Project ID: 722

## Competitive Research Grant (CRG)

# Sub-Project Completion Report

## on

# A field study on the survivability of the vitrified embryos in Bangladeshi native sheep

**Project Duration**

**May 2017 to 30 September 2018**

**Department of Surgery and Obstetrics**
**Bangladesh Agricultural University,**
**Mymensingh-2202**

**Submitted to**
**Project Implementation Unit-BARC, NATP 2**
**Bangladesh Agricultural Research Council**
**Farmgate, Dhaka-1215**

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Bangladesh Agricultural Research Council (BARC)
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Published in: September 2018

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## Acronyms

| | |
|---|---|
| AI | : Artificial Insemination |
| DLO | : District Livestock Officer |
| DMSO | : Dimethyl sulfoxide |
| EG | : Ethylene glycol |
| FBS | : Fetal bovine serum |
| GnRH | : Gonadotropin releasing hormone |
| HM | : Holding medium |
| IETS | : International Embryo Transfer Society |
| MOET | : Multiple Ovulation and Embryo Transfer |
| OPS | : Open pulled straw |
| pFSH | : Porcine follicle stimulating |
| PMSG | : Pregnant mare serum gonadotropin |
| SRF | : Sheep Research Farm |
| TCM | : Tissue culture medium |
| ULO | : Upazila Livestock Officer |
| VFA | : Veterinary Field Assistant |
| VS | : Veterinary Surgeon |

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**Executive Summary**

Indigenous sheep are highly prolific and are mainly reared by the distressed women and poor, marginal, landless and small-scale farmers living mostly in the Char lands of Bangladesh. Sheep could play a major contributing role in food security, poverty reduction and income generation if the genetic merit of the indigenous sheep could be improved through proper breeding management. The principle objective of the present work was therefore breeding management of sheep at farmer's level using MOET, embryo vitrification and direct transfer of vitrified embryos to the recipient ewes. For embryo production, 35 donor ewes underwent estrus with Ovoprost® (Cloprostenol). Superovulation was induced by intramuscular injection of pFSH. Embryos were collected either by inguinal laparotomy or semi-laparoscopic method and graded under sterio-microscope. Grade 1 embryos were vitrified in a medium containing TCM 199, Ethylene glycol, Dimethyl sulfoxide and sucrose solution at different concentrations and duration of time. The vitrified embryos were then stored in liquid nitrogen at -196$^{o}$C until use. A total of 30 sheep farmers were selected and trained for motivation. For transfer of vitrified embryos, 46 ewes (recipient) from the trained farmers were selected, dewormed, vaccinated and given supplementary diet. The recipients were synchronized for estrus and embryos were transferred directly by inserting embryo straw, the open pulled straw (OPS), in the exteriorized tip of the uterine horn. Among the 46 recipients, 16 were treated with 20µg Gonadorelin (Fertagyl®, Intervet, Netherlands) immediately after embryo transfer. Hundred percent ewes showed estrus following injection of Ovoprost®. The Onset of estrus in donor and recipient ewes after 2$^{nd}$ injection of Ovoprost® were 30.2±.0.8 hours and 33.73±0.4 hours whereas, duration of estrus were 27.9± 0.6 hours and 27.5±0.4 hours, respectively. The ewes responded for superovulation induction was 85.7%. The mean of superovulation was 8.5±0.68 and the embryo recovery rate was 82.5%. The embryos recovered per donor were 6.9±0.86. The vitrifiable embryos per donor were 6.5±0.8. The number of Grade 1 embryos were significantly ($p<0.05$) higher (5.50) than other grades (1.00, 0.7 and 0.6 embryos for Grade 1, 2 and 3 respectively). Grade 1 vitrified embryos were transferred to 46 synchronized recipients. Pregnancy rates in non-treated recipients were 56.6% and GnRH treated recipients were 62.5 %, whereas the lambing rates were 76.5 and 80%, respectively. Although a number of obstacle and challenges were faced in the research station as well as in the field the rate of embryo production with MOET was good (82.5%). Pregnancy rate following transfer of vitrified embryos at the field level was 62.5 %. It is hoped that that this study is ready to contribute to increase quality sheep production in Bangladesh at field level on demand.

