# Deposition of Mucin Coat on Rabbit Embryos Cultured *In Vitro* Following Oviductal Transfer

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## ABSTRACT

**Mucin coat is deposited on the embryos during passage through the oviduct in rabbit. When *in vitro* cultured blastocysts were transferred to the recipients, the lack of mucin coat might account in part for failure of pregnancy after transfer. The present study were carried out to investigate whether deposition of mucin coat were induced when *in vitro* cultured blastocysts were transferred to recipients. At 19~20 hours post-coitus one-cell embryos were collected by flushing oviducts. These embryos cultured for 72 hours were reached to blastocyst stage. And these blastocysts were transferred to the oviduct of asynchronized (one day later than the donors) and synchronized recipient. To confirm deposition of the mucin coat, blastocysts transferred to the oviduct were recovered at 24 and 48 hours after the transfer. Fifty eight percent of blastocysts recovered from uterus of asynchronous recipient at 24 hours after transfer and 92.9% of blastocysts recovered from uterus of synchronous recipient were 0~10 μm of mucin coat thickness. And 11.8% of blastocysts of asynchronized recipients and 7.1% of blastocysts from asynchronized recipients were in 11~20 μm of mucin coat thickness. When blastocysts were recovered from uterus at 48 hours after transfer, 87.0% of blastocysts from asynchronized recipients and 5.9% of blastocyst from synchronized recipients were in 0~10 μm of mucin coat thickness. And 76.5% of blastocysts of synchronized recipients and 4.4% of blastocysts from asynchronized recipients were in 11~20 μm of mucin coat thickness. From these results it is speculated that the low implantation rate of *in vitro* cultured rabbit blastocysts transferred to oviduct of recipient was caused by high degeneration of the embryo after transfer and inappropriate deposition of mucin coat.**

**(Key words : Rabbit embryo, *In vitro* culture, Mucin coat, Transfer, Implantation)**

## INTRODUCTION

The rabbit is unique mammals having embryos with thick mucin coats deposited during oviductal passage. The mucin is an acid mucopolysaccharide released from the tubal epithelium after ovulation. Blastocysts developed *in vitro* culture from the one-cell stage do not have mucin coat. The mucin coat prevents the rupture of expanding blastocyst (Denker and Gerdes 1979). Thickness of mucin coat over 20 μm was essential for rabbit embryo to term (Murakami, 1996) and *in vitro* cultured blastocysts did not develop to term when they transferred to the uterus of synchronized recipient. Uterine transfer of *in vitro* cultured embryos may not be successful for inducing pregnancy in rabbits because mucin deposition in the oviduct is needed for implantation of rabbit embryos. Asynchronous embryo transfer can be used to overcome mucin coat problems in implantation (Adams, 1973; Techakumphu et al., 1987; Yang and Foote, 1990). We have reported successful pregnancy following transfer of rabbit blastocysts cultured *in vitro* for 72 hours into oviducts of recipients synchronized one day behind donors (Jin et al. 2000).

To investigate whether rabbit blastocysts cultured *in vitro* for 72 hours can deposit mucin coat following transfer into oviducts of recipients, the blastocysts developed *in vitro* from one-cell rabbit embryos were transferred to the oviducts of recipients synchronized one day behind donors or synchronized the same day as donors. The transferred embryos recovered at 24 to 48 hours after transfer was evaluated for deposition of mucin coat around the embryos in this study.

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## MATERIALS AND METHODS

### Superovulation and Synchronization

Sexually matured (6~9 months) New Zealand White rabbit does were superovulated with subcutaneous injection of 2.7 IU FSH (Sigma) for 3 days at interval of 12 hours (0.3, 0.4, 0.5, 0.5, 0.5, 0.5 IU). At 12 hours after final injection of FSH, The donor was intravenously administered with 50 IU HCG (Yuhan) and mated twice with fertile male. The synchronized recipient was intravenously administered with 50 IU HCG at the same time when the donor was injected with hCG. The asynchronized recipient was intravenously administered with 50 IU hCG at one day later when donor was injected with hCG.

### Embryo Culture and Transfer

At 19~20 hours post-coitus one-cell embryos were recovered by flushing oviducts with 10ml Dulbecco's Phosphate Buffered Saline (DPBS) containing 0.3% bovine serum albumin (BSA). These one-cell embryos were cultured for 72 hours in 50 $\mu$l of RDH(1:1:1 mixture of RPMI 1640, DMEM and Ham's F-10) described by Jin et al. (2000) under mineral oil at 39℃ and 5% $CO_2$ in air. Each drop of medium contained 10~15 embryos. The embryos developed to the blastocyst at 72 hours after culture were transferred surgically to the oviducts of recipients synchronized one day later than the donors (asynchronized group) orsynchronized on the same day as the donors (synchronized group). Ten to 20 embryos were transferred to each oviduct of the recipient.

