Pregnancy rate in indigenous ewes by direct transfer of vitrified embryos

S Ghosh*, MRI Talukder, PK Jha, MGS Alam, NS Juyena and FY Bari
Department of Surgery and Obstetrics, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

Abstract

The effects of PMSG on superovulation, quality of embryos, and pregnancy rate were studied following transfer of vitrified embryos into indigenous ewes. Three donor and nine recipient ewes were synchronized using two intramuscular doses of Cloprostenol (PGF2α) equivalent to 125 µg Prostaglandin F2α (Ovoprost® Bayer, New Zealand) at an interval of nine days. To ensure ovulation donor and recipient ewes were treated with 600 iu and 250 iu Pregnant Mare Serum Gonadotrophin (PMSG; Folligon®, Intervet, Boxmeer, The Netherlands) at the time of 2nd injection of Prostaglandin F2α. All donor ewes were mated by fertile rams. Embryos were collected from donor ewes on day six after mating by inguinal laparotomy. Grade 1 embryos were vitrified using standard procedure. After thawing the embryos were transferred into the exteriorized uterine horn of the recipient by inguinal laparotomy. Pregnancy diagnosis was performed by trans-abdominal ultrasonography on Day 40 of transfer. Oestrus occurred 31.3 ± 8.1 hours after second injection of prostaglandin and 32.6 ± 8.7 hours in recipients. Oestrus lasted 24.7 ± 9.9 hours in donors and 32.8 ± 12.8 hours in recipients. The mean numbers of corpora lutea and embryos of donor ewes were 11.7 ± 4.0 and 8 ± 2.6, respectively. Total embryo recovery rate of donor ewes was 68.6%. The mean values of qualities of embryo yield of donor ewes were 6 ± 1.7 grade 1, 0.3 ± 0.6 grade 2, 1.3 ± 1.2 grade 3, and 0.3 ± 0.6 grade 4. Twenty embryos were vitrified, 16 embryos were transferred and four recipient ewes were found pregnant. The pregnancy rate of recipient ewes was 44.4%. (Bangl. vet. 2017. Vol. 34, No. 1, 27 – 33)

Introduction

The productivity of indigenous sheep is low due to poor genetic merit (Alam et al., 2001), poor nutrition (Alam et al., 2006), and weak management. Multiple Ovulation and Embryo Transfer (MOET) is well accepted and applied worldwide to speed up genetic gain through production of large number of lambs, reducing generation interval and utilization of superior dams (Bari et al., 2003; Bladassarre et al., 2007; Menchaca et al., 2009). The MOET programme, vitrification of embryos for storage followed by direct transfer into recipients, can reduce the cost of embryo transfer (Martinez et al., 2006) with potential applications in field conditions. Direct transfer of fresh embryos is limited in small ruminants compared with cattle, but vitrification in combination with embryo transfer can reduce costs and increase the use of this

*Corresponding author:- E-mail: fybari61@yahoo.com.au
technique in ewes (Baril et al., 2001). The advantages are that warming is easily and rapidly achieved and embryo transfer is directly performed after thawing, without the need for microscope. If the embryos of genetically superior females are preserved by vitrification and transferred into indigenous ewes, the genetic merit of the flock will be improved. For inducing superovulation, PMSG is one of the most commonly used gonadotrophins (Armstrong and Evans, 1983; Bindon and Piper, 1982; Evans and Robinson, 1980) with the advantage that it is relatively inexpensive and requires only one injection. The study was undertaken to observe the effects of PMSG on superovulation and embryo yield in donor ewes, qualities of embryo and pregnancy rate following direct transfer of vitrified embryos into indigenous ewes.

Materials and Methods

Selection of the ewes

Three donors, nine recipients and two rams were selected and maintained at the experimental animal yard of the department and the animals were provided standard food and ad limitum water.

Oestrus synchronization and superovulation of the ewes

All donor and recipient ewes were synchronized using two doses of cloprostenol equivalent to 125 µg prostaglandin F2α (Ovoprost® Bayer, New Zealand) at an interval of nine days by deep intramuscular injection. To induce superovulation, donor and recipient ewes were treated with 600 iu and 250 iu Pregnant Mare Serum Gonadotrophin (PMSG; Folligon®, Intervet, Boxmeer, The Netherlands) at the time of 2nd injection of prostaglandin F2α.