# CRG Sub-Project Completion Report (PCR)

## A. Sub-project Description

1. **Title of the CRG sub-project:**
   A field study on the survivability of the vitrified embryos in Bangladeshi native sheep

2. **Implementing organization:**
   Department of Surgery and Obstetrics
   Bangladesh Agricultural University
   Mymensingh, 2202

3. **Name and full address with phone, cell and E-mail of PI/Co-PI (s):**
   **Principal Investigator:**
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   Bangladesh Agricultural University, Mymensingh,2202.
   Cell: 01745996622, E-mail: fybari61@yahoo.com.au

4. **Sub-project budget (Tk.):**

   4.1 **Total** **:** Tk. 20,11,440.00
   4.2 **Revised (if any)** **:** Tk. 18,97,443.00

5. **Duration of the sub-project**
   5.1 **Start date (based on LoA signed)** **:** May 2017
   5.2 **End date** **:** 30 September 2018

6. **Justification of undertaking the sub-project:**

   Bangladeshi climate is favorable for production and breeding of indigenous sheep. Indigenous sheep are highly prolific and are mainly reared by the distressed women and poor, marginal, landless and small-scale farmers in Char -islands and also in plane land. During recent times, farmers of these areas are showing interest to rear sheep to improve their livelihood and food security since sheep reproductive cycle is short and they are easy to rear. The Government has also given emphasis on sheep production. However, sheep breeding and production at farmers' level is being hampered due to poor genetic merit and management. In addition there is also scarcity of quality stud ram for mating. Furthermore, use of rams born in the same flock (generation after generation) may develop in-breeding depression resulting in dwarfism and increased mortality. The farmers are ignorant of using controlled breeding system and ARTs specially AI and MOET in their farms to speed up the genetic gain. Therefore, the present study was undertaken to increase awareness of sheep farmers about the improved sheep production and to establish and optimize the technology for speeding up the genetic merit through female line.

**7. Sub-project goal:**

Introduction of genetic exploitation technology (MOET, embryo cryopreservation and embryo transfer) in the field to increase and improve sheep production in Bangladesh

**8. Sub-project objective (s):**

a. To produce quality embryos from donor ewes, their successful vitrification and storage.
b. To observe the survivability of vitrified embryos following direct transfer in recipient ewes in field condition.
c. To develop farmer's awareness about sheep breeding, their management, and advantages of using MOET and embryo vitrification.

**9. Implementing location (s):**

- On-station experiment was conducted at the Sheep Research Farm and Reproduction Laboratory, Department of Surgery and Obstetrics, Bangladesh Agricultural University, Mymensingh-2202.

- On-farm research was conducted at Fulpur upazila, Mymensingh.

**10. Methodology in brief:**

**10.1. Production of quality embryos from donor ewes:**

**<u>Embryo production from donor ewes</u>**

**a) Selection, purchase and management of donor ewes and rams**

Selection, purchase and management of donor ewes and rams were conducted at the Sheep Research Farm (SRF), Department of Surgery and Obstetrics, Bangladesh Agricultural University, Mymensingh (Figure 1). Embryo production and vitrification was performed in the Embryo laboratory under the Department of Surgery and Obstetrics, Bangladesh Agricultural University, Mymensingh.

**Figure 1:** Donor ewes management at research station

**b) Synchronization of estrus and superovulation of donor ewes**

Synchronization of estrus was performed by two intramuscular (i.m.) injection of Ovoprost® at the dose rate of 175 µg (Ovuprost® Cloprostenol, Bomac laboratories Ltd, Manukau) at nine days apart. Superovulation was induced using porcine Follicle Stimulating Hormone (pFSH, Folltropin®-v, Bioniche

Animal Health, Belleville, Ontario, Canada) by twice intramuscular injection at 12 hours (hrs) interval beginning from Day 10 after second injection of Ovoprost® for 4 consecutive days. Estrus was detected by the acceptance of teaser ram introducing 24 hrs after second injection of Ovoprost® and on the basis of behavioral and clinical signs (Figure 2).

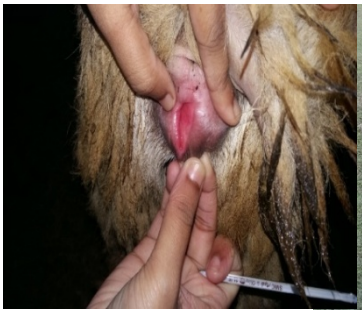

**Figure 2:** Oestrous conformaiton using teaser ram

**Figure 3:** Hand mating of donor ewes

**c) Hand-mating for fertilization of induced ovum**

Estrus ewes were ensured for hand mating using proven fertile ram in the open grazing land. The ewes were then hand mated (Figure 3) twice at 4-6 hrs intervals after 12-14 hrs of the onset of oestrus.

