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Relative Merits of Homo and Heterospermic Bull Semen in Respect of Preservation Quality

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Abstract: The experiment was conducted to compare the relative efficiency of homo and heterospermic bull semen in terms of preservation quality. 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. The quality of semen in terms of mass motility, normal and live sperm content of homo and heterospermic semen were studied at various preservation periods. In total 312 samples were included in the analysis. The average (%) mass motility, normal and live sperm of homospermic semen were 51.77±0.49, 77.55±0.45 and 78.73±0.44 respectively and for heterospermic semen the corresponding values were 59.94±0.85, 83.55±0.78 and 83.69±0.76. The significantly ($p<0.001$) highest mass motility, normal and live sperm percentages were observed in heterospermic semen as compared to homospermic semen. The quality of semen between homo and heterospermic semen in terms of mass motility, normal and live sperm percentage did not differ significantly ($p>0.05$) between groups at first day but differed significantly ($p<0.001$) at second and third day of preservation. Mass motility of homo and heterospermic semen at first day were 60.77±0.55 and 62.31±0.95%, respectively. The corresponding values at third day were 44.04±0.44 and 57.12±0.77%. Normal sperm of homo and heterospermic semen at first day were 86.50±0.43 and 86.31±0.74%, respectively. The corresponding values at third day were 70.36±0.38 and 81.00±0.66%. Live sperm of homo and heterospermic semen at first day were 86.56±0.43 and 86.54±0.75%, respectively. The corresponding values at third day were 71.54±0.46 and 81.42±0.79%. From the above results, it was concluded that heterospermic semen could be better preserved in terms of mass motility, normal and live sperm percentage compared to homospermic ones.

Key words: Homospermic, heterospermic, heterospermic vigour, bull

INTRODUCTION

When spermatozoa from more than one fertile male are mixed a 'heterospermic vigour'[1] develops leading to increased ability in fertilizing egg. Kushner[2] reviewed the works so far been published in Russia and claimed multiple advantages of heterospermic insemination. He demonstrated that four distinct merits could be obtained out of the technique. 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 inherit characteristics from both fathers. He also reviewed that the viability and subsequent breeding performance of progeny born after heterospermic insemination is considerably better than that of the homospermic controls. Like Russian works reports from Hungary state that mixed semen possesses higher fertilizing capacity[3]. Furthermore, Hess *et al.*[4] reported that mixed semen samples maintained their activity *in vitro* longer than did the unmixed controls. They also reported that the motility, viability and fertility of semen could be improved by mixing semen samples from different males. On the contrary, many investigators failed to reproduce the advantages obtainable from heterospermic insemination. Motility and survival of spermatozoa in mixed semen from different bulls were not found to be increased in many works[5-7]. Heterospermic insemination as viewed by Lopyrin and Loginova[8] 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[9]. The

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conflicting results obtained by the various authors mentioned above necessitate to undertake further research to examine the effects of heterospermiation. A practical application of this line of research may contribute in the improvement of male fertility. On the above perspective the present experiment with heterospermiation of bull semen was, therefore, designed aiming to compare the relative preservation efficiency of homospermic and heterospermic semen.

MATERIALS AND METHODS

Site of experimentation: Maintenance of bulls, collection of semen, its evaluation following processing and preservation were accomplished at the Bangladesh Agricultural University Artificial Insemination Center (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, normal sperm, live sperm and sperm concentration. 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. Each kinds of semen was kept in separate vial and preserved in the refrigerator at 4°C for a period not exceeding 3 days.

Mass motility (%): Percentage of motility (Mass movement) of spermatozoa was estimated by microscopic examination at first, second and third day of preservation for both homo and heterospermic semen.

Morphology of spermatozoa (Normal %): Morphology of spermatozoa was studied under microscope by Rose Bengal Staining technique[10] at first, second and third day of preservation for both types of semen. Spermatozoa with normal morphology was expressed in percent of total number of spermatozoa.

Live spermatozoa (%): Percentage of live spermatozoa was studied by differential staining technique[10] under microscope for both types of semen (homo and heterospermic) at first, second and third day of

