Zygote **12** (August), pp. 215–219. © 2004 Cambridge University Press
DOI:10.1017/S0967199404002795 Printed in the United Kingdom

Effects of transferring *in vitro*-cultured rabbit embryos to recipient oviducts on mucin coat deposition, implantation and development

So Young Joung[1], *Hyun Joo Kim, Wha Sik Choi*[2], *Kyung Soon Im*[1], *Seong Ho Lee*[3], *Chang Sik Park and Dong Il Jin*

Division of Animal Science and Resources, Research Center for Transgenic Cloned Pigs, Chungnam National University, Daejeon City, Korea

Date submitted: 02.02.04. Date accepted: 23.04.04

Summary

A mucin coat is deposited on rabbit embryos during passage through the oviduct; rabbit blastocysts cultured from the 1-cell stage *in vitro* have no mucin coat. When cultured blastocysts are transferred to recipients, the lack of mucin coat might account in part for subsequent failure of pregnancy. We have investigated the possibility that mucin coat deposition is induced following transfer of *in vitro* 72 h-cultured blastocysts to oviducts of asynchronous or synchronous recipients. One-cell embryos were collected by flushing oviducts 19–20 h post-coitus and were cultured *in vitro* for 72 h until they reached the blastocyst stage. The blastocysts were transferred to the oviducts of recipients that were synchronized either with the donors (synchronous) or 1 day later than the donors (asynchronous). They were recovered after 24–48 h and the mucin coat thickness and embryo degeneration rate were measured. The degeneration rate of blastocysts recovered from uteri of synchronous recipients was higher than that from asynchronous recipients (72.2% vs 40.0%). The mucin coats around embryos recovered from oviducts of asynchronous recipients after 48 h were thicker than those from synchronous recipients. More asynchronous recipients were pregnant and gave birth to more pups than synchronous recipients. These results indicate that the oviducts of asynchronous recipients secreted more mucin around the transferred embryos, causing higher rates of implantation of the *in vitro*-cultured blastocysts.

Keywords: Implantation, *In vitro* culture, Mucin coat, Rabbit embryo, Transfer

Introduction

The rabbit is a unique mammal in that its embryos have thick mucin coats deposited during oviductal passage. Mucin is an acid mucopolysaccharide released from the tubal epithelium after ovulation and may have a role in preventing blastocysts from expanding to the point of rupture (Denker & Gerdes 1979). Lack of a mucin coat results in failure to maintain pregnancy after transfer of *in vitro*-cultured rabbit embryos to recipient uteri (Mauer *et al.*, 1970; Binkerd & Anderson, 1979; Seidel *et al.*, 1976). However, compact morulae or early blastocysts cultured for 48 h developed to term after transfer to oviducts of synchronized recipients, in which mucin might be deposited during passage through the oviduct (Murakami & Imai, 1996; Li *et al.*, 1997). Murakami & Imai (1996) demonstrated that mucin coat thickness is important for rabbit embryo implantation: *in vitro*-cultured blastocysts did not develop to term when transferred to uteri of synchronized recipients. Asynchronous embryo transfer can be used to overcome mucin coat problems in implantation (Adams, 1973; Techakumphu *et al.*, 1987; Yang & Foote, 1990). We have reported successful pregnancy following transfer of rabbit blastocysts cultured *in vitro* for 72 h into the oviducts of recipients synchronized 1 day behind donors (Jin *et al.*, 2000).

All correspondence to: Dong IL Jin, Divison of Animal Science and Resources, Research Center for Transgenic Cloned Pigs, Chungnam National University, Daejeon City, 338–708, Korea. Tel: +82 42 821 5876. Fax: +82 42 822 6712. e-mail: dijin@cnu.ac.kr
[1] Department of Animal Science and Technology, College of Agriculture and Life Sciences, Seoul National University, Suweon 441-744, Korea.
[2] Department of Clinical Pathology, Kimchun College, Korea.
[3] College of Visual Image & Health, Kongju National University, Korea.