**d) Superovulation, embryo collection and grading of donor ewes**

The activities related to superovulation, embryo collection and grading of embryos are presented in Figures 4, 5, 6 and 7. Embryos, 6.5-7.0 days old were collected from donor ewes by inguinal laparotomy with 3.81 centimeter (cm) incision. Donor ewes were fasted for 24 hrs before surgery. All donor ewes were pre-medicated using Acepromazine Maleate (ACP, Delvet PTY. Ltd) 0.1 mg/kg body weight approximately 15-30 min before surgery for induction of general anaesthesia. General anaesthesia was induced by i.m. injection of Xylazine 0.22 mg/kg body weight (Xylazine, Indian Immunological Ltd, Hydrabad). A local anesthesia was achieved by subcutaneous injection of 10 ml 2% lidocaine on the incision site. The donor ewes were placed on the laparoscopic cradle in a head down position. About 3.81 cm incision was given in inguinal region on in front of the udder, 4-5 cm away from the ventral mid line. Following exteriorization of the reproductive tract, an 8-G two ways Foley catheter was introduced into the lumen of the uterine horn through a hole made in front of bifurcation. The number of ovulation was determined by counting the number of corpus luteum in the ovary. The embryo collection medium (Tissue culture medium 199 + 10% fetal bovine serum) was flushed through the horn using a 19- blunt needle inserted into the tip of the horn. The flushed out medium was collected in a sterile pot through the Foley catheter outlet. The same process was then repeated for the other uterine horn. The embryos were evaluated and graded as per guideline of International Embryo Transfer Society (IETS). Only the grade 1 embryos were vitrified.

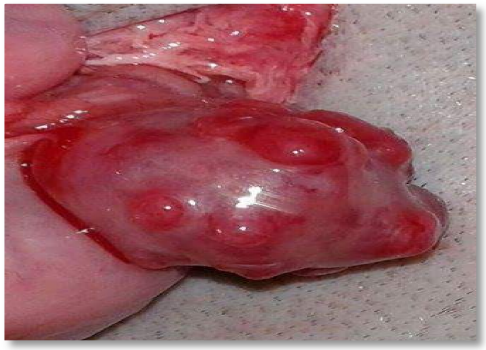

**Figure 4:** Superovulation response in donor ewes

**Figure 5:** Collection of embyros from donor ewes

**Figure 6:** Searching and grading of embryos

**Figure 7:** Graded embryos for vitrification

**e. Embryo vitrification**

The embryos were vitrified in open pulled straw (OPS) using a cryopreservation solution of dimethyl sulfoxide (DMSO) and ethylene glycol (EG) by the technique as described by Mara *et al*. (2013). The embryos were initially equilibrated for at least 10 min in holding medium (HM) consisting of Tissue culture medium (TCM) 199+20% FBS. After initial equilibration, the embryos were exposed to equilibration solution containing 10% EG+ 10% DMSO in HM at room temperature for 5 minutes and then successively passed the vitrification solution containing 20% EG + 20% DMSO + 0.5 M sucrose in HM for ≤40 Sec (Kuwayama *et al*., 2005). The embryos were then loaded into OPS (2 embryos per OPS) and then immediately plunged into liquid nitrogen. The OPS with the vitrified embryos was stored and maintained in liquid nitrogen till thawing and transfer. Embryo vitrification process and storing of OPS in liquid nitrogen are shown in Figures 8, 9 and 10.

**Figure 8:** Embryos in vitrification process

**Figure 9:** Embryos in vitrification solution


**Figure 10:** Liquid nitrogen can containing vitrified embryos

**10.2. Survivability of vitrified embryos following direct transfer in recipient ewes:**

**<u>Vitrified embryo transfer in recipient ewes</u>**

**i) Farmer selection, training and motivation**

To implement the project at the field level a day long training program was organized with the help of DLO (District Livestock Office) Mymensingh, Upazila Livestock Officer (ULO), Veterinary Surgeons (VS), Veterinary Field Assistant (VFA) of Fulpur upazila, Mymensingh along with Master's students, Department of Surgery and Obstetrics, Bangladesh Agricultural University (BAU). A total of 30 sheep Farmers, having minimum 5 to maximum 15 sheep from Kandapara, Ramvadrapur Union Porishad under Fulpur upazila, Mymensingh participated in the training programme. Each farmer had registration with address and contact mobile number (enclosed). The DLS officers actively participated in the training program as resource persons and gave their valuable lectures about the benefits of sheep farming. They also encouraged the farmers to participate in the project work to use better management, manipulative reproduction as a breeding tool for increasing their sheep production and socio-economic condition. The PI of the project delivered audio-visual presentation on the advantages of sheep farming in Bangladesh. The PI discussed about the project concept, its objectives, mode of work with the farmers and intervention which was going to provide by the project and the outcomes/benefits which would get by

the farmers. The presentation emphasized the benefits of using MOET and vitrified embryos for speeding up of genetic improvement in the farmer's ewe. The farmers were keen to accept the technology. Activities related farmer selection, training and motivation are shown in Figures 11, 12, 13 and 14.

**Figure 11:** Farmers training at Fulpur livestock office

**Figure 12:** Farmers attending the training


**Figure 13:** Officials and farmers at training programme

**Figure 14:** Field visit by the PI and her team

**ii) Recipient ewe selection, rearing and intervention with medicines and vaccines:**

Estimated 100 ewes from the 30 selected sheep farmers were targeted for the study at the farmer's field for the entire duration of the project. The ewes were ultrasound scanned for non-pregnancy and selected for the project. The ewes were then dewarmed by subcutaneous (s. c.) injection of Amectin Plus (Ivermection® plus chlorsulon) and vaccinated against PPR and Rabbies. They were also supplemented with diet. Activities related to selection and management (deworming and vaccination) of recipient ewes at farmers field are shown in Figure 15.