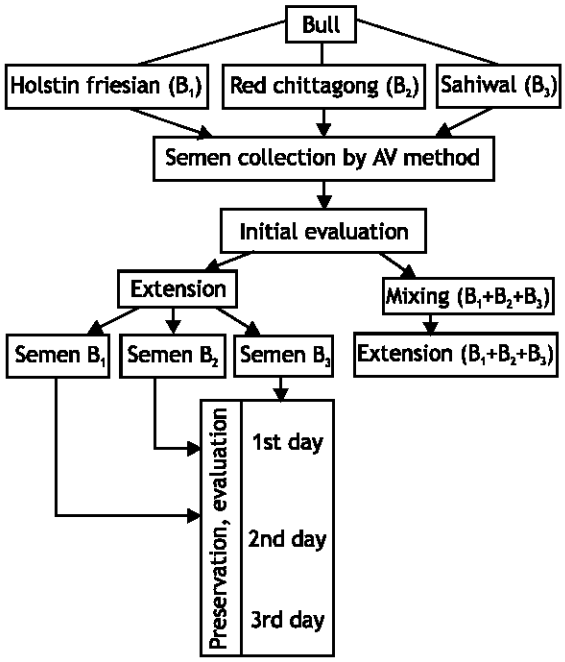

Fig. 1: Flow diagram showing activities of the experiment

Statistical analyses of data: Data collected as per CRD 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[11]. For separation of subclass means Duncun's Multiple Range Test was performed to compare statistical variations among treatments where ANOVA showed significant difference[12].

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 semen on the preservation quality of semen (age combined): Percentage of mass motility, normal sperm and live sperm differed significantly ($p<0.001$) between homospermic and heterospermic semen when age of semen was combined. Heterospermic semen was found to be better preserved in

terms of mass motility of semen, normal sperm and live sperm percentage than that of homospermic one (Table 1). The average values (%) for mass motility of semen, normal sperm and live sperm of homospermic semen were 51.77±0.49, 77.55±0.45 and 78.73±0.44 respectively, where as for heterospermic semen the corresponding values (%) were 59.94±0.85, 83.55±0.78 and 83.69±0.76. The result of this study partially agrees with the findings of Hess *et al.*[4] who reported that the motility and viability of semen could be improved by mixing semen samples from different males. They also reported that mixed samples of bull semen had greater motility, survival and fertilizing capacity than unmixed samples. These results are in close agreement with the findings of the results of the present study. However, conflicting results about the claimed improvement of motility and longevity of mixed semen samples of bull were not confirmed by Frappell and williams[5], Dott and Walton[7]. These results contradict with the findings of the present study. If mixing causes a change in motility as was actually claimed by Hess *et al.*[4] this might be due to an interaction between spermatozoon and spermatozoon, or between spermatozoa and seminal plasma and higher activity or survival might result in an increase or decrease of conception rates. In a standardized environment no mutual influence of bull spermatozoa from different origin occurs with respect to motility characteristics, nor can any effect on longevity demonstrated[13].

Effect of individual bull semen and heterospermic semen on preservation quality (age combined): The average mass motility of semen, normal sperm content and live sperm content differed significantly ($p<0.001$) between individual bull semen and heterospermic semen when age of semen was combined. Table 2 clearly shows that heterospermic semen was found to be better preserved in terms of mass motility of semen, normal sperm and live sperm percentage than did individual bull semen. Bulls did not differ ($p<0.05$) among themselves when compared for the parameters studied. The results of this study partially agrees with the findings of Hess *et al.*[4,14] who concluded that heterospermic motility and viability of the spermatozoa in mixed ejaculates were better maintained for a longer period in vitro than with the unmixed samples. These results are in close agreement with the present findings. However, mixed semen did not always exhibit conducive results in preservation. Motility and viability remained unaffected when semen from different bulls were mixed and preserved[5,7]. These results disagree with the findings of the present study. Differences between the expected values for either motility characteristics, as found by Hess *et al.*[4] and Dott and Walton[7], in comparison with the values actually found in mixed ejaculates must therefore be due to changes in the environment of the spermatozoa. Spermatozoa of low motility from oligospermic human semen samples showed an average increase of 30% motility when resuspended in seminal plasma of normal semen[15]. In addition, the effect was proved in actual insemination experiments where two out of five childless women were successfully inseminated with their husband's spermatozoa suspended in heterologous seminal plasma from normal semen.

Effect of type of semen on preservation quality at different days: The quality of semen in terms of mass motility, normal sperm and live sperm between homospermic and heterospermic semen did not varied significantly ($p>0.05$) at first day but varied significantly ($p<0.001$) at second and third day of preservation (Table 3). The results projected above support the proposition of heterospermic vigour[1]. On the contrary the finding of the present study disagrees with the results obtained by Campbell and Jaffe[6] and Dott and Walton[7] who reported that the mixed samples of bull semen frequently showed less motility than the better of their unmixed controls

Effect of individual bull semen and heterospermic semen on preservation quality at different days: The quality of semen decreased as the age of semen increased but the rate of deterioration in semen quality was higher in case of individual bull semen as compared to heterospermic semen (Table 4). Percentage of mass motility, normal sperm and live sperm content of individual bull semen and heterospermic semen differed significantly ($p<0.05$) at first day and at subsequent two days ($p<0.001$. At first day

