service either with fresh or

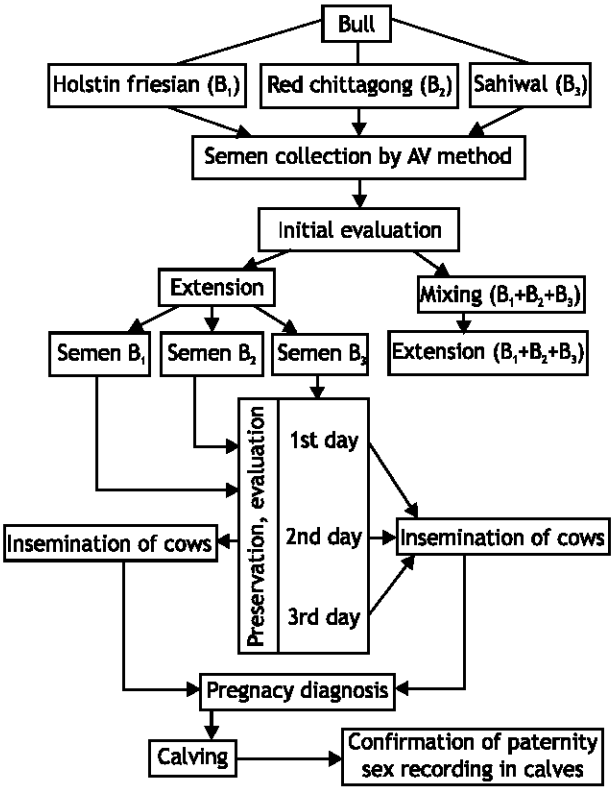

Fig. 1: Flow diagram showing activities of the experiment

preserved homo or heterospermic semen. Four types (three from three bulls and one heterospermic) of semen were used at random in inseminating cows. A total of 168 cows were inseminated in this experiment. The heterospermic semen inseminated out of these 42 cows and 126 cows were inseminated with homospermic semen. Amongst them 42 cows were inseminated by the semen from each of the three bulls (Holstein Friesian, Red Chittagong and Sahiwal). The insemination was carried out regularly for a period of 3 months (May to July, 2001). For ease of interpretation only first service records were taken into account. Detail records were maintained from the time of insemination to calving for each cow inseminated. Flow diagram showing activities of the experiment was shown in Fig. 1.

Examination of cows inseminated for pregnancy: Pregnancy diagnosis was accomplished by per rectal palpation of the genital tract at 3 month or onward from the date of insemination.

Statistical Analyses of data: Data collected as per Completely Randomized Design mass motility, morphology (normal) and live percent of sperm, conception rate, calving rate, paternity of calves and sex of calves etc. were statistically analysed using MSTAT computer package program in accordance with the principle of CRD[15]. For separation of subclass means Duncun's Multiple Range Test (DMRT) was performed to compare statistical variations among treatments where ANOVA showed significant difference[16].

Statistical model: One way statistical model used in the analyses of data are as follows:

$$Y_{ij} = \mu + t_i + e_{ij}$$

Where:

Y_{ij} = Individual observation
μ = General mean
t_i = Treatment effect
e_{ij} = Random error term, normally and independently distributed with mean '0' and variance $\sigma^2 e$

RESULTS AND DISCUSSION

Effect of homospermic and heterospermic insemination on conception rate and calving rate: Difference between homospermic and heterospermic insemination on conception rate and calving rate was not significant. However, average of the both parameters was insignificantly ($p>0.05$) higher in heterospermic insemination compared to its homospermic counterparts. The means±SE of conception rate and calving rate after homospermic insemination were 66.67±0.04 and 50.79±0.04%, respectively. After heterospermic insemination the corresponding values were 80.95±0.07 and 64.29±0.08% (Table 1). Hess *et al.*[1,7] obtained a higher conception rate with mixed bull semen. Stewart *et al.*[7] reported the heterospermic non-return rate to be 86.4% at 4 weeks and 71.9% at 16 weeks, the homospermic rates being 79% at 4 weeks and 63% at 16 weeks in cows and the heterospermic calving rate was at the satisfactory level of 66.00% in cows. Further, Beattly *et al.*[6] concluded the heterospermic 16-week non-return rate was 68.1%, the homospermic rate being 65.5% and the heterospermic calving rate as at the adequate level of 53.7% in Friesian cows. Small number of cows per sire in the present experiment might constrict the difference between homospermic and heterospermic insemination resulting insignificant variation between the two. In fact mixing the semen from two or more males prior to insemination might possibly produce interactions between: (1) spermatozoon and spermatozoon, (2) spermatozoa and seminal plasma and (3) seminal plasma and seminal plasma. The results of these interactions