Field visit for selection of farmers ewes and selection of non-pregnant recipient ewes by ultrasonography

Deworming and vaccination of recipient ewes

**Figure 15:** Activities related to selection and management of recipient ewes at farmers field

**iii) Estrus synchronization of recipient ewes**

The recipient ewes were synchronized for estrus in the same way as the donor ewes. The recipient ewes were injected with 500 I.U. PMSG (Pregnant mare serum gonadotropin) for minor stimulation of folliculogenesis (Figure 16) and were regularly monitored for the onset of estrus using teaser ram (Figure 17).


**Figure 16:** Injection of Ovoprost® in recipient ewes **Figure 17:** Teaser-detection of oestrus in recipient ewes

**iv) Direct transfer of embryos in the synchronized recipient ewes**

The vitrified embryos were transferred directly to the synchronized recipient ewes with the similar stage of development of uterus of the donor. The ewes were fasted for 24 hrs before transfer and the tip of the uterine horn was exteriorised through minor inguinal surgery or laparoscopy. The vitrified embryos within OPS was warmed in warming solution and directly transferred to the tip of the exteriorised horn without microscopic evaluation. The OPS was attached to Tom Cat catheter which was further attached with 1ml syringe to introduce embryos within horn. Two embryos were transferred per recipient ewe. Activities related to direct transfer of vitrified embryos in the synchronized recipient ewes are shown in Figure 18.

Taking out of vitrified embryos from liquid nitrogen can and warming up of the embryos

Preparation of recipient ewes for embryo transfer

Transfer of vitrified embryos in the horns

**Figure 18:** Activities showing direct transfer of vitrified embryos in the synchronized recipient ewes

**v. Attempt to increase pregnancy rate through GnRH injection:**

Among 46 recipients, 16 ewes were infected intramuscularly (i.m.) with 20 µg of Gonadorelin (0.2 ml Inj. Fertagyl®, Intervet, Boxmeer, The Netherlands) immediately after transfer of vitrified embryos.

**vi. Pregnancy diagnosis:**

Pregnancy was determined by ultrasonography (USG) at day 40-50 post embryo transfer to recipients. Pregnant ewes and the lambs born following transfer of vitrified embryos are shown in Figure 19.

Farmers with pregnant ewes

Lamb born following transfer of vitrified embryos (non-treated group)

Lamb born following transfer of vitrified embryos (GnRH treated group)

**Figure 19:** Pregnant ewes and the lambs born following transfer of vitrified embryos

**vii. Statistical analysis:**

The collected data were entered in Microsoft Excel program. The values relating to all parameters were expressed as mean ±Standard error of mean (SEM). The comparison of grade 1, grade 2, grade 3 and grade 4 embryos was performed using ANOVA. The Percentages of embryos were calculated. This statistical analysis was done using SPSS (Statistical Package for the Social Sciences) Version 20.0.

**10. 3. Farmer's awareness development:**

For motivation and awareness development related to sheep breeding, their management, advantages of using MOET, embryo vitrification, etc. a total of 30 sheep farmers were selected from Fulpur upazila of Mymensingh and a training program was arranged for the farmers.

**11. Results and discussion:**

**11.1. Production of quality embryos from donor ewes**

Multiple Ovulation and Embryo Transfer (MOET) is the manipulative reproductive technology used to speed up genetic gain by production of large number of lambs within short period of time through utilization of genetic superiority of sire and dam (Bladassarre *et al.*, 2007; Menchaca *et al.*, 2009). It involves the synchronization of estrus in donors and recipients, superovulation, fertilization, embryo collection, grading and subsequent transfer to the recipient ewes to be carried up to term (Bari *et al*., 2003). In the present study, the results of estrus synchronization of donor and recipient ewes induced with $PGF_2\alpha$ hormones are placed in Table 1. Thirty five donor and 46 recipient ewes were induced for synchronization of estrus. Eestrus induction was seen in 100% ewes. Onset of estrus in donor and recipient ewes after 2nd injection of Ovoprost® were 30.20 ± 0.80 hrs and 33.73±0.40 hrs, respectively. The duration of estrus was 27.90±0.60 hrs and 27.50±0.40 hrs, respectively. This response is similar to other published works in the same laboratory and abroad (Zahara *et al*., 2014; Hackett *et al*., 1981 and Kusina *et al*., 2000). Therefore, to achieve responsive estrus synchronization on indigenous ewes two injections of $PGF_2\alpha$ at the dose rate of 175 mg should be given 9 days apart. The donor ewes showed estrus significantly earlier than recipient ewes, however, no difference was observed for the duration of estrus. About 3 hrs asynchrony of estrus was excellent environment in the uterus of recipient ewes for embryo survival.