Table 1: Effect of type of semen on preservation quality (age combined)

		Parameters		
Types of semen	No. of observation	Mass motility of semen (%)	Normal sperm (%)	Live sperm (%)
Homospermic	234	51.77±0.49	77.55±0.45	78.73±0.44
Heterospermic	78	59.94±0.85	83.55±0.78	83.69±0.76
Level of significance		***	***	***

***, ($p<0.001$)

Table 2: Effect of individual bull semen and heterospermic semen on preservation quality (age combined)

		Parameters		
Bull	No. of observation	Mass motility of semen (%)	Normal sperm (%)	Live sperm (%)
Holstein friesian (B_1)	78	53.01±0.84b	78.63±0.78b	79.36±0.76b
Red Chittagong (B_2)	78	51.86±0.84b	77.12±0.78b	77.74±0.76b
Sahiwal (B_3)	78	50.45±0.84b	76.96±0.78b	79.09±0.76b
Heterospermic ($B_1+B_2+B_3$)	78	59.94±0.84a	83.53±0.78a	83.69±0.76a
Level of significance		***	***	***

Means with uncommon superscripts in the same column differ significantly ($p<0.05$) from each other ***, ($p<0.001$)

Table 3: Effect of type of semen on preservation quality at different days

Age of semen (day)	Type of semen	No. of observation	Parameters: Mass motility of semen (%)	Normal sperm (%)	Live sperm (%)
1	Homospermic	78	60.77±0.55	86.50±0.43	86.56±0.43
	Heterospermic	26	62.31±0.95	86.31±0.74	86.54±0.75
			NS	NS	NS
2	Homospermic	78	50.51±0.49	75.91±0.41	78.09±0.47
	Heterospermic	26	60.39±0.85	83.35±0.70	83.12±0.81
			***	***	***
3	Homospermic	78	44.04±0.44	70.36±0.38	71.54±0.46
	Heterospermic	26	57.12±0.77	81.00±0.66	81.42±0.79
			***	***	***

***, ($p<0.001$) NS = Non- significant

Table 4: Effect of individual bull semen and heterospermic semen on preservation quality at different days

Age of semen (day)	Bulls	No. of obser-vation	Parameters: Mass motility of semen (%)	Normal sperm (%)	Live sperm (%)
1	Holstein friesian (B_1)	26	62.31±0.94a	88.31±0.70a	89.50±0.64a
	Red Chittagong (B_2)	26	60.96±0.94b	85.04±0.70b	83.92±0.64c
	Sahiwal (B_3)	26	59.04±0.94b	86.00±0.70b	86.27±0.64b
	Heterospermic ($B_1+B_2+B_3$)	26	62.31±0.94a	86.36±0.70b	86.58±0.64b
			*	*	*
2	Holstein Friesian (B_1)	26	52.31±0.83b	76.42±0.71b	77.65±0.81b
	Red Chittagong (B_2)	26	50.19±0.83b	75.58±0.71b	77.73±0.81b
	Sahiwal (B_3)	26	49.04±0.83b	75.73±0.71b	78.89±0.81b
	Heterospermic ($B_1+B_2+B_3$)	26	60.39±0.83a	83.35±0.71a	83.12±0.81a
			***	***	***
3	Holstein Friesian (B_1)	26	44.42±0.77b	71.15±0.65b	70.92±0.79b
	Red Chittagong (B_2)	26	44.42±0.77b	70.35±0.65b	71.58±0.79b
	Sahiwal (B_3)	26	43.27±0.77b	69.56±0.65b	72.12±0.79b
	Heterospermic ($B_1+B_2+B_3$)	26	57.12±0.77a	81.00±0.65a	81.42±0.79a
			***	***	***

Means with uncommon superscripts within column in each cell differ significantly ($p<0.05$) *, ($p<0.05$). ***, ($p<0.001$)

mass motility of heterospermic semen and that obtained from Holstein-Friesian (HF) bull were higher than its other counter parts. Normal sperm percent was higher in HF semen and the other groups were statistically similar ($p>0.05$). Highest and lowest live sperm percent was shown by HF semen and Red Chittagong bull semen where as the other two groups were almost similar ($p<0.05$). At second and third day mass motility of semen, normal sperm and live sperm content of heterospermic semen were interestingly higher than they were in homospermic semen. Banu *et al.*[16] reported that the Buck semen preserved at varying periods differ significantly ($p<0.05$) and also reported that the semen quality decreased as the age of semen increased. These results are more or less similar to the present study.

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