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Fertility of Cow in Using Locally Produced Chilled and Imported Frozen Semen

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Abstract: The experiment was carried out at Central Cattle Breeding Station and Dairy farm, Savar, Dhaka, and 3 sub- station and 9 points of Chandpur District in Bangladesh to evaluate the quality and fertilizing capacity of locally produced chilled and imported frozen semen. Motility, sperm concentration and mass activity of semen from different experimental bulls were almost similar. Quality of imported frozen semen was better than that of locally produced chilled semen in respect of motility, motile sperm/ Insemination dose and spermatozoa with normal head. Motility and pH value of semen decreased significantly for transportation and prolongation of preservation duration. Average conception rate of imported frozen semen (57.33) was found to be higher than locally produced chilled semen (45.33). But it was similar between imported frozen (57.33) and average of 1st & 2nd day preserved semen (57%).

Key words: Cow, chilled semen, frozen semen, semen quality, microscope, transportation, preservation, ice, fertility

Introduction

Artificial insemination (AI) was introduced during the latter part of 1950th decade for upgrading indigenous cattle with infusion Bos Taurus blood in Bangladesh. Quality of semen is one of the key factors to have better fertility. Therefore preservation of semen either as chilled or frozen is needed to get maximum conception rate. Morphologically abnormal spermatozoa are unable to fertilize Oocyte (ovum) (Shamsuddin and Rodriguez-Martiez, 1994). Quality of semen even from good, proven fertile bull may be affected by collection, transportation and environmental temperature during semen collection (Sekoni and Gustafsson, 1987).

Imported frozen semen in the bovine artificial insemination programme in Bangladesh is limited, mostly in urban areas and in some commercial farms (Shamsuddin *et al.,* 1997). Most of the cows in rural areas at present are bred AI using method with chilled semen. The chilled semen is usually preserved at 4 to 5 °C temperature, maintained by keeping in the refrigerator or ice-containing thermoflask.

After preservation at chilling temperature the post warm sperm motility, total motile spermatozoa per AI dose and the proportion of normal spermatozoa may be inconsistent due to fluctuating temperature. In addition, long time preservation of chilled semen may cause the exhaustion of reserved energy of spermatozoa due to slow metabolic activities, which affects to reduce viability and motility of spermatozoa (Salisbury and Flerchinger, 1976; Shamsuddin *et al.,* 1987). With this idea in view, the present study was aimed at to compare the fertility of cow in inseminating by imported frozen and locally produced chilled semen.

Materials and Methods

The experiment was conducted between July, 1998 and June 1999 at Central Cattle Breeding Station. (CCBS) and Dairy Farm, Savar, Dhaka, 3 sub-centers and 9 points of Chandpur District, Bangladesh.

Locally produced chilled semen: Semen from different crossbred bulls (Table 1) was collected using artificial vagina once in a week at CCBS, and twice in a week at Chandpur district AI center, and diluted with egg yolk citrate extender remaining 20 x 10^6 progressively motile spermatozoa per million. Individual insemination doses were transferred in glass vials covered by cork and preserved at 4-8 °C until used.

Transportation and distribution of semen: Imported frozen semen (Table 1) was used for study. Individual doses in vials were placed in a thermoflask containing ice for transportation to different AI sub- centers and points, and preserved usually for two days in the refrigerator or in ice containing thermoflask. Imported frozen semen straws were distributed from CCBS to district AI centers and from there to sub-centers and AI points using public transport. Frozen semen always remained in liquid nitrogen container during transportation and local preservation.