**Table 1.** Oestrus synchronization of donor & recipient ewes (Mean ± SE)

| **Parameter** | **donor (n=35)** | **Recipient (n=46)** | **P-value** |
|---|---|---|---|
| Onset of estrus (hrs) | 30.20 ±0.80 | 33.73±0.41 | 0.001 |
| Estrus duration (hrs) | 27.90 ± 0.60 | 27.50±0.42 | 0.584 |

MOET is well accepted and applied worldwide to speed up of genetic gain of economic traits (Bladassarre*et al*., 2007; Menchaca*et al*., 2009). MOET protocol has been developed, routinely used in our laboratory and found feasible for indigenous sheep (Zohara *et al*., 2014). The animal is said to be superovulated when it produces more than or equal to 3 oocytes. The result of superovulation and embryo production in donor ewes are shown in Table 2. In the present study, among thirty five, 30 ewes responded to superovulation induction (85.70%). This response was excellent. The success of MOET has been shown to be limited by wide variation between donor ewes on responses. That's why the program requires the involvement of large number of donor ewes. In the present study, the number of superovulation per donor ewes was 8.50±0.68. The number of recovered embryos per donor was 6.93±0.86. The percent of embryo recovery was 82.50%. The number of superovulation in this present project was good, similar with many cited references. The mean number of superovulation would be more if it was not affected by season and monsoon in the present study. The embryos were collected either by semi-laparoscopy or inguinal surgical method. The number of embryos recovered was related to the number of superovulation per donor which was excellent.

**Table 2.** Superovulation and embryo production in donor ewes

| **Parameter** | **(Mean ± SE)** |
|---|---|
| % ewes responded superovulation (n=30) | 85.70% |
| No. of corpus luteum/donor | 8.50 ±0.68 |
| No. of recovered embryo/donor | 6.90 ±0.86 |
| Embryo recovery rate (%) | 82.50% |

The number of different quality of yielded embryos is shown in the Table 3. Most of the collected embryos were Grade 1 and significantly higher than other grades ($p<0.05$). Therefore, it could facilitate greater embryosurvival rate following direct transfer.

Vitrification is a method of producing cryo-preserved embryos that could be preserved for long time. Vitrification does not require any special equipment but could provide quality embryos to farmers on demand, and make the MOET technique cost effective for genetic improvement (Green *et al*., 2009; Baril *et al*., 2001)**.** The mean number of vitrified embryos in the present study was 6.50 ±0.80, which was consistent with the number of ovulation and embryo recovery rate.

**Table 3:** Embryo grading after collection and vitrification of embryos

| **Embryo Grading and vitrification** | **Mean ± SE** |
|---|---|
| Grade 1 | 5.50 ±0.77* |
| Grade 2 | 1.00 ±0.33 |
| Grade 3 | 0.70±0.25 |
| Grade 4 | 0.60±0.17 |
| No. of vitrifiable embryo/donor | 6.50 ±0.80 |

**11.2. Survivability of vitrified embryos following direct transfer in recipient ewes:**

Vitrification in open-pulled straw and direct plunging into the liquid nitrogen for rapid cooling, fast thawing of embryos and microscopic-free transfer with potential application to the field indicates the novel technology for speed up of genetic improvement (Isachenko *et al*., 2003). The survival of vitrified embryos by direct transfer is shown in Table 4. The vitrified embryos were transferred to 46 recipient ewes of farmers. Two embryos were transferred to each recipient. The pregnancy and lambing rates were 58.70% and 77.80%, which were comparable to other studies (Baril *et al.*, 2001; Isachenko *et al*., 2003). This increased survival of embryos could be due to advantages of direct transfer in recipients. Baril *et al*. (2001), stated that direct transfer of vitrified embryos represent a potential gain of 7 to 8% in terms of lamb born which could increase the genetic gain in sheep MOET. They explained that the morphological evaluation of frozen thawed embryos in indirect transfer may lead to the elimination of

viable embryos or the methods used for cryo-protactant removal are less efficient than direct transfer. The success of the present project is the production of lambs (n= 21) through transfer of cryo-preserved embryos in field sheep by direct transfer.