Table 1: Effect of homospermic and heterospermic insemination on conception rate and calving rate

Types of insemination	No. of observation	Parameters: Conception rate	Parameters: Calving rate
Homospermic	126	66.67±0.04	50.79±0.04
Heterospermic	42	80.95±0.07	64.29±0.08
Level of significance		NS	NS

NS = Non-significant (p>0.05)

Table 2: Effect of insemination with individual bull semen and heterospermic semen on conception rate and calving rate

Bulls	No. of observation	Parameters: Conception rate	Parameters: Calving rate
Holstein Friesian (B_1)	42	71.43±0.07	54.76±0.08
Red chittagong (B_2)	42	64.29±0.07	47.62±0.08
Sahiwal (B_3)	42	64.29±0.07	50.00±0.08
Heterospermic (B_1+B_2+B_3)	42	80.95±0.07	64.29±0.08
Level of significance		NS	NS

NS= Non- significant (p>0.05)

Table 3: Effect of homospermic and heterospermic insemination at different days on conception rate and calving rate

Age of semen (day)	Type of insemination	No. of observation	Parameters: Conception rate	Parameters: Calving rate
1	Homospermic	42	76.19±0.07	57.14±0.08
	Heterospermic	14	85.71±0.12	64.29±0.13
			NS	NS
2	Homospermic	42	64.29±0.07	50.00±0.08
	Heterospermic	14	78.57±0.13	64.29±0.13
			NS	NS
3	Homospermic	42	59.52±0.07	45.24±0.08
	Heterospermic	14	78.57±0.13	64.29±0.13
			NS	NS

NS = Non- significant (p>0.05)

Table 4: Effect of insemination with individual bull semen and heterospermic semen at different days on conception rate and calving rate

Age of semen (day)	Bulls	No. of observation	Parameters: Conception rate	Parameters: Calvingrate
1	Holstein Friesian (B_1)	14	78.57±0.0.11	64.29±0.13
	Red Chittagong (B_2)	14	71.43±0.13	50.00±0.14
	Sahiwal (B_3)	14	78.57±0.11	57.14±0.14
	Heterospermic (B_1+B_2+B_3)	14	85.71±0.10	64.29±0.13
			NS	NS
2	Holstein Friesian (B_1)	14	71.43±0.13	50.00±0.14
	Red Chittagong (B_2)	14	64.29±0.13	50.00±0.14
	Sahiwal (B_3)	14	57.14±0.14	50.00±0.14
	Heterospermic (B_1+B_2+B_3)	14	78.57±0.11	64.29±0.13
			NS	NS
3	Holstein Friesian (B_1)	14	64.29±0.13	50.00±0.14
	Red Chittagong (B_2)	14	57.14±0.14	42.86±0.13
	Sahiwal (B_3)	14	57.14±0.14	42.86±0.13
	Heterospermic (B_1+B_2+B_3)	14	78.57±0.11	64.29±0.13
			NS	NS

NS= Non- significant (p>0.05)

Table 5: Chi-squire (χ^2) test for numbers of calves sired by heterospermic insemination

	HF	RC	SL	Total
Observed Frequency : O	12	8	7	27
Expected frequency : E	9	9	9	27
:O-E	+3	-1	-2	0
χ^2	1	0.11	0.44	1.55 NS

Table 6: Chi –square (χ^2) test for goodness of fit in sex ratio among progeny

	Male	Female	Total
Observed Frequency : O	12	15	27
Expected frequency : E	13.5	13.5	27
:O-E	-1.5	+1.5	0
χ^2	0.17	0.17	0.34 NS

might affect fertilization and embryonic development in the female resulting a variation in conception rate, pre-and post-natal growth and other characters of the offspring.