Detection of oestrus

The ewes were kept with teaser rams, and observed for signs of oestrus twice a day for 60 minutes in each observation from 24 hours after second injection of prostaglandin F2α. All donor ewes were mated during oestrus by fertile rams with high vigour.

Embryo recovery

Embryos were collected from donor ewes six days after mating, by inguinal laparotomy using standard procedure (Munoz et al., 2010).

Assessment of embryos

After collection embryos were assessed on morphological aspects and graded on a scale of 1 - 4 under Stereo microscope (Olympus, SZX2-TR30, Tokyo, Japan) with 10 to 25X.

Vitrification of embryos

The vitrified embryos were produced using the procedure as described by Mara et al.
The grade 1 embryos were equilibrated in holding medium (HM) for 10 minutes. Two to four embryos were again equilibrated in the equilibrium solution for five minutes, followed by verification solution for 45 seconds. Two embryos were loaded by capillary action into the narrow end of an open pulled straw (OPS) and plunged into liquid nitrogen using forceps.

**Thawing of vitrified embryos**

For thawing, the open pulled straw containing vitrified embryos was withdrawn from cryocan using forceps and held in air for six seconds. The narrow end of the straw was immersed in thawing solution comprising HM with 0.5 M sucrose for six seconds at 37°C.

**Transfer of embryos**

After thawing, the embryos were transferred into the exteriorized uterine horn ipsilateral to the ovary bearing at least one functional corpora lutea of a recipient by inguinal laparotomy. The tip of a Tom Cat catheter, attached to a 1 mL syringe was inserted into the open pulled straw containing two thawed embryos. The tip of the straw was inserted through a hole in the uterine horn and advanced 2 - 3 cm. The plunger of the syringe was depressed to discharge the whole content of the straw. The straw was withdrawn and the uterine horn was sprayed with normal saline and returned into the abdomen.

**Pregnancy diagnosis**

Pregnancy diagnosis was performed by trans-abdominal ultrasonography 40 days after embryo transfer in recipient ewes that did not return to oestrus.

**Statistical analysis**

The data were expressed as mean ± standard deviation (SD) in Excel program. This statistical analysis was done using SPSS (Statistical Package for the Social Sciences) Version 20.0. The comparison of grades of embryos was performed using Anova. The difference was regarded as insignificant at P>0.05.

**Results and Discussion**

**Synchronization of donor and recipient ewes**

In three donor ewes, oestrus occurred 24 – 40 hours after second injection of prostaglandin (mean 31.3 ± 8.1) and at 24 – 47 hours in five recipients (mean 32.6 ± 8.7). Oestrus lasted 18 – 36 hours in donors (mean 24.7 ± 9.9) and 16 – 48 hours in recipients (mean 32.8 ± 12.8) (Table 1).

Hackett *et al.* (1981) showed that 2 injections given 9 to 11 days apart resulted in oestrus in all ewes, with increased fertility. Beck *et al.* (1993) reported that two doses of 125 µg cloprostenol with 11-day interval and short-term progestagen (MAP, 5 days) combined with single dose PGF$_{2\alpha}$ in Clun Forest sheep produced similar oestrus
recurrence rates. Therefore, to achieve oestrus synchronization, two injections of PGF$_2$α 9 days apart make sure that every ewe is at the correct stage of cycle to respond.

Table 1: Synchronization of donor and recipient ewes within MOET programme (Mean ± SD)

| Parameters                                | Donor (n = 3) | Recipient (n = 5) |
|-------------------------------------------|---------------|-------------------|
| Onset of oestrus after 2nd injection of PGF$_2$α (Hour) | 31.3 ± 8.1    | 32.6 ± 8.7        |
| Duration of oestrus (hour)                | 24.7 ± 9.9    | 32.8 ± 12.8       |

