Protective role of mucin coat

Grygoruk C.[1*], Mrugacz G.[1], Modliński JA.[2], Gajda B.[3], Grad I.[3], Grusza M.[1], Sieczynski P.[4]

[1] Center for Reproductive Medicine BOCIAN, Akademicka 26, 15-267 Bialystok, Poland;
[2] Institute of Genetics and Animal Breeding of the Polish Academy of Sciences, Jastrzebiec, Postepu 1, 05-552 Wolka Kosowska, Poland
[3] National Research Institute of Animal Production, Department of Biotechnology of Animal Reproduction, Krakowska 1, 32-083 Balice/Krakow, Poland
[4] Center for Reproductive Medicine KRIOBANK, Stoleczna 11, 15-879 Bialystok, Poland

ABSTRACT

Purpose: The present study was designed to examine the protective property of the mucin coat against pressure fluctuation.
Materials and Methods: Thirty non-hatched rabbit blastocysts containing mucin coat and thirty mouse blastocysts were exposed to pressure fluctuations in *in-vitro* conditions. Morphological response of the blastocysts was assessed 5 and 60 minutes after exposition to pressure fluctuation.
Results: Out of thirty mouse blastocysts there were 4 collapsed in 5th minute and 8 collapsed in the 60th minute. There were no collapsed rabbit blastocysts in 5th minute and only one in 60th minute. The difference between groups was statistically significant; $p<0.05$.
Conclusions: Mucin coat has a unique property of protecting embryo from a deleterious effect of the rapid pressure fluctuations.
Key words: blastocyst, mucin, mucin coat, pressure, rabbit embryo

***Corresponding author:**
Cezary Grygoruk, Center for Reproductive Medicine Bocian
26 Akademicka str., 15-267 Bialystok, Poland
Tel/Fax: +48857447700
e-mail: cezary.grygoruk@gmail.com

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INTRODUCTION

Mammalian oocyte are surrounded by a non-cellular layer called the zona pellucida at the time of ovulation. This layer has a neutral or weakly acid mucopolysaccharide composition. Rabbit ooocyte, in contrast to those of almost all other mammals including mouse, acquire a second covering in the oviduct and uterus called the mucin coat. Mucins are a family of high molecular weight, heavily glycosylated proteins (glycoconjugates) produced by epithelial tissues in most metazoans [1]. Mucins' key characteristic is their ability to form gels; therefore they are a key component in most gel-like secretions, serving functions from lubrication to cell signaling to forming chemical barriers [1]. Mucins belong to the family of glycoproteins identified as the oviduct-specific glycoproteins (OGPs) [2]. Expression of OGPs appears to be regulated and is estrogen-dependent or estrogen-associated.

However, differences in species and effectors are apparent [3]. At least eight OGPs have been cloned and a high degree of conservation in both nucleotide and amino acid sequence demonstrated across species. A universal characteristic of OGPs is their association with the zona pellucida and perivitelline space of oocytes and embryos, with the possible exception of the mouse zona pellucida.

A variety of functional roles for OGPs have been proposed, but relatively few studies have been performed. The role and the function of the mucin coat of the rabbit embryo is not well understood. It is assumed that it helps choosing the valuable healthy embryos to implant, secures timely appropriated implantation and prevents the embryo from exposure to pathogenic viruses [4].

Another possible hypothesis is that the mucin layer may protect the rabbit embryos from direct impact of the pressure fluctuations generated by the contractile activity of the rabbit uterus.

The present study was designed to examine the protective property of the mucin coat against pressure fluctuation.

MATERIAL AND METHODS

Collection and culture of embryos

The experiment was carried out, after receiving consent from the local ethics committee, in 60 mouse and 60 rabbit non-hatched blastocysts.

The mice originated from the mouse-raising facility in the Department of Experimental Embryology. Mice were kept under a 12-h day starting at 7 am. Food (Labofeed H, Poland) and water were available ad libitum. Inbred DBA/2 or MIZ (Swiss albino) females aged 2–3 months mated to 3- to 10-month-old males of the same breeds served as donors of embryos. Females were superovulated by injection of 7.5 IU of pregnant mare serum gonadotrophin (PMSG—Folligon, Intervet) followed by 7.5 IU of human chorionic gonadotrophin (hCG—Chorulon, Intervet, Boxmeer, Holland) 48 hr later and mated with F1 (C57Bl10_CBA/H) males. To obtain eight-cell embryos, females were caged with males in the evening and next morning they were inspected for vaginal plugs. Those females that mated were killed by cervical dislocation 48–50 h later. Oviducts were excised into M2 manipulation medium (HEPES-buffered M16; Fulton & Whittingham 1978) and their contents were flushed with the same medium using a pipette introduced into the infundibulum 68-70 hrs after hCG injection. After thorough rinsing with M2 without CD, the embryos were placed in drops of KSOM medium (Specialty Media, Phillipsburg, NJ, USA) under paraffin oil (Sigma) in Petri dishes (Corning) and were cultured at 37°C, in an atmosphere of 5% CO_2 in air until they reached blastocyst stage.