Table 1 Recovery rates of blastocysts 24 and 48 h after asynchronized and synchronized transfer

	Time recovered after transfer					
	24 h			48 h		
Recipients	No. (%) of embryos recovered/transferred	No. (%) of embryos recovered from: Oviduct	Uterus	No. (%) of embryos recovered/transferred	No. (%) of embryos recovered from: Oviduct	Uterus
Asynchronous	92/108 (85.2)	55 (59.8)	37 (40.2)	73/94 (77.6)	21 (28.8)	52 (71.2)[a]
Synchronous	76/90 (84.4)	47 (61.8)	29 (38.2)	71/100 (71.0)	30 (42.3)	41 (47.7)[b]

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

To investigate whether rabbit blastocysts cultured *in vitro* for 72 h acquire a mucin coat during such manipulations, blastocysts grown from 1-cell embryos were transferred to the oviducts of recipients synchronized either on the same day as, or 1 day after, the donors. The transferred embryos were recovered 24–48 h after transfer, and pregnancy rates and deposition of mucin coats were evaluated.

Materials and methods

Animals and embryos

Sexually mature (6–9 months) New Zealand White rabbit does were induced to superovulate by subcutaneous injection of 2.7 IU FSH (follicle stimulating hormone, Sigma) for 3 days at intervals of 12 h (0.3, 0.4, 0.5, 0.5, 0.5 and 0.5 IU). Twelve hours after the final injection, 50 IU hCG was intravenously administered and the donor was mated twice with a fertile male. One-cell embryos were recovered 19–20 h post-coitus by flushing the oviducts with 10 ml Dulbecco's phosphate-buffered saline (DPBS) containing 0.3% bovine serum albumin (BSA). These embryos were cultured for 72 h in 50 µl of RDH (1:1:1 mixture of RPMI 1640, DMEM and Ham's F-10) under mineral oil at 39 °C and 5% CO_2 in air. Each drop of medium contained 10–15 embryos.

Embryo transfer and recovery

Embryos developed to the blastocyst stage after 72 h culture were surgically transferred to the oviducts of recipients synchronized 1 day later than the donors (asynchronous group) or synchronized on the same day as the donors (synchronous group). Recipient synchronization was induced by intravenous injection of 30–40 IU hCG and stimulation of vagina at the time of donor mating. Ten to 20 embryos were transferred to each oviduct. To confirm deposition of a mucin coat, the blastocysts were recovered by flushing the oviducts or uteri 24-48 h after the transfer and examining them microscopically. The thickness of the mucin coat was measured. After the full term of pregnancy, the offspring number was recorded.

Data analysis

Data (percentiles) were analysed by chi-square tests for significant differences. Differences in average mucin thickness among experimental groups were analysed by Student's *t*-test (Snedecor & Cochran, 1967).

Results

Recovery rate of transferred blastocysts

As shown in Table 1, the recovery rates of blastocysts from asynchronous and synchronous recipients 24 h after transfer were 85.2% and 84.4%, respectively. There were no significant differences between the recovery rates from asynchronous and synchronous recipients either from oviducts (59.8% and 61.8% respectively) or from uteri (40.2% and 38.2% respectively). However, 48 h after transfer, the recovery rate of embryos from uteri of asynchronous recipients (71.2%) was significantly higher than that from synchronous recipients (47.7%); the recovery rates from oviducts (asynchronous recipients 28.8%; synchronous recipients 42.3%) were not significantly different at 48 h.

Degeneration rate of recovered blastocysts

The recovered embryos were examined morphologically (Fig. 1) and degenerated embryos were counted (Table 2). The degeneration rates of blastocysts recovered 24 h after transfer from the oviducts of asynchronous and synchronous recipients were 38.0% and 43.5%, respectively. The corresponding figures for uteri were 17.1% and 21.4%. Forty-eight hours after transfer,

https://doi.org/10.1017/S0967199404002795 Published online by Cambridge University Press