Effects of PMSG on superovulation and embryo yield in donor ewes

The mean values of numbers of corpus luteum, embryos and total embryo recovery rate of donor ewes are shown in Table 2. The number of corpora lutea and embryos recovered varied from 8-16 and 6-11, respectively. However, their mean values were 11.7 ± 4.0 and 8 ± 2.6, respectively. The embryo recovery rate varied from 63.6 - 75 (68.6%). Collection of embryos from donor ewes on day 6 of insemination by inguinal laparotomy resulted in more than 80% embryo recovery (Wallace, 1992) at the first flush. However, a post-operative adhesion compromises the repeated use of genetically valuable donors (Mobine et al., 2002; Wright, 1988; Youngs et al., 1989) and lowers their future fertility (Pereira et al., 1998; Thibier and Guerin, 2000). The use of a modified mid ventral laparotomy, which include an inguinal cut combined with the non-suture of peritoneum helps to prevent abdominal herniation as well as to avoid adhesions and post-operative complications (Munoz et al., 2010). In the present study the percent of embryos recovered varied from 63 - 75% with an average of 68.6%. This is excellent since the recovery percentages could be more if larger number of donor could be used. This method is inexpensive and could be applied in field conditions.

Table 2: Effects of PMSG on superovulation and embryo yield in donor ewes.

| Parameters                                | Donor (N = 3) |
|-------------------------------------------|---------------|
| No. of corpora lutea (Mean ± SD)          | 11.7 ± 4.0    |
| No. of collected embryos per donor (Mean ± SD) | 8 ± 2.6     |
| Total embryo recovery rate (%)            | 68.6 %        |

Quality of embryos

The quality of embryos is shown in Table 3. The number of grade 1, 2, 3 and 4 varied from 5 - 8, 0 - 1, 0 - 2 and 0 - 1, respectively. Their mean values were 6 ± 1.7, 0.3 ± 0.6, 1.3 ± 1.2 and 0.3 ± 0.6, respectively. The mean number of grade 1 embryos was significantly higher (P<0.05) than grade 2, grade 3 and grade 4 embryos, respectively. This facilitated the verification of grade 1 embryos within the project in the research station.
Table 3: Qualities of embryo yield

| Donor ID | No. of collected embryos (Total) | Grading of embryos |
|----------|----------------------------------|---------------------|
|          |                                  | Grade 1 | Grade 2 | Grade 3 | Grade 4 |
| 1        | 7                                | 5       | 0       | 2       | 0       |
| 2        | 11                               | 8       | 1       | 2       | 0       |
| 3        | 6                                | 5       | 0       | 0       | 1       |
| (Mean ± SD) |                              | 6 ± 1.7* | 0.3 ± 0.6 | 1.3 ± 1.2 | 0.3 ± 0.6 |

**Effects of direct transfer of vitrified embryos on pregnancy rate**

Total numbers of embryos vitrified, recipient ewes, vitrified embryos transferred and pregnancy rate are shown in Table 4. Only grade 1 embryos of donor ewes were vitrified and only two embryos were transferred to each recipient. Total numbers of embryos vitrified, recipient ewes, embryo transferred and pregnant recipient ewe were 20, 9, 18 and 4, respectively. The pregnancy rate of recipients was 44.4%. The pregnancy rates is good and similar with published works (Baril et al., 2001; Isachenko et al., 2003).

Table 4: Effects of direct transfer of vitrified embryos on pregnancy rate

| Parameters                          | Values |
|-------------------------------------|--------|
| Total No. of embryos vitrified      | 20     |
| No. of recipient ewes               | 9      |
| Total No. of vitrified embryos transferred | 18     |
| No. of pregnant recipient ewe       | 4      |
| Pregnancy rate (%)                  | 44.4%  |

**Conclusions**

We can conclude that the effects of PMSG on superovulatory response of donor and recipient ewes, embryo yield and their qualities were according to expectation. The pregnancy rate following direct transfer in recipient ewes in the research station was 40%. Further study involving direct transfer of large number of vitrified embryos in recipient ewes is needed to observe the consistency of the present experiment to be applied this technology in the field recipient to speed up the process of genetic improvement.

