A SIMPLE METHOD FOR NON-SURGICAL BLASTOCYST TRANSFER IN MICE

L. MARSK AND K. S. LARSSON

Laboratory of Teratology, Karolinska Institute, S-104 01 Stockholm 60, Sweden

(Received 24th May 1973)

Summary. An improved non-surgical blastocyst transfer technique in mice is described. The advantage of using this simple method in teratological experiments and in studies in fetal pharmacology is discussed.

INTRODUCTION

In a recent book on methods in embryology, a whole chapter is devoted to egg-transfer technique (Dickmann, 1971). The reviewer states that “it is obvious that a non-surgical technique for transferring eggs into the uterus is preferable to a surgical one”. The poor results from the non-surgical transfers of mouse eggs reported by Beatty (1951) and Tarkowski (1959) have apparently made many researchers reluctant to try this method, though Vickery, Erickson & Bennett (1969) described a fair success with non-surgical egg transfer in the rat.

In our studies on the nature of the matroclinous influence on cortisone-induced cleft palate in mice of the A/Jax strain, the vaginally transferred blastocyst technique was tried (Marsk, Theorell & Larsson, 1971). Since we have been fairly successful with the simple non-surgical blastocyst transfer, it seems appropriate to describe the method and give a summary of our results thus far.

MATERIALS AND METHODS

Animal material

Seventy-nine CBA-strain mouse recipients have received 521 A/Jax blastocysts and thirty-five A/Jax-strain recipients have been given 241 CBA blastocysts. These animals represent a major part of the transfers made during the past 2 years at our laboratory. In addition to untreated recipients, animals which were treated with salicylate (500 mg/kg intramuscularly) on Day 9 of gestation are included (results to be published elsewhere). A group of animals treated with cortisone on Days 11 to 14 (Marsk et al., 1971) is not listed here since this treatment caused a high incidence of resorptions among which transferred or native embryos could not be distinguished with certainty.

The A/Jax-strain mice have been inbred at our laboratory since 1958 (Larsson, 1962) and the inbred CBA strain was obtained from the Department of Genetics, University of Stockholm. The animals were kept in macrolone cages in a room with constant temperature (23°C) and automatically regulated...
exposure to light from 06.00 to 18.00 hours. They were freely fed a standard laboratory diet (Astra-Ewos, Södertälje, Sweden), and water. The females were mated overnight with fertile males of their own strain and checked for vaginal plugs the next morning, denoted as Day 0 of pregnancy.

Donors were killed by dislocation of the neck on Day 3½ of gestation. The uterine horns were dissected out and each horn flushed separately with about five droplets of saline (10°C). The blastocysts were collected in a watch-glass and allowed to remain in the fluid for 5 to 15 min at room temperature.

Recipients of the reciprocal strain were taken on Day 2½ of gestation (McLaren & Michie, 1956). It was possible to transfer the blastocysts to an unanaesthetized recipient but to facilitate the procedure, the females were given approximately 0.2 ml of a 2% tribromoethanol (TBE) solution intraperitoneally. This imparted full anaesthesia for 5 min, and caused the animals to sleep or rest for at least 30 min. A 10-mm piece of plastic tubing with an outer diameter of 4 mm was introduced into the vagina as a speculum.

Transfer was performed with a micrometer syringe (e.g. Agla, Burroughs Wellcome & Co., London) connected to a commercially available micropipette ('Microcaps', Drummond Scientific Co., Broomall, Pa., U.S.A.) with a fine-bore polyethylene tubing (see Pl. 1, Fig. 1). The micropipette had an outer diameter of 0.6 mm and contained a maximum of 2 µl fluid. The micrometer syringe was prepared with a straightened rubber band around the plunger resulting in withdrawal of the plunger when the micrometer was unscrewed. Air was used as a medium in the syringe and in the connecting polyethylene tubing. The blastocysts were sucked up in the micropipette from the watch-glass under any kind of light microscope or dissecting microscope at a magnification at which the condition of blastocysts could be safely judged. It was important to have some fluid both in front of and behind the blastocyst-containing portion (five to ten blastocysts) which could be delineated by two small air bubbles in order to have full control over the position in the system of the eggs (Text-fig. 1). The sleeping recipient was lifted with forceps just in front of the clitoris and the micropipette was inserted into the vagina (Pl. 2, fig. 2). With gentle 'pushing', the cervical opening was blindy searched for and usually rapidly found. The pipette was then slid into the uterus. There is a great difference between the pressure needed to perforate the vaginal fornix and the pressure needed to slide the pipette into the cervical canal. With the pipette inserted about 10 mm into the uterus, the blastocysts were expelled by increasing the pressure in the microsyringe. To avoid flushing too much air into the uterus, it is recommended that unscrewing of the micrometer shall be stopped when some fluid remains in the micropipette above the blastocyst-containing portion. The excess fluid below this portion will prevent leakage of blastocysts from the pipette since, when searching for the uterine entrance, some amount of fluid at the tip of the pipette might be lost at every 'push'. The pipette and speculum were then withdrawn and the recipient brought to the cage for awakening under the warmth of a lamp.

Recording of results

The animals were killed on Day 14 or 16 of gestation according to the design
Fig. 1. The transfer equipment described, including a micrometer syringe connected to a micropipette with polyethylene tubing and a dissecting microscope.

(Facing p. 394)
Fig. 2. An anaesthetized recipient with a 10-mm plastic tube (4 mm o.d.) inserted as a speculum in the vagina.

(Facing p. 397)
of the different experiments. The number and position of living and resorbed embryos were recorded. Native and transferred embryos could easily be distinguished by eye marker. The number of resorptions given in Table 1 indicates only resorptions in the horn where transferred embryos were found.