Rabbit females (New Zealand and California, N = 4) 4.5– 6.5 months of age and weighing 3–4 kgs were used as donors. Females were superovulated by IM injection of 100 IU of PMSG (Serogonadotropin; Biowet, Poland), followed by 100 IU of hCG (Biogonadyl, Biomed, Poland), injected 72 hours later. Immediately, after hCG injection, donors were inseminated. The embryos at the one-cell stage were collected at 18 to 20 hours after insemination. The embryos were recovered at room temperature by flushing the Fallopian tubes with 10 mL of Dulbecco's solution (PBS, Sigma) supplemented with 20% fetal calf serum (Sigma). The recovered embryos were examined morphologically under a stereo-microscope. All the embryos were washed in a B2 medium (INRA, Ménézo, bioMerieux, France) [5] and cultured in 1.0mL of the medium in four-well multidishes (Nunc, Denmark) in a CO_2 incubator (5% CO_2 in an air at 38°C) for 3 to 4 days until they reached a blastocyst stage. Assessments of embryos were carried out under a stereomicroscope (Nikon SMZ800, Nikon, Tokyo, Japan) [6].

Morphologically normal non-hatched mouse and rabbit blastocysts were included into the study.

Experimental groups

The mouse blastocysts were randomly divided into an experimental group (M) and a control group (MC). Similarly, the rabbit blastocysts were randomly divided into an experimental group (R) and a control group (RC). The mouse and rabbit blastocysts from the experimental groups were exposed into the pressure fluctuation. The mouse and rabbit blastocysts from the control groups were not exposed into the pressure fluctuations.

Experimental set up

The pressure fluctuations were generated with the use of the standard catheter for embryo transfer (Labotect, Bovender-Gottingen, Germany) connected to 1 ml insulin syringe (Polfa Lublin, Poland). The reference loading of the syringe-catheter complex was arranged in the following order from the catheter tip: 0.1 µl air, 1.5 µl liquid (embryo culture medium), 0.2 µl air, 1.5 µl liquid (mouse/rabbit blastocyst in embryo culture medium), 0.2 µl air, 1.5 µl liquid (embryo culture medium) and 25 µl air, (Figure 1).

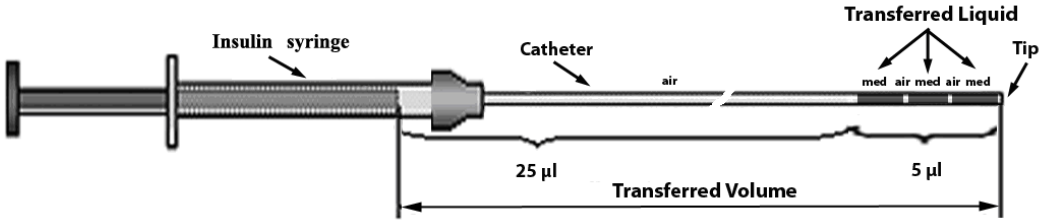

Figure 1. The experimental set for pressure fluctuation generation composed of the insulin syringe and embryo transfer catheter.

According to our previous study, the parameters of the pressure wave created by the catheter and insulin syringe complex during ejection of the transferred load were following: the mean value of peak pressure 76 mmHg (SD 37), the mean pressure increase slope 26 682 mmHg/s (SD 16 595), the mean pressure decrease slope was 61 742 mmHg/s (SD 34 209) [7, 8]. The mean time of the pressure wave duration was 0,021 sec (SD 0,006). The mean speed of injection of transferred volume 12.1 m/s (SD 3.5). The exemplary recording of pressure wave is presented on Figure 2.