**Acknowledgements**

The authors are grateful to the Ministry of Science and Technology (MOST), Government of Peoples’ Republic of Bangladesh for financial support and Bangladesh Academy of Sciences- The United States Department of Agriculture (BAS-USDA) (LS-02) for financial support.
References

Alam MGS, Azam MSU, Khan MJ 2006: supplementation with urea molasses and body weight, milk yield and onset of ovarian cyclicity in cows. *Journal of Reproduction and Development* **52** 529-535.

Alam MGS, Ghosh A, Mondal AK, Akbar MA 2001: Supplementation and puberty of zebu calves of Bangladesh. *The Bangladesh Veterinarian* **18** 1-8.

Armstrong DT, Evans G 1983: Factors influencing success of embryo transfer in sheep and goats. *Theriogenology* **19** 31-42.

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 AL, Cognie Y, Leboeuf B, Beckers JF, Mermirld P 2001: Successful direct transfer of vitrified sheep embryos. *Theriogenology* **56** 299-305.

Beck NFG, Davies GB, Williams SP 1993: Oestrus synchronization in ewes- the effect of combining a prostaglandin analogue with a 5-day progestagen treatment. *Animal Production* **56** 207-210.

Bindon BM, Piper LR 1982: *Physiological basis of ovarian response to PMSG in sheep and cattle*. In: Embryo transfer in cattle, sheep and goats. Eds. JN Shelton, AO Trouson, NW Moore, JW James. Australian Society for Reproductive Biology, pp. 1-5.

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.

Evans G, Robinson TJ 1980: The control of fertility in sheep: Endocrine and ovarian response to progestagen-PMSG treatment in the breeding season and in anoestrus. *Journal of Agricultural Science* **94** 69-88.

Hackett AJ, Robertson HA, Wolymotz MS 1981: Effects of prostaglandin F2α and pregnant mare serum on the reproductive performance of fluorogestone acetate PMSG treated ewes. *Journal of Animal Science* **53** 154-159.

Isachenko, Vladimir, Evgenialsachenko, Markus Montag, Victoria Zaeva, Irina Krivokharchenko, Frank Nawroth, Salvatore Dessole, Igor I. Katkov, Hans van der Ven 2005: "Clean technique for cryoprotectant-free vitrification of human spermatozoa." *Reproductive Biomedicine online* **10** 350-354.

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

Martinez AG, Valcarcel A, Furnus CC, De Matos DG, Iorio G, De Las Heras MA 2006: Cryopreservation of in vitroproduced ovine embryos. *Small Ruminant Research* **63** 288-296.

Menchaca A, Vilarino M, Pinczak A, Kmjd S, Saldana JM 2009: P4treatment, FSH plus eCG, GnRH administration, and day 0 Protocol for MOET programmes in sheep. *Theriogenology* **72** 477-483.
Mobine S, Heath AM, Pugh DG 2002: Theriogenology of sheep and goat. In: DG Pugh (Edition) Sheep and Goat Medicine. Saunders Company, Philadelphia, USA. pp. 129-186.

Munoz IMM, Mara L, Sanna D, Dattena M 2010: The use of inguinal laparotomy combined with the “STARK TECHNIQUE” to improve surgery in sheep MOET programs. XXVI World Buiatrics Congress, Santiago, Chile.

Pereira RJTA, Sohnrey B, Holtz W 1998: Non-surgical embryo collection in goats treated with prostaglandin F2α and oxytocin. Journal of Animal Science 76 360-363.

Thibier M, Guerin B 2000: Embryo transfer in small ruminants: the method of choice for health control in germplasm exchanges. Livestock Production Science 62 253-270.

Wallace JM 1992: Artificial insemination and embryo transfer. In: Progress in Sheep and Goat Research. Edition Speedy, AW, CAB International, UK, Chapter I.

Wright RW Jr 1988: Embryo collection, evaluation and transfer. In: JA Laing, WJ Brinley Morgan, WC Wagner (Editors), Fertility and Infertility in Veterinary Practice, Balliere Tindall, London, UK. pp. 52.

Youngs CR, McGinnis LK, Duplantis SCJ 1989: An overview of sheep embryo transfer technologies. In: K Erskine (Editor) Colored Sheep and Wool: Exploring Their Beauty and Function. The Proceedings of the World Congress on Colored Sheep, Black Sheep Press, Ashland, USA. pp. 210.