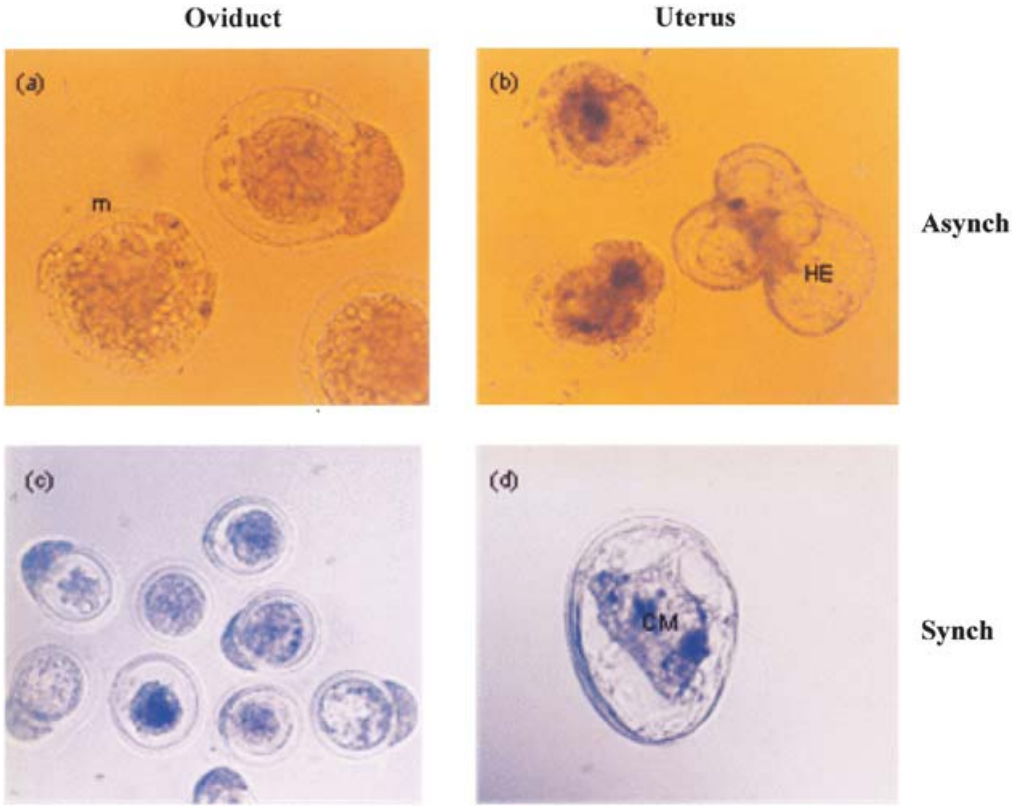

Figure 1 Embryos recovered 48 h after asychronous (Asynch) and synchronous (Synch) transfer. (*a*) Blastocysts recovered from the oviduct of an asynchronous recipient. m, mucin coat. (*b*) Blastocysts recovered from the uterus of an asynchronous recipient. HE, hatched embryo. (*c*) Blastocysts recovered from the oviduct of a synchronous recipient. (*d*) Abnormally expanded blastocyst recovered from the uterus of a synchronous recipient. CM, degenerated cellular material.

Table 2 Degeneration rates of blastocysts recovered 24 and 48 h after asynchronous and synchronous transfer

	Time recovered after transfer			
	24 h		48 h	
	No. (%) of embryos degenerated/ recovered from:		No. (%) of embryos degenerated/ recovered from:	
Recipients	Oviduct	Uterus	Oviduct	Uterus
Asynchronous	19/50 (38.0)	6/35 (17.1)	9/20 (45.0)	20/50 (40.0)[a]
Synchronous	20/46 (43.5)	3/14 (21.4)	18/27 (66.6)	39/54 (72.2)[b]

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

the degeneration rates from oviducts had risen to 45.0% and 66.6% for asynchronous and synchronous recipients respectively (no significant difference), but the corresponding figures for uteri were 40.0% and 72.2% ($p < 0.05$).