### Recovery and Examination of Embryos

The blastocysts transferred to the oviduct were recovered from oviduct or uterine at 24 and 48 hours after the transfer and examined microscopically the development stage and appearance. The thickness of mucin coat was measured. Data were analysed by Chi-square test for significant difference.

## RESULTS

### Recovery Rate of Blastocyst Transferred

One-cell embryos recovered at 19 hours post coitus were *in vitro* cultured for 72 hours. The blastocysts were transferred to the oviduct of asynchronized and synchronized recipients and recovered from oviduct and uterine at 24 and 48 hr after transfer.

As shown in Table 1, when embryos were recovered at 24 hours after transfer, recovery rate did not differ between asynchronized and synchronized recipients (85.2 avs 91.1%, respectively). In recovery from oviduct, synchronized recipient (82.9%) showed significantly ($p< 0.05$) higher recovery rate than asynchronized recipient (59.8 %). The recovery rate of embryos from uterus was significantly different($p<0.05$) between asynchronized recipient (40.2%) and synchronized recipient (17.1%). At 48 hours after transfer recovery rate of blastocysts was significantly different($p<0.05$) between asynchronization (80.9%) and synchronization(91.0%). At 48 hours after transfer, the recovery rate of embryos from oviduct was not significantly different($p<0.05$) between asynchronized recipient (69.7 %) and synchronized recipient (78.0%). The recovery rate of embryos from uterus was not significantly different($p<0.05$) between asynchronized recipient (30.3 %) and synchronized recipient (22.0%).

### Degeneration Rate of Blastocysts Recovered

The degeneration rate of the blastocyst recovered at 24 and 48 hours after transfer is presented in Table 2 and Fig. 5. At 24 hours after transfer, the degeneration rate of blastocysts recovered from oviduct and uterus in asynchronized and synchronized recipient was 41.7 and 42.3%, 16.2% and 7.1%, respectively. The degeneration rate of blastocysts recovered from oviduct and uterus was significantly ($p<0.05$) higher in asynchronization than synchronization.

At 48 hours after transfer, the degeneration rate of blastocysts recovered from oviduct in asynchronized and synchronized recipient was 66.2 and 74.4%, respectively. And also degeneration rate of blastocysts recovered from uterus of asynchronized and synchronized recipient was 66.7% and 82.4%, respectively. The degeneration rate of blastocysts recovered from oviduct and uterus was not significantly different ($p<0.05$) between asynchronization than synchronization.

### Thickness of Mucin Coat of Blastocyst Recovered after Transfer

**Table 1. Recovery rate of blastocysts transferred at 24 and 48 hours after asynchronous and synchronous transfer**

| Synchronization | Time recovered after transfer | | | | | |
|---|---|---|---|---|---|---|
| | 24 hr | | | 48 hr | | |
| | No.(%) of embryos recovered/ transferred | No.(%) of embryos recovered from | | No.(%) of embryos recovered/ transferred | No.(%) of embryos recovered from | |
| | | Oviduct | Uterus | | Oviduct | Uterus |
| Asynch. | 92/108 (85.2)[a] | 55 (59.8)[b] | 37 (40.2)[d] | 76/94 (80.9)[f] | 53 (69.7)[h] | 23 (30.3)[i] |
| Synch. | 82/90 (91.1)[a] | 68 (82.9)[c] | 14 (17.1)[e] | 91/100 (91.0)[g] | 71 (78.0)[h] | 20 (22.0)[i] |

Values with different superscripts within column are significantly different.
($p < 0.05$).

**Table 2. Degeneration rate of blastocysts recovered at 24 and 48 hours after asynchronous and synchronous transfer**

| Synchro-nization | Time recovered after transfer | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 24hr | | | | 48hr | | | |
| | No. of embryos recovered | | No.(%) of embryos degenerated | | No. of embryos recovered | | No.(%) of embryos degenerated | |
| | O | U | O | U | O | U | O | U |
| Asynch. | 60 | 26 | 25 (41.7)[a] | 11 (42.3)[a] | 65 | 15 | 43 (66.2)[c] | 10 (66.7)[c] |
| Synch. | 68 | 14 | 11 (16.2)[b] | 1 ( 7.1)[b] | 78 | 17 | 58 (74.4)[c] | 14 (82.4)[c] |

O : Oviduct, U : Uterus.
Values with different superscripts within column are significantly different. ($p < 0.05$).