Monitoring of semen quality: Volume, colour, mass activity of semen, sperm concentration and motility of fresh ejaculated semen from locally bred experimental bulls were recorded. The mass activity of semen was evaluated in a drop of fresh undiluted semen placing on a warm slide without cover slip at

Table 1: Identification of semen and doner bulls

Semen types	Producers	Bulls		Age (years)
		Bulls Name/ID No	Breed	
Imported semen	Semex, Canada	Rocket	Friesian	♦
		Tutor	Friesian	♦
		Tyrone	Friesian	♦
	Sercia, France	Permis	Friesian	♦
		Mount	Friesian	♦
Locally produced Chilled semen	District AI Center, Chandpur	5670	Friesian x Sahiwal	6.0
		5615	Friesian x Sindhi	7.5
		6718	Friesian x Local	8.0
		7567	Friesian x Local	9.0
		6887	Friesian x Sahiwal	10.0

♦Date on the age of bulls were not available.

low magnification (400X). The mass activity was graded as (+ 1 to+ 4,+ 1= no activity ,+ 2= slow wave motion, + 3= rapid wave motion with formation of eddis at the end of waves,+ 4= eddies). Concentration of spermatozoa (million/ml) in fresh semen was determined by Kagras scale.

Motility of spermatozoa in diluted semen: A small drop of diluted semen was placed on a microscopic slide, covered with a cover slip and examined at magnification of 400 X under light microscope. Only sperm moving in a straight forward direction were included in the motility count. Both mass activities were examined at body temperature of 37°C.

pH: Semen pH was measured using indicator paper strips (nitrazene paper).

Morphological examination of spermatozoa: It was done by staining the smear of semen with William's method. Five hundred cells were counted under microscope of oil emersion objective (X100). Five hundred normal sperms from individual smears were recorded. Big, narrow, pear shaped, short, broad and abnormal contour head or any other deformities of sperm were treated as abnormal sperms.

Insemination and pregnancy diagnosis: Fifteen villages under 3 sub-centres having 50 cows in each were selected.After detection the sign of estrus, the cows were inseminated using locally produced chilled or imported frozen semen by the inseminators. To test the accurate pregnancy, milk samples were collected from the experimental cows and determined the progesterone level immediately before palpation. A decline level of progesterone at about days 17 - 21 after insemination indicated the non pregnancy, while a stable or rising level indicated pregnancy. In addition 60-90 days after insemination, pregnancy was confirmed by per rectal palpation of the genital tract.

Statistical analysis: Data were analyzed using MSTAT computer Package program. The quality of locally produced and imported frozen semen were tested using F-test. Data for transportation and preservation effect on quality of locally chilled semen, and conception rate between locally chilled and imported frozen semen were tested using t- test.

Results and Discussion

Significant difference was found for semen volume ($P< 0.01$) but not significant ($P> 0.05$) for colour, mass activity motility and sperm concentration of fresh semen produced by locally crossbred bulls (Table 2). Non significant differences were found for the quality of semen from locally produced chilled and imported frozen semen ($P> 0.05$). However motility, motile sperm/insemination dose, and spermatozoa with normal head tended to increase in imported frozen semen compared

Table 2: Semen characteristics of experimental bulls

Bull ID No.	Volume (ml)	Colour	Mass activity (+ 1 to + 4)	Motility%	Sperm concentration (x 10^6 ml)
5670	6.00	2.00	3.00	70.00	1162.50
5615	5.00	2.00	3.00	70.00	1068.75
6718	4.50	2.00	3.00	70.00	1100.00
7567	7.75	2.13	3.00	66.88	1075.00
6887	5.50	2.00	3.00	66.25	1262.50
Level of significance	**	NS	NS	NS	NS

NS, $P> 0.05$; **, $P< 0.01$

Table 3: Quality of imported frozen and locally produced chilled semen

Semen type	Bull name	Motility %	Motile Spermatozoa/ Insemination dose (x 10^6 ml	Spermatozoa with normal head morphology
Imported semen	Rocket	72.0	15.5	97.0
	Tutor	70.0	16.0	95.0
	Tyrone	68.0	12.0	95.0
	Permis	71.0	11.5	97.0
	Mount	69.0	20.0	96.0
Average		70.0	15.0	96.0
Level of significance		NS	NS	NS
Locally chilled semen	5670	67.0	10.0	94.0
	5615	65.0	12.0	92.0
	6718	66.0	11.5	91.0
	7567	62.0	11.0	94.0
	6889	63.0	15.5	95.0
Average		65.0	12.00	93.0
Level of significance		NS	NS	NS