Thickness of mucin coats of recovered blastocysts

Mucin coat thicknesses on embryos recovered from oviducts or uteri were measured by photomicroscopy (Fig. 1, Table 3). Coat thicknesses from synchronous recipient oviducts after 24 h (2.9 ± 0.89 μm) were considerably lower than those from asynchronous recipient oviducts (6.8 ± 0.91 μm); the corresponding figures for uteri were 4.7 ± 1.05 μm and 3.4 ± 0.84 μm. Coat thicknesses from asynchronous recipient oviducts after 48 h (19.5 ± 2.59 μm) were higher than those from synchronous recipient oviducts (10.5 ± 1.65 μm) and the corresponding figures for uteri were 12.3 ± 1.7 μm and 6.4 ± 1.34 μm.

https://doi.org/10.1017/S0967199404002795 Published online by Cambridge University Press

Table 3 Mean mucin coat thicknesses of blastocysts recovered 24 and 48 h after asynchronous and synchronous transfer

	Time recovered after transfer			
	24 h		48 h	
	Mucin thickness ± SE (μm)		Mucin thickness ± SE (μm)	
Recipients	Oviduct	Uterus	Oviduct	Uterus
Asynchronous	6.8 ± 0.91^a	4.7 ± 1.05	19.5 ± 2.59^a	12.3 ± 1.73^a
Synchronous	2.9 ± 0.89^b	3.4 ± 0.84	10.5 ± 1.65^b	6.4 ± 1.34^b

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

Table 4 Implantation of *in vitro*-cultured blastocysts transferred to the oviducts of asynchronous and synchronous recipients

Recipients	No. of embryos transfered	No. of recipients	No. of pregnant recipients	No. of pups (%)
Asynchronous	260	13	4	$27\ (10.4)^a$
Synchronous	240	12	1	$5\ (1.9)^b$

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

Pregnancy rates after blastocyst transfer

Pregnancy and delivery rates after transfer of *in vitro*-cultured blastocysts to the oviducts of asynchronous and synchronous recipients are summarized in Table 4. Four of 13 asynchronous recipients were pregnant and a total 27 pups were born, while 1 of 12 synchronous recipients was pregnant and gave birth to a total of 5 pups. The delivery rate of asynchronous recipients (10.4%) was significantly higher than that of synchronous recipients (1.9%).

Discussion

Our results indicate that the oviducts of asynchronous recipients deposit a mucin coat around embryos to facilitate implantation. To confirm deposition of the mucin coat, blastocysts were recovered from oviducts 24–48 h after transfer. The recovery rate from asynchronous recipients uteri was higher than that from synchronous recipients after 48 h (Table 1), perhaps implying that asynchronous recipient oviducts (day 2 post-coitus) transport the transferred embryos more actively than those of synchronous recipients (day 3 post-coitus). The degeneration rate was higher in synchronous recipients (Table 2), probably because of the relative reproductive tract inactivity. Overall degeneration rates of transferred embryos were high in this experiment. Rabbit embryos without or with only traces of mucin could rupture easily around the zona and hatch during subsequent *in vitro* culture (Kane & Foot, 1971; Kane, 1975). Presumably the mucin coats deposited during this study were not adequate to offset this fragility. A longer residence time in the oviduct results in a thicker mucin coat around the zona pellucida (Table 3).

No mucin coat, or only a thin one, is deposited on the zona pellucidae during early tubal passage, as indicated for example by embryo recovery soon after fertilization. Deposition of the mucin coat is essential for successful implantation. The blastocysts recovered from oviducts or uteri of asynchronous recipients 24 and 48 h after transfer had thicker mucin coats than those of synchronous recipients. Asynchronous recipients had an oestrus cycle 1 day later than the donors (day 2 pc) when the *in vitro*-cultured blastocyts were transferred. Rabbit oviduct secretes mucin from about 20 h pc (Seidel *et al.*, 1976; Binkerd & Anderson, 1979). In this experiment the asynchronous recipient oviducts deposited more mucin onto the transferred blastocysts; synchronous ones might not actively produce mucin (Table 3). This experiment indicates that asynchrony resulted in higher pregnancy and birth rates than synchrony (Table 4). Implantation must be related to appropriate mucin deposition in recipient oviducts; reduction of the mucin layer in rabbit embryos is incompatible with normal implantation and reduces the rate of successful implantation (Greenwald, 1962). Murakami & Imai (1996) observed that only embryos with mucin layers were implanted when rabbit embryos recovered at different times post-coitus and cultured *in vitro* were transferred to the uterus of a synchronized recipient; a mucin coat thickness over 20 μm may be needed for rabbit embryos to produce live pups. Mucin thickness in this experiments was 19.5 ± 2.59 μm in embryos recovered from oviducts of asynchronous recipients 48 h after transfer, probably close to threshold for implantation. Embryos in recipient uteri tended to reduce mucin coat thickness, probably due to expansion of the embryos and degradation of the mucin coat.

