

## **Use of Dye Deposition in Cows' Excised Genital Tract to Evaluate Inseminators' and Refreshment Training to Improve Their Skill**

**S. Mohammed<sup>†</sup>, S. H. Mohammad, A. R. S. Mohammad and A.H.M. S. I. Khan**  
*Field Fertility Clinic, Department of Surgery and Obstetrics, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh*

### **SUMMARY**

To find out the possible inefficiencies of artificial inseminators at rectovaginal insemination in cows, inseminators' skill were evaluated by controlling the semen thawing procedure adopted and by using the technique of dye deposition in the genital tract of slaughtered cows. This was followed by refreshment training for the inseminators. Thirty seven artificial insemination technicians regularly working in the government, cooperative and NGO (Non Government Organization) artificial insemination programmes at different places of Bangladesh were included in the study. Individual technicians were asked to thaw a semen straw and deposit dye in the genital tract of slaughtered cows following the procedures they would have adopted in their actual practices of insemination. The time and water temperature adopted by technicians were recorded and genital tract after sham artificial insemination was dissected to determine the site of dye deposition. Then, the inseminators took part in a three days intensive training program. The training program was ended up with the same tests for thawing frozen semen straw and dye deposition in the genital tract of slaughtered cows. At pre training evaluation, only 25% and 72% (n=36) inseminators adopted correct thawing time and temperature, respectively. At post training evaluation, all inseminators thawed semen straws for proper time and temperature. At pretraining evaluation, 21 (57%), 11 (30%) and 3 (8%) inseminators deposited dye at the body of uterus, in the vagina or in cervix, and into the horn of uterus, respectively. In 2 (5%) cases dye did not pass into the genital tract, instead back flowed through the space between the barrel of insemination gun and sheath. At post training evaluation, all inseminators successfully deposited dye in the body of uterus. Frequent evaluation of inseminators' skill and subsequent training would help improvement of the artificial insemination technicians' skill.

(Key words : artificial insemination technician, cows excised genital organ, dye deposition, refreshment training)

### **INTRODUCTION**

Man's intervention in the natural processes of reproduction with the use of artificial insemination

has allowed rapid genetic improvement in dairy industry and has resulted in a marked increase in livestock productivity. However, the conception rates after artificial insemination is not more than

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<sup>†</sup> Correspondence : E-mail : mohammedshamsuddin@hotmail.com

50% in Bangladesh and 60% worldwide. The technical expertise and careful attention to semen handling, and correct placement of semen in the uterus are among others important factors that ensure the success of artificial insemination. The conception rate greatly varies with technicians because of differences in professional skill with regard to achieve high conception rate (O'Connor and Jana, 1985; Barth, 1993; Shamsuddin *et al.*, 2001). Inseminators sometime do things, because of their convenience in doing artificial insemination, that often lead to a situation detrimental to semen and/ or artificial insemination procedure. Therefore, frequent monitoring and evaluation of the artificial insemination efficiency of the professional technicians is essential and if necessary, refreshment training should be carried out. A good training programme should emphasize on pre evaluation of the technician's skill on thawing of semen and its deposition on correct site of female genital organs. This helps sorting out the inseminators with less confident in artificial insemination. Such inseminators may be given refreshment training to overcome their problems. The aim of the present study was to evaluate the skill of inseminators by dye deposition in the excised bovine female genital tract and subsequently improve their skills through refreshment training.

## MATERIALS AND METHODS

Thirty seven regularly working artificial insemination technicians of government, cooperative and NGO (Non Government Organization) artificial insemination programmes at different places of Bangladesh were asked to participate in the test. For evaluating the skill on artificial insemination, inseminators were asked individually to thaw a 0.25 ml frozen semen straw and do an insemination in the excised female genital organ. One inseminator did not take part in semen thawing as he has

been doing insemination with liquid semen (semen preserved at 4°C).