Effect of insemination with individual bull semen and heterospermic semen on conception rate and calving rate: Conception rate and calving rate from individual bulls semen and a mixture of them did not differ significantly (p>0.05) each other. The means±SE of conception rate and calving rate after insemination with individual bull semen and heterospermic semen are presented in Table 2. Literature pertaining to the effect of heterospermic insemination is abundant. It was reported that the rate of fertility following heterospermic insemination is generally superior to the mean rate following homospermic insemination with the same males[1,6,7]. Beatty[18] concluded that the conception rate or percentage of inseminations yielding a litter, was shown in one experiment to increase as the males contributing to the inseminate increased, even though the net total number of spermatozoa per inseminate was held constant. These results do not support the present findings.

Effect of homospermic and heterospermic insemination at different days on conception rate and calving rate: The conception rate and calving rate from heterospermic insemination was insignificantly (p>0.05) different from that of homospermic insemination separately at first, second and third day of preservation (Table 3). Here it is seen that the conception rate and calving rate declined progressively with the increasing age of semen from 76.19±0.07 and 57.14±0.08%, respectively at first day to 59.52±0.07 and 45.24±0.08%, respectively at third day for homospermic insemination and 85.71±0.12 and 64.29±0.13%, respectively at first day to 78.57±0.13 and 64.29±0.13%, respectively at third day for heterospermic insemination. But the rate of deterioration in conception rate and calving rate was numerically higher in case of homospermic insemination as compared to heterospermic insemination. The result obtained in the present study agrees with the results obtained by Boyd and Reed[19]. They reported the conception rate to be declined progressively with increasing age of semen from 65.5% for first day semen to 45.68% for over third day and 46.3% for deep frozen semen. Similar results were obtained by Almquist *et al.*[20], Salisbury and Flerchinger[21] who stated

the fertility of freshly collected bull semen was not as high as that semen kept for a while. These results are in close to the findings of the present study in the sense of aging.

Effect of insemination with individual bull semen and heterospermic semen at different days on conception rate and calving rate: The mean conception rate and calving rate for insemination with individual bull semen and heterospermic semen did not differ significantly ($p>0.05$) each other at any day (Table 4). The result obtained in the present study agrees with the results obtained by Boyd and Reed[19] stated that the conception rate declined progressively with increasing age of semen from 65% in first day to 45.68% in third-day and 46.3% for deep-frozen semen. Similar results were obtained by Shamsuddin *et al.*[22] and reported that the preservation period of diluted semen at refrigerator temperature significantly affect the fertility, fertilizing capacity of spermatozoa drops after the third day of preservation at 4-7°C. The highest (55.61%) and the lowest (50.58%) conception rates were observed in semen preserved for 0-24 and 48-72 h, respectively. The conception rates were negatively correlated with the increasing age of semen.

Paternity of calves: In this study a total of 27 calves were born from heterospermic insemination. Calves sired by Holstein Friesian, Red Chittagong and Sahiwal bull were 12, 8 and 7, respectively. The paternity of the calves born from heterospermic semen was determined by coat colour and conformation phenotype of the calves comparing with those of component sires (Plate 1, 2 and 3 show the Photograph of HF, RC and SL bull each with their calf). The numbers of offspring (12:8:7) sired by the three bulls (HF: RC: SL) after using heterospermic semen did not differ significantly from an assumed 1:1:1 ratio ($\chi^2 = 1.55$: $p>0.05$). The χ^2 test shows that calves produced from heterospermic semen fitted well with expected 1:1:1 ratio (Table 5). It means that all the three bulls contributed equally in the occurrence of fertilization. Stewart *et al.*[7] mixed spermatozoa from four Friesian bulls in equal numbers. After insemination of the fresh mixed semen, the paternity of calves was scored by blood typing and the number of calves sired per bull was in a ratio close to 1:1:1:1. On the other hand Beatty[8] reported that the mixing of semen from different male rabbit frequently resulted in a ratio of offspring different from 1:1, even though equal numbers of viable sperm were used. Former result agrees but the later contradicts with the present finding.

Sex ratio among progeny: In this study a sum of 27 calves were born from heterospermic insemination and the sex distribution for male and female calves were 12 and 15, respectively. The over all sex ratios for heterospermic semen (15♂: 12♀) did not differ significantly ($\chi^2 = 0.34$: $p>0.05$). The χ^2 test show that calves produced form heterospermic semen fitted well with expected 1:1 ratio (Table 6). It means that sex ratio was unaffected when mixed from different sources.