https://doi.org/10.1017/S0967199404002795 Published online by Cambridge University Press

The results of the present study demonstrate that oviducts of asynchronous recipients produce more mucin around 72 h-cultured rabbit blastocysts and affect implantation of the transferred blastocysts. Transformation of mucin secreted from oviducts onto rabbit embryo zonae and the role of the mucin coat in development need further investigation.

Acknowledgements

This work was supported by grant no. R11-2002-100-01000-0 from the ERC programme of the Korean Science and Engineering Foundation.

References

Adams, C.E. (1973). Asynchronous egg transfer in the rabbit. *J. Reprod. Fertil*. **35**, 613–14.

Binkerd P.E. & Anderson G.B. (1979). Transfer of cultured rabbit embryos. *Gamete Res*. **2**, 65–73.

Carney, E.W. & Foote, R.H. (1990). Effects of superovulation, embryo recovery, culture system and embryo transfer on development of rabbit embryos *in vivo* and *in vitro*. *J. Reprod. Fertil*. **89**, 543–51.

Denker, H.W. & Gerdes, H.J. (1979). The dynamic structure of rabbit blastocyst coverings. I. Transformation during regular preimplantation development. *Anat. Embryol*. **157**, 15–34.

Greenwald, G.S. (1962). The role of the mucin layer in development of the rabbit blastocyst. *Anat. Rec*. **142**, 407–15.

Jin, D.I., Kim, D.K., Im, K.S. & Choi, W.S. (2000). Successful pregnancy after transfer of rabbit blastocysts grown *in vitro* from single-cell zygotes. *Theriogenology* **54**, 1109–16.

Kane, M.T. (1975). Inhibition of zona shedding of rabbit blastocysts in culture by the presence of a mucin coat. *J. Reprod. Fertil*. **44**, 539–42.

Kane, M.T. & Foote, R.T. (1971). Factors affecting blastocyst expansion of rabbit zygotes and young embryos in defined media. *Biol. Reprod*. **4**, 41–7.

Li, J., Foote, R.H., Liu, Z. & Giles, J.R. (1997). Development of rabbit zygotes into blastocysts in defined protein-free medium and offspring born following culture and embryo transfer. *Theriogenology* **47**, 1103–13.

Mauer, R.R., Onuma, H. & Foote, R.H. (1970). Viability of cultured and transferred rabbit embryos. *J. Reprod. Fertil*. **21**, 417–22.

Murakami, H. & Imai, H. (1996). Successful implantation of *in vitro* cultured rabbit embryos after uterine transfer: a role for mucin. *Mol. Reprod. Dev*. **43**, 167–70.

Seidel, G.E. Jr, Bowen, R.A. & Kane, M.T. (1976). *In vitro* fertilization, culture and transfer of rabbit ova. *Fertil. Steril*. **27**, 861–70.

Snedecor, G.W. & Cochran, W.G. (1967). *Statistical Methods*. Ames, Iowa: lowa State University Press.

Techakumphu, M., Wintenberger-Torres, S. & Sevellec, C. (1987). Survival of rabbit embryos after synchronous or asynchronous transfer. *Anim. Reprod. Sci*. **12**, 297–304.

Yang, X. & Foote, R.H. (1990). Survival of bisected rabbit morulae transferred to synchronous and asynchronous recipients. *Mol. Reprod. Dev*. **26**, 6–11.