To confirm deposition of mucin coat of blastocysts recovered at 24 and 48 hours after the transfer, the thickness of mucin coat were measured by a photograph. Distribution of embryos according to thickness of the mucin coat of blastocysts recovered from oviduct or uterus at different hours after transfer is shown in Fig. 1~4. Fig. 1 is shown that the 75.4% of blastocysts recovered from the oviduct of asynchronized recipients at 24 hours after transfer was in 0~10 $\mu$m of mucin coat thickness. And 41.2% of blastocysts from oviduct of synchronized recipients at 24 hours after transfer were in 0~10 $\mu$m of mucin coat thickness. As shown in Fig. 2, 58.1% of blastocysts recovered from uterus of asynchronous recipient at 24 hours after transfer and 92.9% of blastocysts recovered from uterus of synchronous recipient were 0~10 $\mu$m of mucin coat thickness. As shown in Fig. 3, when blastocysts were recovered from oviduct at 48 hours after transfer, 75.3% of blastocysts from asynchronized recipients and 38.2% of blastocysts from synchronized recipients were in 0~10 $\mu$m of mucin coat thickness. And 38.2% of blastocysts of synchronized recipients and 6.7% of blastocysts from asynchronized recipients were in 11~20 $\mu$m of mucin coat thickness. As shown in Fig. 4, when blastocysts were recovered from uterus at 48 hours after transfer, 87.0% of blastocysts from asynchronized recipients and 5.9% of blastocyst from synchronized recipients were in 0~10 $\mu$m of mucin coat thickness. And 76.5% of blastocysts of synchronized recipients and 4.4% of blastocyst of asynchronized recipients were in 11~20 $\mu$m of mucin coat thickness.

The average thickness of mucin coat of embryos from that of oviduct of synchronous recipient (12.9 $\mu$m) at 24 hours after transfer was considerably higher than in

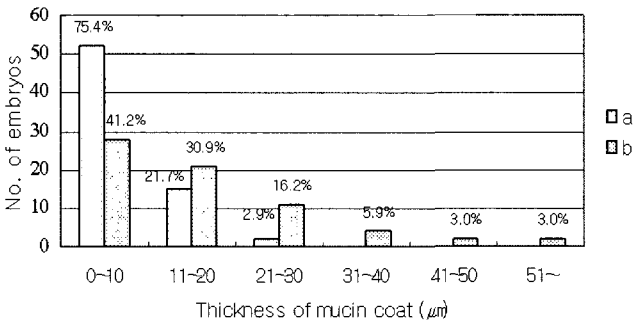


**Fig. 1. The thickness of mucin coat of blastocyst recovered from oviduct after transfer at 24hr.**

a : Recovered from asynchronized recipient
b : Recovered from synchronized recipient

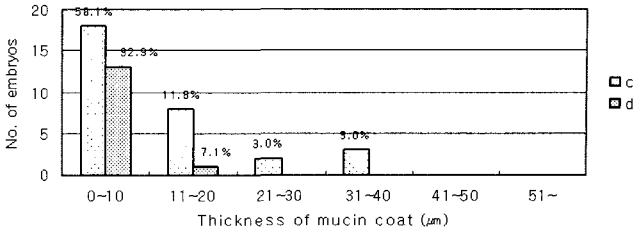


**Fig. 2. The thickness of mucin coat of blastocyst recovered from uterus after transfer at 24hr.**

c : Recovered from asynchronized recipient
d : Recovered from synchronized recipient

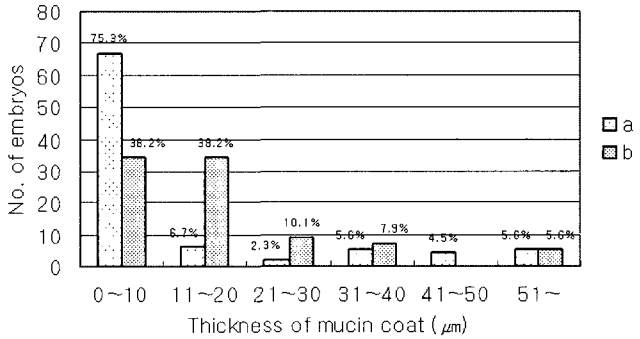


**Fig. 3. The thickness of mucin coat of blastocyst recovered from oviduct after transfer at 48hr.**

a : Recovered from asynchronized recipient
b : Recovered from synchronized recipient

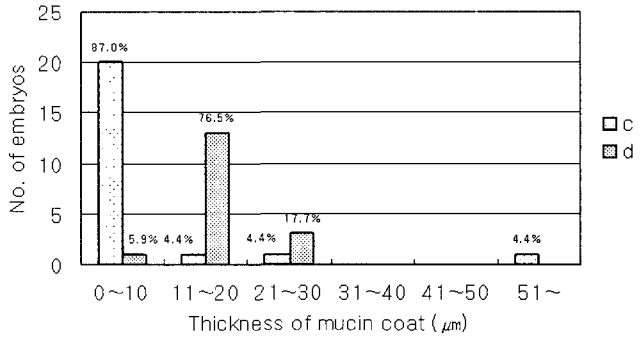


**Fig. 4. The thickness of mucin coat of blastocyst recovered from uterus after transfer at 48hr.**

c : Recovered from asynchronized recipient
d : Recovered from synchronized recipient