**Table 4:** Pregnancy and lambing rate following direct transfer of vitrified embryos to recipient ewes

| **Parameters** | **Values** |
|---|---|
| No. of recipient ewes | 46 |
| No. of vitrified embryos transferred | 92 |
| No. of pregnant ewes | 27 |
| Pregnancy rate (Pooled data, non-treated +treated) | 58.70% (27/46) |
| Number recipient delivered lambs | 21 |
| Lambing rate | 77.80% (21/27) |

** One pregnant ewe died due to carbohydrate engorgement.*

GnRH has been shown to increase the pregnancy rate by inducing luteal function (Reyna *et al.,* 2007). Effects of GnRH on pregnancy rate following direct transfer of vitrified embryos to recipient ewes are shown in Table 5. In GnRH treated group and non-treated group, number of pregnant recipient ewes were 10 and 36. Although the pregnancy and lambing rates were tended to increase in GnRH treatment group (62.50% and 80%) compared with non-treatment group (56.70% and76.50%) however, the difference was not significant. This could be due to the fact that the number of recipients in treatment group was smaller compared with non-treatment group.

**Table 5:** Effects of GnRH on Survivability of vitrified embryos following direct transfer to recipient

| **Parameters** | **Treated group (n=16)** | **Non-treated group (n=30)** |
|---|---|---|
| No. of pregnant ewes | 10 | 17 |
| Pregnancy rate | 62.50% | 56.70% |
| % recipient lambed | 80% (8/10) | 76.50% (13/17) |

**11.3. Farmer's awareness development:**

A total of 30 sheep farmers (08 male and 22 female) attended the daylong training program held on 11.1.2017. Along with the experts from Bangladesh Agricultural University, Mymensingh District Livestock Officials from Fulpur attended the program as resource persons. The topics of the training included advantages of improve management of sheep, controlled sheep breeding, vitrified embryo transfer in the sheep, etc.

**12. Research highlight/findings:**

- The ewes responded for superovulation induction was 85.7%. The mean of superovulation was 8.5±0.68 and the embryo recovery rate was 82.5%. The embryos recovered per donor were 6.9±0.86. The vitrifiable embryos per donor were 6.5±0.8. The number of Grade 1 embryos were significantly ($p<0.05$) higher (5.50) than other grades.

- MOET (Multiple Ovulation and Embryo Transfer) in indigenous sheep has been established. The rate of embryo production with MOET was 82.5%. Pregnancy rates in non-treated recipients were 56.6% and in GnRH treated recipients were 62.5 %, whereas the lambing rates were 76.5 and 80%, respectively.

- Embryo vitrification protocol has been developed and optimized.

- Protocol for direct transfer of vitrified embryos in the recipient ewes at farmer's level has been developed with the satisfactory pregnancy rate of 56.70%.

- First time recorded in Bangladesh, the birth of lambs by direct transfer of vitrified embryos in the recipient ewes at farmer's level.

**B. Implementation Position**

**1. Procurement:**

| Description of equipment and capital items | PP Target | | Achievement | | Remarks |
|---|---|---|---|---|---|
| | Phy (#) | Fin (Tk) | Phy (#) | Fin (Tk) | |
| (a) Office equipment | - | - | - | - | - |
| (b) Lab &field equipment | Liquid Nitrogen can (01) | 55,000.00 | 01 | 54,900.00 | Purchase was completed. |
| (c) Other capitalitems | - | - | - | - | - |

**2. Establishment/renovation facilities:**

| Description of facilities | Newly established | | Upgraded/refurbished | | Remarks |
|---|---|---|---|---|---|
| | PP Target | Achievement | PP Target | Achievement | |
| | | | | | |
| Animal shed renovation | - | - | 20,000.00 | 20,000.00 | Renovation was completed |

**3. Training/study tour/ seminar/workshop/conference organized:**

| Description | Number of participant | | | Duration (Days/weeks/ months) | Remarks |
|---|---|---|---|---|---|
| | Male | Female | Total | | |
| (a) Training | 08 | 22 | 30 | 1day Dated: 11.11.2017 | Excellent participation and coordination. |
| (b) Workshop | - | - | - | - | - |

**C. Financial and physical progress**

**Fig in Tk**

| Items of expenditure/ activities | Total approved budget | Fund received | Actual expenditure | Balance/ unspent | Physical progress (%) | Reasons for deviation |
|---|---|---|---|---|---|---|
| A. Contractual staff salary | 5,65,254.00 | 4,88,764.00 | 4,88,764.00 | 00 | 100.00 | |
| B. Field research/ lab expenses and supplies | 11,17,686.00 | 11,04,898.00 | 11,04,898.00 | 00 | 100.00 | |
| C. Operating expenses | 1,20,000.00 | 1,20,000.00 | 1,19,855.00 | 145.00 | 99.89 | |
| D. Vehicle hire and fuel, oil & maintenance | 00 | 00 | 00 | 00 | 00 | |
| E. Training/ workshop/seminar etc. | 50,000.00 | 50,000.00 | 50,000.00 | 00 | 100.00 | |
| F. Publications and printing | 85,000.00 | 60,282.00 | 25,000.00 | 35,282.00 | 41.47 | |
| G. Miscellaneous | 18,500.00 | 18,500.00 | 18,500.00 | 00 | 100.00 | |
| H. Capital expenses | 55,000.00 | 55,000.00 | 54,900.00 | 100.00.00 | 99.82 | |
| Total TK | 20,11,440.00 | 1,897,443.00 | 1,860,841.00 | 35,527.00 | 98.07 | |

