BRIEF NOTE

Non-Surgical Collection and Surgical Transfer of Bovine Embryos

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Recent developments in non-surgical embryo collection techniques in cattle show that, under most circumstances, the inherent risks of surgery such as adhesions are no longer warranted. Several authors have described procedures for non-surgical embryo collection via the cervix from superovulated cows, however, there remains such difficulties in recovering the embryos as the need for skillful manipulating of the uterus and a lack of reliable catheter [1, 5-7]. With these in mind, the authors have devised a catheter with stylet for guiding the insertion catheter into the cervix.

Our device has been used successfully in embryo transfer experiments done Holstein Friesian cows in Hokkaido.

Superovulation: Following the confirmation of two normal estrus cycles, 5 high yielding Holstein Friesian cows were subjected to superovulation treatment with 3,000 IU of PMSG (Pregnant Mare's Serum Gonadotrophin, Sankyo Company Ltd.), which was administered intramuscularly during postestral period day 10, designating the day of the onset of standing estrus as day 0. Forty-two hours after the gonadotrophin injection, 25 mg of prostaglandin F2α (Pronalgon F, Upjohn Company) was injected intramuscularly. The onset of standing estrus was observed approximately 40 hours after the prostaglandin injection. During estrus, artificial insemination was performed three times at 12 hours intervals each with one dose of frozen semen which was obtained from a local artificial insemination center. Seven days after the first insemination, the embryos were recovered from the uterus by non-surgical flushing with tissue culture medium (TCM-199, Grand Island Biological Company) at 37°C.

Embryo collection technique: The donors were placed in stocks in a standing position. To prevent straining the donor was given a caudal epidural injection (5 ml, 2% xylcocain). The vulva was washed with an antiseptic solution and disinfected with alcohol. Then, the cervix was dilated by a set of dilators designed for uterine irrigation to allow easy passage through the cervix. The 14, 16, 18 and 20 French size two-way Foley catheters with 30 ml balloons (C. R. Bard International Ltd.) were modified in length and in the tip of the hole. To lengthen the catheter, a silicone tube of approximately 20 cm in length was connected to the main tube and to the balloon tube respectively. A tip of the stainless steel stylet was inserted into the first hole of the catheter with a silicone sealant.
to prevent the withdrawal of the stylet during manipulation (Figs. 1–4). The catheter was manipulated through the cervix into one of the uterine horns by rectal palpation, and then the balloon was inflated with 16–20 ml of air. About 50 ml of flushing medium (TCM-199) was infused three or four times into one of the uterine horns using a plastic syringe attached to the main tube. The recovery was achieved by gravity flow and/or by the siphon effect created when the syringe is removed after each infusion, and by grasping and elevating the distal end of the uterine horn by a hand through the rectal wall. After recovery, the flushing medium were subdivided into plastic Petri dishes and examined under a stereomicroscope (Table 1).

Embryo transfer: After recovery, the embryos were kept in small glass dishes with Brinster’s medium (BMOC-3, Grand Island Biological Company) until being transferred to the recipient animals. Each recipient animal was used on days 6, 7 or 8 following standing estrus, which was synchronized by prostaglandin injection if necessary. The animals were prepared by withholding food and water for one day before the operation in order to reduce rumen volume and to allow free movement of the genital tract. The operation was performed in the upper flank on the side of the exist-
Table 2. Summary results of embryo transfer

<table>
<thead>
<tr>
<th>Donor No.</th>
<th>Recipient No.</th>
<th>Synchronization</th>
<th>Day of transfer</th>
<th>Synchronization from donor (day)</th>
<th>Hours between collection and transfer (hours)</th>
<th>Newborns</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>PG$^{(1)}$ 15 mg</td>
<td>6</td>
<td>-1</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>PG$^{(1)}$ 15 mg</td>
<td>6</td>
<td>-1</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>PG$^{(2)}$ 5 mg</td>
<td>6</td>
<td>-1</td>
<td>2.0 female</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>PG$^{(2)}$ 5 mg</td>
<td>7</td>
<td>0</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>PG$^{(2)}$ 5 mg</td>
<td>7</td>
<td>0</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>NE —</td>
<td>7</td>
<td>0</td>
<td>3.0 female</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>NE —</td>
<td>6</td>
<td>-1</td>
<td>3.5 female</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>PG$^{(2)}$ 15 mg</td>
<td>7</td>
<td>0</td>
<td>4.0</td>
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<tr>
<td>9</td>
<td>9</td>
<td>PG$^{(2)}$ 15 mg</td>
<td>7</td>
<td>0</td>
<td>5.0</td>
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</tr>
<tr>
<td>10</td>
<td>10</td>
<td>NE —</td>
<td>8</td>
<td>+1</td>
<td>7.5 female</td>
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<tr>
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<td>NE —</td>
<td>8</td>
<td>+1</td>
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<td></td>
</tr>
<tr>
<td>12</td>
<td>12</td>
<td>PG$^{(2)}$ 15 mg</td>
<td>6</td>
<td>-1</td>
<td>2.0</td>
<td></td>
</tr>
</tbody>
</table>

$^{(1)}$ Injected intramuscularly with prostaglandin.
$^{(2)}$ Injected into the intra-mucous membrane of the dorsal commissure of the vulva with prostaglandin.
$^{(3)}$ -1, one day before donor; 0, same day as donor; +1, one day after donor.
NE: Natural estrus.

ing corpus luteum under local and epidural anaesthesia with 2% xylocain solution. A 10–15 cm incision was made to allow easy access to the uterine horn. The wall of the distal end of the uterine horn was pushed through the myometrium into the lumen by a blunt needle, avoiding the uterine vessels, and then withdrawn. The fine blunt needle, avoiding the uterine vessels, and then withdrawn. The fine blunt glass pipette containing an embryo in a minimal volume (about 0.2 ml) of BMOC-3 was inserted into the uterine lumen of the recipient via a puncture wound in the uterine wall. The site of insertion of the embryo was about 3–5 cm from the uterotubal junction. To close the incision wound in the flank, the peritoneum was sutured to the linea alba using No. 2 chrome gut with continuous sutures and the skin was closed with simple interrupted sutures. However the puncture wound in the uterus was not sutured.

For non-surgical collection of the embryos, many researchers have used a balloon type catheter with a metal stylet [2–6]. The metal stylet is usually inserted into the balloon catheter to help it to penetrate the cervix. After the catheter is located inside the uterus, the stylet is removed. With this type of catheter we have experienced some technical difficulties in the manipulation of the catheter in the uterus after the removal of the stylet.

In the present study, however, we have modified the catheter by connecting the tip of the catheter and the stylet, and the stylet was not inserted in the catheter. Also, the stylet was not removed after insertion of the catheter into the uterus in order to monitor the position of the catheter with ease and to replace it to the other uterine horn. The search for embryos was not hampered by 300–400 ml of flushing medium. In this way, easy manipulation of the uterus from the rectum was practised, and collection of the embryos was guaranteed. In addition, leakage of flushing medium was prevented. In our experiments 22 embryos from 29 ova, estimated by number of cor-
The results of the embryo transfer are shown in Table 2. The four calves were born from the 12 recipients. By chance, all of them were female.

References


Explanation of Figures

Fig. 3. Modified Foley catheter.
1: Main tube for medium inflow and collection.
2: Air tube for inflation of balloon.
3: Stylet.

Fig. 4. Tip of catheter. Medium inflow and collection hole (arrows). Stylet and tip of catheter bound together with silicone sealant (S).