NS, $P> 0.05$

Table 4: Effect of transportation and duration of preservation on quality of locally produced chilled semen

Semen group	Motility %			pH value		
	Day-1	Day-2	Day-3	Day-1	Day-2	Day-3
Without transportation (AI Centre)	65.00	57.5	41.5	6.55	6.2	4.69^a
After transportation (AI sub Centre)	57.0	45.7	26.0	6.20	6.02	3.77^b

Different superscript in the same colour differ significantly ($P< 0.05$)

Table 5: Conception rate of imported frozen and locally produced chilled semen

Parameters	Frozen semen			Total	Locally produced chilled semen			Total
	Semen type				Age of semen (hrs)			
	Rocket+ Permis+ Tutor	Rocket+ Permis+ Mount	Tutor+ Tyrone + Permis		24	48	72	
No. of AI cows	50	50	50	150	50	50	50	150
No. of cows conceived	29	28	29	86	30	27	11	68
Percent conceived	58	56	58	*57.33	60	54	22	*45.33

*, P< 0.05

to locally chilled semen (Table 3). Motility of spermatozoa and the pH value of semen were significantly (P< 0.05) decreased for transportation and prolongation of preservation time (Table 4).

Conception rate of imported frozen semen was significantly higher (57.33%) than that of locally produced chilled semen (45.33%) (P< 0.05). There was no significant difference between 1st and 2nd groups of chilled semen (P> 0.05). The conception were significantly higher in cows inseminated by 1st and 2nd groups than those of inseminated by 3rd groups of chilled semen (Table 5). Nevertheless the difference in the proportion of conception rate between imported frozen and two groups (1st and 2nd) of locally chilled semen was not significant (P> 0.05).

Individual experimental genotype differed with regards to the semen volume, but almost similar for colour, mass activity, motility and sperm concentration (million /ml). Variation in semen volume may be caused for different ages of bull and genotype. Imported frozen semen was found to be better than the locally produced chilled semen in respect of motility, motile spermatozoa per AI dose and normal head morphology. Abnormal sperm occur due to semen collection, processing, storage, transportation, frequent semen collection and prolong sexual rest supported by Sullivan (1978), Amann (1986), Watson (1990). On the other hand quality of frozen semen may be detorated due to transfer of frozen semen, changing temperature straws from one container to another different centres and sub- centres, and exposure to ambient temperature (Berdtson *et al.,* 1976). But in the present study frozen semen thawed at 37°C for 12 seconds and stored in liquid nitrogen at -196°C (Foot, 1978). Locally produced chilled semen showed the decreasing motility and pH value after transportation and prolongation the period of preservation, may be caused for exhaustion of reserved energy due to metabolism of spermatozoa reported by (Shamsuddin *et al.,* 1987).

Conception rate of chilled semen was significantly lower than that of imported frozen semen due to inconsistency in cooling during transportation and storage of chilled semen, supported by Shamsuddin *et al.* (1997). They also indicated that low milk producing cows with poor nutritional condition traditional management and often used for drought purpose may be the reasons to reduce conception rate. Conception rate of 1st and 2nd day preserved semen in thermoflask or in ice was found almost similar due to the presence of more normal motile spermatozoa per insemination dose. This means chilled semen can be used for AI up to two days for better conception rate.

The findings revealed that imported frozen semen may get prime consideration for quality and better fertilizing ability particularly in transportation causes. Locally produced chilled semen may be used as AI programme up to two days without any adverse effect on conception rate. More attention will be given for collection, dilution, transportation and preservation of locally produced semen. In maintaining the quality of locally produced chilled semen provide hygienic condition and transportation facilities which will bring the better outcomes, and save the foreign currency in this regards.

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