If no living transferred embryos were recorded, the numbers of resorbed embryos in the right horn were noted since 85 out of 95 dams with any transferred embryos had them in the right horn.

**RESULTS**

Out of a total of 521 transferred A/Jax-strain blastocysts to seventy-nine CBA-strain dams, 262 (50%) were recovered as living embryos (Table 1). This material included a group of salicylate-treated animals and if this group was excluded, the yield of living embryos was 54%. In Table 1, it can be seen that sixty-seven out of seventy-nine dams developed at least one A/Jax-strain fetus, giving a ‘take’ rate of 85%.

Of a total of 241 CBA blastocysts transferred to thirty-five A/Jax-strain dams, seventy-five were recovered as living embryos, i.e. 31% (Table 1). In the A/Jax-strain recipients, the yield of living embryos was higher in the untreated group (36%). The ‘take’ rate in the A/Jax strain of CBA fetuses was 80%.

**DISCUSSION**

The non-surgical method described above seems to be as efficient as the surgical method with a yield of 20 to 50%. Moreover, it offers by its simplicity advantages in teratological experiments where a certain loss of embryos can be predicted due to the action of the teratogen. The high ‘take’ rate seems to be of special value in fetal pharmacology experiments where strains with different suscepti-
Table 1. The 'take' rate and number of resorptions following transfer of mouse blastocysts of one strain to mouse recipients of another strain

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of recipients</th>
<th>No. of recipients with at least one blastocyst as living embryo</th>
<th>'Take' rate (%)</th>
<th>No. of transferred blastocysts</th>
<th>No. of transferred blastocysts recovered as living embryos</th>
<th>Yield (%)</th>
<th>No. of resorptions in horn with transferred embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/Jax blastocysts to CBA mice</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>51</td>
<td>40</td>
<td>78</td>
<td>345</td>
<td>185</td>
<td>54</td>
<td>25</td>
</tr>
<tr>
<td>Salicylate on Day 9</td>
<td>28</td>
<td>27</td>
<td>96</td>
<td>176</td>
<td>77</td>
<td>44</td>
<td>23</td>
</tr>
<tr>
<td>Total</td>
<td>79</td>
<td>67</td>
<td>85</td>
<td>521</td>
<td>262</td>
<td>50</td>
<td>48</td>
</tr>
<tr>
<td>CBA blastocysts to A/Jax mice</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>14</td>
<td>10</td>
<td>71</td>
<td>108</td>
<td>39</td>
<td>36</td>
<td>1</td>
</tr>
<tr>
<td>Salicylate on Day 9</td>
<td>21</td>
<td>18</td>
<td>86</td>
<td>133</td>
<td>36</td>
<td>27</td>
<td>21</td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>28</td>
<td>80</td>
<td>241</td>
<td>75</td>
<td>31</td>
<td>22</td>
</tr>
</tbody>
</table>

Five to ten blastocysts, 3½ days old, were transferred to recipients on Day 2½ of gestation. Autopsy was performed on Day 14 or 16.
bility to drugs which damage the fetus can be investigated within the same environment.

In earlier non-surgical trials in the mouse, Beatty (1951) could only recover five young out of fifty-nine morulae and blastocysts transferred to thirteen recipients and Tarkowski (1959) reported in a larger series that only 8.4% of transferred blastocysts developed normally. It is impossible to distinguish the particular factors contributing to the sudden fair success of a non-surgical blastocyst transfer, but several minor technical improvements might have contributed. The micrometer syringe offers a safe control during the moment of transfer of blastocysts to the uterus. Kirby (1962) used similar equipment for transfer of mouse eggs to extracorporeal sites. One possible reason for earlier loss of eggs could be the loss of fluid from the tip of the pipette at the time of transfer during the search for and penetration through the cervical canal. The additional fluid sucked up and separated by an air bubble will minimize the risk of the blastocyst-containing portion being expelled into the vagina. Vickery et al. (1969) ascribed the success of their non-surgical transfer experiments in the rat to the adequate visualization of the cervix and consequent reduction of the risk of blastocyst loss at this step. It has been suggested that the blastocysts could be lost by slipping out through the cervix after transfer (Briones & Beatty, 1954; Tarkowski, 1959). If such an escape could be facilitated by a dilatation of the canal during insertion of the pipette, it is important, as in the present study, to insert a pipette with the same diameter throughout its length and to expel the eggs as near to the oviduct as possible. Due to the efficient anaesthesia, the blastocysts seem to have some time to settle down in the uterus of the sleeping recipient while the transfer medium is resorbed. Obviously, no complicated transfer media are needed. Tarkowski (1959) tentatively ascribed some of his poor success with a vaginal transfer to the wrong type of transfer media.

The fact that most transferred embryos were in the right horn might be due to the fact that the operator was right-handed and tended to direct the pipette to the left in the vagina and cervix. The rôle of using pregnant recipients compared to pseudopregnant females for this type of transfer has to be evaluated in further studies.

As seen from the present result, there are not only differences in methods and techniques giving variations in the result but the results also depend on the strains used as donor and/or recipient. The CBA-strain dams were better recipients than A/Jax-strain dams or A/Jax eggs were better transfer eggs than CBA eggs. There are some other indications that CBA to A/Jax is a bad match since Vetter (1971) did not succeed in this type of transfer using a surgical method. One should, therefore, be careful in comparing different transfer results. The simple method described here would seem to be worth testing in many strain combinations.

ACKNOWLEDGMENTS

This study was supported by the Swedish Medical Research Council (Nos. 14X- and P-993-08) and Expressen’s Fund for Prenatal Research.
398

L. Marsk and K. S. Larsson

REFERENCES