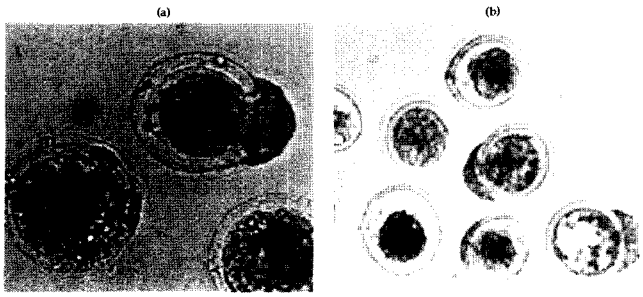


**Fig. 5. Rabbit embryos recovered at 24 hours following asynchronous and synchronous transfer:** (a) hatching blastocysts recovered from oviduct asynchronized recipient (× 200); (b) abnormally expanded blastocysts recovered from uterus of asynchronous recipient (× 100). m: mucin coat.

asynchronous recipient (1.8 $\mu$m). However mean thickness of embryos from uterus of asynchronous recipient (4.7 $\mu$m) was considerably higher than that of synchronous recipient (0.4 $\mu$m). At 48 hours after transfer, the average thickness of mucin coat of embryos from oviduct of synchronous recipient (18.5 $\mu$m) was relatively higher than that of asynchronous recipient (14.5 $\mu$m). In embryos from uterus, mean thickness of mucin coat of synchronous recipient (11.4 $\mu$m) was relatively higher than that of asynchronous transfer (7.3 $\mu$m).

## DISCUSSION

The purpose of this study is to investigate the effect of mucin coat on implantation. Deposition of mucin coat around zona pellucida during passage through oviduct is thought important to study mechanism on implantation of rabbit embryos. The function of mucin coat following ovulation is not well understood. Several theories have been offered to account for the extensive deposition of mucin around the rabbit embryos (Seidal et al., 1976; Binkerd and Anderson, 1979). The mucin layer by releasing its water content might be responsible for the expansion of the rabbit blastocyst. The findings in relation to the problem of ovulation, suggest that depolymerization of acid mucopolysaccharides may increase intra-follicular osmotic pressure; this may account for the final spurt of growth of the preovulatory follicle. Another theory assigned a purely protective role to the mcuin layer. The expansion of the rabbit embryo is dependent most likely on hydrostatic pressure and the thin wall of the blastocysts may be incapable of withstanding the build-up in pressure. The rigid tough mucin layer could act to prevent the rupture of the expanding blastocyst.

To confirm deposition of the mucin coat, blastocysts were recovered from oviduct at 24 and 48 hours after transfer. The recovery rate of embryos from the oviduct was higher than that from the uterus. It is confirmed that 59.8~91.1% of blastocysts transferred were remained in oviduct during 24~48 hours after transfer (Table 1). It is supposed that mucin coat would be deposited to the blastocysts during their passage through oviduct. The role of the mucin coat for transformation of the coverings and for development is difficult to evaluate. As shown in Fig. 1 and 3, prolonged stay of embryos in the oviduct allowed deposition of a mucin coat on the surface of the zona pellucida. It is apparent that reduction of the mucin layer to a thickness of only 12 $\mu$m is not incompatible with normal implantation development of the rabbit embryos. However, the rate of successful implantation was sharply reduced (Gilbert 1962).

The smaller amount of mucin did not alter the rate of embryo development (Carney and Foot, 1990). If the mucin coat has not been deposited on the zona pellucida during tubal passage or is present only in traces, for example by embryo recovery soon after fertilization, embryos can rupture the zona and hatch during subsequent *in vitro* culture (Kane and Foot, 1971 ; Kane, 1975).

As shown in this experiment, 58.1~87.0% of blastocysts recovered from oviduct or uterus of asynchronized recipient after transfer at 24 and 48 hour showed 0~10 $\mu$m of mucin coat thickness and 5.9~92.9% of blastocysts from synchronized recipient showed 0~10 $\mu$m of mucin coat thickness. The role and function of mucin coat on implantation of rabbit embryos are not clear. A simple explanation may be that the mucin coat could physically prevent the blastocysts from direct exposure to a deleterious uterine environment and allow them to expand until the appropriate time for implantation. Another possible explanation may be that the mucin coat may slow the rate of blastocysts expansion, resulting in more cells in the inner cell mass (Murakami, 1996). Although implantation rate might be low, offsprings could be produced from the *in vitro* cultured blastocysts transferred to the oviduct of asynchronized recipients.

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