The inseminators were provided with straws in the cryocan filled with liquid nitrogen, forceps, scissors, kidney tray, warm and tape water, thermometer, insemination gun, sheath and tissue papers. They were asked individually to thaw a semen straw following the procedures they would have adopted to thaw semen during their actual practices of artificial insemination. Unnoticed to the inseminators, the time and water temperature adopted by technicians during thawing semen were recorded.

The deposition of dye by the inseminators in the excised genital tract of cows was used in the study. The reproductive tracts were collected from the local slaughterhouse immediately after slaughter. The straws were filled with gentian violet and the laboratory ends were sealed. The technicians were individually asked to cut a straw, load insemination gun and deposit the dye in the part of the genital tract where they would have deposited semen at their routine artificial insemination practices. The genital tract was then opened and the site of semen deposition was shown to the technicians. An evaluator followed the procedure and recorded the findings.

After the evaluation, the inseminators took part in three days refreshment training. The training included theory lectures and insemination practices on numerous reproductive tracts and in live cows. The lecture topic included functional anatomy of reproductive system, relationship between various organ of reproductive system, physiology of female reproduction, sanitation in artificial insemination practices and standard procedure of doing artificial insemination with frozen semen. On the third day, inseminators were asked again to thaw a frozen straw and do an artificial insemination in the excised bovine female genital organ. The procedure adopted by the inseminators were observed, recor-

ded and compared with the pre-training evaluation to judge the success of training.

## RESULTS

The part of cows' genital tract where the inseminators deposited dye in relation to the frequencies of the inseminators at pre and post training evaluation are shown in the Table 1. At pretraining evaluation, twenty one of 37 (57%) inseminators deposited dye at the body of uterus (Fig. 1), 11 (30%) inseminators deposited dye either in the va-

Table 1. Distribution of artificial inseminator technicians ( $n=37$ ) in relation to the site of gentian violet in the bovine genital tract.

Places of dye deposition	Pretraining evaluation	
	Number	%
Anterior vagina	4	44.00
Between the ring of cervix	7	19.00
Body of uterus	21	57.00
Horn of uterus	3	8.00
Back flow*	2	5.00

\* No dye found in the genital tract after open.



Fig. 1. Dye deposited in the body of the uterus, body of uterus encircled.

gina or in some parts of cervix (Fig. 2~4), in 3 (8%) cases dye was found deep into one of the horn of uterus (Fig. 5) and in 2 (5%) cases no dye was found any where in the genital tract. On inspection of the loaded artificial insemination gun, dye was found between the artificial insemination sheath and outer surface of the artificial insemination gun. In both cases, the cut end of the straws was like pen's nip. Therefore, dye did not pass in to the genital tract but poured back between the sheath and the outer surface of the insemination gun.



Fig. 2. Dye deposited in the fornix of vagina (arrow), cervix encircled.



Fig. 3. Dye in the anterior vagina, cervix encircled.



Fig. 4. Dye in front of first cervical ring, cervix encircled, arrow indicate the first ring.



Fig. 5. Dye deep into the left horn of uterus, body of uterus encircled.

At post training evaluation, all inseminators successfully deposited dye in the body of uterus.

The time and temperature of thawing of semen straw at pre and post training is shown in the Table 2. About 75% inseminators thawed the semen straws either for shorter or longer time (42.00% and 33.00%, respectively) than 10~12 seconds, the proper time of thawing. After training, all inseminators thawed semen for proper time. At pretraining evaluation, the thawing temperature ranged

Table 2. Distribution of technicians ( $n=36$ ) in relation to thawing time and temperature

Places of dye deposition	Pretraining evaluation	
	Number	%
Thawing time		
3~ 9 sec.	15	42.00
10~12 sec.	9	25.00
12~32 sec.	12	33.00
Thawing temperature		
31~34°C	3	8.00
35~38°C	26	72.00
39~46°C	7	20.00

from 31°C to 46°C. The correct temperature for thawing semen was considered to be 35~38°C. The temperature was incorrect for 28% inseminators. However, at post training evaluation all inseminators' thawed semen straw at proper temperature.