**D. Achievement of sub-project by objectives:**

| Specific objectives of the sub-project | Major technical activities performed in respect of the set objectives | Output (i.e. product obtained, visible, measurable) | Outcome (short term effect of the research) |
|---|---|---|---|
| To produce quality embryos from donor ewes, their successful vitrification and storage | i. Estrus synchronization of donor by i.m. injection of PGF2α.<br>ii. Superovulation by i.m injection of pFSH for 4 consecutive days.<br>iii. Hand mating to fertilize the superovulated ovum.<br>iv. Embryo collection by inguinal laparotomy from ewes on day 6-7 of hand mating.<br>v. Embryo grading to obtain the quality embryos.<br>vi. Vitrification of quality embryos in OPS and storage in liquid nitrogen | i. 100% donor ewes showed estrus.<br>ii. 85.70% responded for superovulation.<br>iii. 82.50 % embryos were recovered.<br>iv. 80.0% of the recovered embryos ware grade 1.<br>v. Vitrification was excellent observed by pregnancy rates.<br>vi. Development of Mutiple Ovulation and Embryo Transfer program for quality embryo production. | i. The knowledge developed through this project will help to preserve genetic resources resulting in protection of biodiversity<br>ii. The knowledge will help in infertility treatment following transfer of cryo-preserved embryos |
| To observe the survivability of vitrified embryos following direct transfer in recipient ewes in field condition | i. Selection, deworming and vaccination of farmer's ewes.<br>ii. Synchronization of estrus of farmer's ewes (recipients) by i.m. injection of $PGF_2\alpha$.<br>iii. Warming of vitrified embryos in OPS.<br>iv. Direct transfer of the embryos without observing under microscope in the tip of uterine horn with tom cat catheter with inguinal laparotomy.<br>v. Injection of GnRH hormone immediately after embryo transfer in recipient ewes (treatment group). | i. 100% responded to estrus induction.<br>ii. 100% attempt was successful to transfer the embryos.<br>iii. Pregnancy rate was 56.60% following transfer.<br>iv. Pregnancy rate was 62.85 following stimulation of luteal development.<br>v. Developed the protocol for direct transfer of vitrified embryos in to the field recipient ewes without observing the existence of embryos within the open pulled straw. | |
| To develop farmer's awareness | Selection, interaction and day long workshop on advantages of improve management, controlled sheep breeding, vitrified embryo transfer in their sheep. | Training programme was 100% successful with the help of District Livestock Officials. | |

**E. Materials Development/Publication made under the Sub-project:**

| Publication | Number of publication | | Remarks (e.g. paper title, name of journal, conference name, etc.) |
|---|---|---|---|
| | Under preparation | Completed and published | |
| Technology bulletin/booklet/leaflet/flyer etc. | | | |
| Journal publication | **1** | | |
| Information development | | | |
| Other publications, if any | **Abstract 1** | **1** | Effects of GnRH treatment on survivability of direct transferred vitrified embryos in recipient ewes |

**F. Technology/Knowledge generation/Policy Support (as applied):**

**i. Generation of technology (Commodity )**

i. Embryo vitrification protocol for MOET program optimized.
ii. Protocol developed for direct transfer of vitrified embryos in to the field recipient ewes without observing the existence of embryos within the open pulled straws.

ii. **Generation of new knowledge that help in developing more technology in future**

i. The knowledge developed through this project will help to preserve genetic resources resulting in protection of biodiversity
ii. The knowledge will help in infertility treatment following transfer of cryo-preserved embryos

**iii. Technology transferred that help increased agricultural productivity and farmers' income**

None

iv. **Policy Support**

None

**G. Information regarding Desk and Field Monitoring**

**i) Desk Monitoring:**

| SL no. | Desk Monitoring parameters | description & output of consultation meeting, monitoring workshops/seminars |
|---|---|---|
| **1** | Visit by the member director of BARC, Livestock PIU-BARC, NATP-2 | The member director and his team directly visited the PI office, laboratory and sheep shed. Through discussion with the PI, MS student and laboratory attendant evaluation was done on the type and amount of research. The results and future work direction was discussed with the PI. The report submitted following the visit as ''Good progress''. |
| **3** | Visit by, Director and his team, PIU-BARC, NATP-2 | There was progress report presentation during visiting by director of BARC, Livestock PIU-BARC, NATP-2. The remarks were excellent. The team also visited the sheep farm and was happy about the work and management. |
| **4** | Two workshop and seminar- **six monthly and annually.** | **Six monthly workshop**: Excellent presentation. The only comment was to increase the pregnancy rate following direct transfer (56.70%). Thereafter, attempts were undertaken to increase the pregnancy rate by injecting GnRH immediately after embryo transfer. The increased pregnancy rate was 62.50%.<br>**Annual Report**: Excellent presentation. The participants were happy with the answer of all questions. The comments were about the cost of embryo transfer, selection criteria of donor and recipients, collection and transfer methodology.<br>Cost benefit analysis was not included in the PP, selection of donor and recipient was on the basis of phenotypic characteristics, collection and transfer of embryos by inguinal laparotomy. |

