

Pregnancy rate in indigenous ewes by direct transfer of vitrified embryos

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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

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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 libitum* 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 F_{2α} (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 F_{2α}.

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 F_{2α}. 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.*

(2013). 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 \pm 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 μ g cloprostenol with 11-day interval and short-term progestagen (MAP, 5 days) combined with single dose $\text{PGF}_2\alpha$ in Clun Forest sheep produced similar oestrus

recurrence rates. Therefore, to achieve oestrus synchronization, two injections of PGF₂α 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 2 nd 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 overage 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 virification 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 \pm SD) | | 6 \pm 1.7* | 0.3 \pm 0.6 | 1.3 \pm 1.2 | 0.3 \pm 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 quality 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.

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