Stewart *et al*[7] stated that spermatozoa from four Friesian bulls were mixed in equal numbers and inseminated. But the sex ratio of progeny remained unaffected. The overall sex ratios for fresh semen (73♂: 51♀) and for frozen semen (56♂: 55♀) did not differ significantly ($p<0.1$). The results shown are in agreement with the result observed by Beatty[23] in rabbit.

REFERENCES

1. Hess, E.A., T. Ludwick, H.C. Rickard and F. Ely, 1954. some of the influences of mixed ejaculates upon bovine fertility. J. Dairy Sci., 37: 649.
2. Maclaren, A. and D. Michie, 1954. Current trends of genetic research in hungary. Nature, 174: 390.
3. Beatty, R.A., 1956. Relation between genetic constitution of an offspring and weight of its litter-mates. Nature, 178: 48.
4. Napier, R.A.N., 1961a. Fertility in the male rabbit. II. Variation in the percentage of eggs fertilized. J. Reprod. and Fert., 2: 260.
5. Napier, R.A.N., 1961b. Fertility in the male rabbit. III. The estimation of spermatozoa quality by mixed insemination and the inheritance of spermatozoan characters. J. Reprod. and Fert., 2: 273-289.
6. Beatty, R.A., G.H. Bennett, J.G. Hall, J.L. Hancock and D.L. Stewart, 1969. An experiment with heterospermic insemination in cattle. J. Reprod. Fert., 19: 491-502.
7. Stewart, D.L., R.L. Spooner, G.H. Bennett, R.A. Beatty and J.L. Hancock, 1974. A second experiment with heterospermic insemination in cattle. J. Reprod. and Fert., 36: 107-116.
8. Beatty, R.A., 1970. The genetics of the mammalian gamete. Biol. Rev., 45: 73-119.
9. Edwards, R.G., 1955. Selective fertilization following the use of sperm mixtures in the mouse. Nature, 175: 215.
10. Beatty, R.A., 1960a. The birth weight of rabbits born after heterospermic insemination. Genet. Res. Cambridge, 1: 39-49.
11. Kushner, K.F., 1954. The effect of heterospermic insemination in animals and its biological nature (translated). Bull. Acad. Sci. U.S.S. R. (Ser. Biol.), 1: 32-52.

12. Frappell, J.P. and G. Williams, 1956. A study of heterospermic insemination in cattle. Proc. IIIrd intl. Congr. Anim. Reprod. Cambridge, Section, 1: 65-67.
13. Lopyrin, A.I. and N.V. Loginova, 1955. The theory and practice of inseminating females with mixed semen. Usp. Sovr. Biol., 39: 212.
14. Gomes, W.R., 1977. Artificial Insemination. Reproduction in Farm Animals. (3rd Edn.). (Eds.), Cole, H.H. and P.T. Cupps. Academic Press New York, San Fransisco, London.
15. Steel, R.G.D. and J.H. Torrie, 1980. Principles and Procedures of Statistics 2nd Ed. McGraw Hill Book Company. INC, New York.
16. Snedecor, G.W. and W.G. Cochran, 1980. Statistical Methods, 7th Edn. The Iowa State University press. Ames. U.S.A., pp: 271-273.
17. Hess, E.A., T.M. Ludwick, H.C. Rickard and F. Ely, 1958. Fertil. and Steril., 9: 239.
18. Beatty, R.A., 1960b. Fertility of Mixed semen from different rabbits. J. Reprod Fert., 1: 52-60.
19. Boyd, H. and H.C.B. Reed, 1961. Investigation into the incidence and causes of infertility in dairy cattle. Fertility variations. Brit. Vet. J., 117: 18-31.
20. Almquist, J.O., 1954. Diluters of bovine semen. V.A. comparison of heated milk and egg yolk citrate as diluters for semen from bulls of high and low fertility. J. Dairy Sci., 37: 1308-1215.
21. Salisbury, G.W. and F.H. Flerchinger, 1967. Aging phenomena in spermatozoa. II. Estrus cycle length after successful insemination with spermatozoa of varying age. J. Dairy Sci., 50: 1679-1682.
22. Shamsuddin, M., J.U. Ahmed, M.G.S. Alam and P.C. Modak, 1987. Effect of age of semen on conception rate in cattle under farm condition. Bangladesh Vet. J., 21: 51-58.
23. Beatty, R.A., 1957. A pilot of experiment with heterospermic insemination in the rabbit. J. Genet., 55: 325-347.