Descriptive statistics were used to present the data.

## DISCUSSION

The results of the present study reveal that more attention should be paid on the refreshment training of technicians to develop their skill. About 75% inseminators in Bangladesh are likely to achieve poor conception rate only for improper thawing of frozen semen straw and/or incorrect deposition of semen in the female genital tract.

In Bangladesh, there has been a quick shift away from the natural insemination to artificial insemination (Shamsuddin *et al.*, 2002). Such a trend should not suggest that artificial insemination is a easy technique or all inseminators are efficient in artificial insemination. The conception rate is still very low only 43% with frozen semen (Shamsuddin *et al.*, 2001). Low conception rate after artificial insemination is contributed among others by the skills of inseminator (Dyrendahl, 1980; Barth, 1993).

High conception rates require proper insemina-

tion technique. It is likely that conception will not establish although artificial insemination is done with good quality semen at the proper time of estrus if insemination technique is not in up to par. The mechanism of passing insemination gun through the cervix requires practice and periodical reviewed with the aid of professionals. Sanitary practices are critical to good insemination technique (Nebel, 1997). Good insemination technique also mean proper time of insemination at estrous. Such a training programme is essential to review inseminators' skill and habituate them to follow a step-by-step procedure of insemination to achieve good conception rate.

In an earlier study, Shamsuddin *et al.* (2001) found that conception rate varied greatly with the inseminators ranging from 31% to 61% in Bangladesh. This finding simply indicated that about 30% conception rate can be increased only by developing inseminators skill on doing artificial insemination in the existing situation prevailing in Bangladesh. Therefore, such evaluation and training is important to find out the possible malpractices in artificial insemination and to correct that.

It has been difficult to evaluate critically the accurate site of semen deposition by the technicians. Electrocauterization, evaluating radiographic plate after doing artificial insemination in living animals, linear array ultrasonography, biological dye deposition in the live animals or in excised female reproductive tract are widely used procedure for this purpose (Beal *et al.*, 1989; Peters and Senger, 1983; Peter *et al.*, 1984; O'Connor and Jana, 1985). In the present study, dye deposition in the excised genital tract of cows was used to determine the site of semen deposition by the artificial insemination technicians. The location of the dye within the tract indicated the site of semen deposition. For many years, deposition of dye method has been in use because of its accuracy, easier application and less cost involvement (O'Connor and Jana,

1985).

There are some limitations to the dye deposition methods. The location of insemination gun tip cannot be determined in this method. If the method is applied in live animals, distortion of dye distribution can occur due to manipulation of reproductive tract of cows during slaughter and/ or dissection. However, in the present study use of excised genital tract and small amount of dye (0.25 ml) minimized the problem.

One may raise question that the failure of technicians to pass the cervix may be due to unfamiliarity with phantom artificial insemination because the animals might have not been in estrus at slaughter and therefore the cervix might have been closed. Testing and training artificial insemination technicians using slaughterhouse specimen is a well accepted procedure. Because some technicians succeed to do the artificial insemination successfully, others should have done that if they were sufficiently skilled.

## CONCLUSION

Dye deposition in the excised genital tract of cows is an effective tool to evaluate artificial insemination technicians skills and training of inseminators technicians on semen thawing and depositing in the female excised genital tract is of great important to refresh their skills.

## ACKNOWLEDGEMENT

The research project has been funded by the United States Department of Agriculture, USA and the International Atomic Energy Agency, Vienna, Austria. We thankfully acknowledge the help of the Department of Livestock Services, Bangladesh, Dhaka, Bangladesh Milk Producers' Cooperative Union Limited and Bangladesh Rural Advancement Committee for their help in organizing the training.

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(접수일: 2005. 6. 12 / 채택일: 2005. 7. 24)