**ii. Field Monitoring (time& No. of visit, Team visit and output):**

| Sl. No | Time | No. of visit | Team visit | Output |
|---|---|---|---|---|
| 1. | Dated 4.03.2018 | 01 | Visited by the member director and his team. BARC, Livestock PIU-BARC, NATP-2 | The sheep field was visited by the member director of BARC, Livestock PIU-BARC, NATP-2. Their comment was good. |
| 2. | Dated. 25.03.2018 | 01 | Visited by, Director and his team, PIU-BARC, NATP-2 | The sheep field was visited by Director of BARC, Livestock PIU-BARC, NATP-2. The team was happy about the work and management in the farm. They also took photograph of the sheep and farm. The remark was excellent. |

**I. Lesson Learned (if any)**

i. Farmers are not aware of economic return – just keep the sheep for food and income.

**J. Challenges:**

- Natural disaster (flood and heavy rain) at the beginning delayed the project activities with the farmers.
- Farmer's motivation and co-operation was challenging for the work at the field level.
- Small farm size.

25/02/2019

Signature of the Principal Investigator
Date .....
Seal

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**References:**


Bari FY, Khalid M, Haresign W, Murray A, Merrell B. 2003. Factors affecting the survival of sheep embryos after transfer within a MOET program. Theriogenology. 59: 1265-1275.

Baril G, Traldi A-L, Cognie Y, Leboeuf B, Beckers JF, Mermillod P. 2001. Successful direct transfer of vitrified sheep embryo. Theriogenology. 56:299-305.

Bladassarre H, Rao KM, Neveu N, Brochu E, Begin I, Behboodi E, Hockley DK. 2007. Laparoscopic ovum pick-up followed by in vitro embryo production for the reproductive rescue of aged goats of high genetic value. Reproduction fertility and development. 19: 612-616.

Green RE, Santos BFS, Sicherle CC, Landim-Alvarenga FC and Bicudo SD. 2009. Viability of OPS vitrified sheep embryos after direct transfer. Reproduction in Domestic Animals. 44, 406-410.

Greyling JPC, Van Der Westhuysen JM 1980: The synchronization of oestrus in sheep. The use of intravaginalprogestagen and/ or prostaglandin. South African journal of animal Science 10 65-68.

Hackett AJ, Robertson HA, Wolymotz MS.1981. Effects of prostaglandin F2α and pregnant mare serum on the reproductive performance of flourogestone acetate PMSG treated ewes. Journal of Animal Science. 53: 154.

Isachenko, Vladimir, EvgeniaIsachenko, Markus Montag, Victoria Zaeva, Irina Krivokharchenko, Frank Nawroth, Salvatore Dessole, Igor I. Katkov, and Hans van der Ven. "Clean technique for cryoprotectant-free vitrification of human spermatozoa." *Reproductive biomedicine online*10, no. 3 (2005): 350-354.

Kusina NT, Tarwirei F, Hamudikuwanda H, Agumba G, Mukwena J. 2000. A comparison of the effects of progesterone sponges and ear implants, PGF2alpha, and their combination on efficacy of oestrus synchronization and fertility of Mashona goat does. Theriogenology. 53: 1567-1580.

Kuwayama M, Vajta G, Ieda S, Kato O. 2005. Comparison of open and closed methods for vitrification of human embryos and the elimination of potential contamination. 11: 608-14.

Mara L, Sanna D, Casu S, Dattena M and Mayorga Munoz IM. 2013. Blastocyst rate of in vitro embryo production in sheep is affected by season. Zygote 22: 366-371.

Menchaca A, Vilarino M, Pinczak A, Kmid S, Saldana JM. 2009. P4 treatment, FSH plus eCG, GnRH administration, and day 0 Protocol for MOET programmes in sheep. Theriogenology. 72: 477-483.

Reyna J, Thomson PC, Evans G 2007: Synchrony of ovulation and follicular dynamics in Merino ewes treated with GnRH in the breeding and non-breeding seasons. *Reproduction of Domestic Animals***42** 410-417

Zohara BF, Azizunnesa, Islam F, Alam MGS, Bari FY. 2014. Comparison of estrus synchronization by $PGF_2\alpha$ and progestogen sponge with PMSG in indigenous ewes in Bangladesh. GSTF International Journal of Veterinary Science (JVET).1 (1): 27-37.