The blastocyst from the experimental groups either M or R was loaded individually into the embryo transfer catheter. Then, a tip of a loaded syringe catheter-complex was positioned in the center of a well (four-well multidishe, Nunc, Denmark) filled with 1.0mL of the B2 medium (INRA, Ménézo, bioMerieux, France) pre-incubated in a CO_2 incubator (5% CO_2 in the air at 38°C). Then after that each blastocyst was injected into the medium and left in the well, one embryo per one well, and cultured in a CO_2 incubator (5% CO_2 in the air at 38°C) for 1 hr. The embryos were investigated for the morphological changes 5 minutes and 60 minutes after exposition to the pressure fluctuations.

The blastocysts from the control groups MC and RC were not exposed into the pressure fluctuations. The blastocysts were investigated for the morphological changes five minutes and one hour after beginning of the experiment.

Embryo assessment

A natural response of the blastocyst to a stressful environmental factor is a linkage. Therefore, we decided to use the blastocysts as an experimental model in order to follow the shrinkage response of embryos in reaction to the pressure fluctuations.

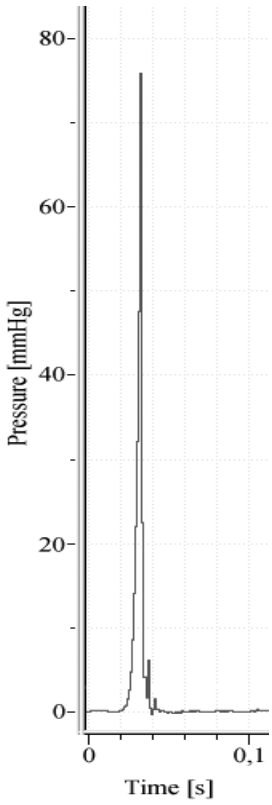

Figure 2. An exemplary recording of pressure wave generated with the use of the insulin syringe and the catheter for embryo transfer. The pressure sensor was positioned in a distance of 1 mm from the tip of the embryo transfer catheter. The registered pressure values were as follows: peak pressure 76 mmHg, pressure increase slope 26 120 mmHg/s, pressure decrease slope 50 106 mmHg, pressure wave duration 0.021 s.

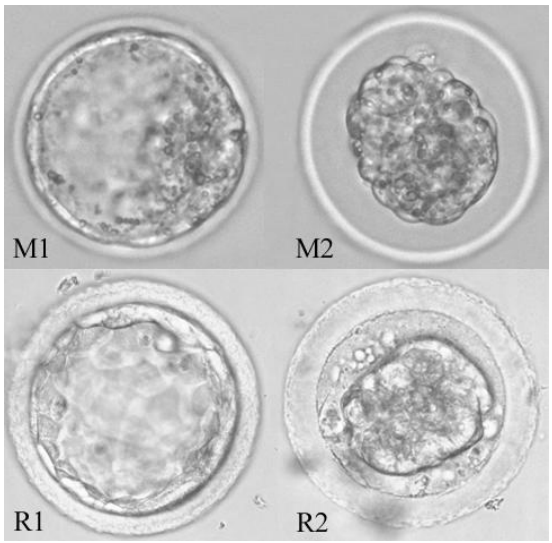

Figure 2a. Blastocyst morphological response to a pressure impulse. M1 – unchanged mouse blastocyst before exposition to pressure wave, M2- collapsed mouse blastocyst, no blastocele observed, after exposition to pressure wave, R1 – unchanged rabbit blastocyst before exposition to pressure wave, R2- collapsed rabbit blastocyst, no blastocele observed, after exposition to pressure wave.

The blastocysts shape and the blastocele sizes were assessed before, 5 and 60 minutes after the beginning of the experiment. The shrunken blastocysts with no visible blastocele were classified as a collapsed.

Statistical analysis

Statistical analysis was performed using chi-square test. Differences were considered significant at $p < 0.05$. Statistical analyses were performed with the SPSS package (IBM, Chicago, USA).

RESULTS

There were 30 blastocysts in each of four groups. Five minutes after the exposition to the

All together, the collapsed blastocysts constituted 27% of all blastocysts in group M and only 3% in the group R. The detailed data on the blastocysts shrinkage response, in particular, groups were presented in Table 1.

pressure fluctuations, there were four collapsed blastocysts in the mouse experimental group and none in the rabbit experimental; $p<0.05$. All blastocysts remained expanded in the rabbit and mouse control groups in the fifth minute of the experiment. One hour after the exposition to the pressure fluctuations, there were significantly more collapsed blastocyst in the M group than R group; $p<0.05$. There were eight collapsed mouse blastocysts in group M and only one collapsed blastocyst in group R, (Figure 3).

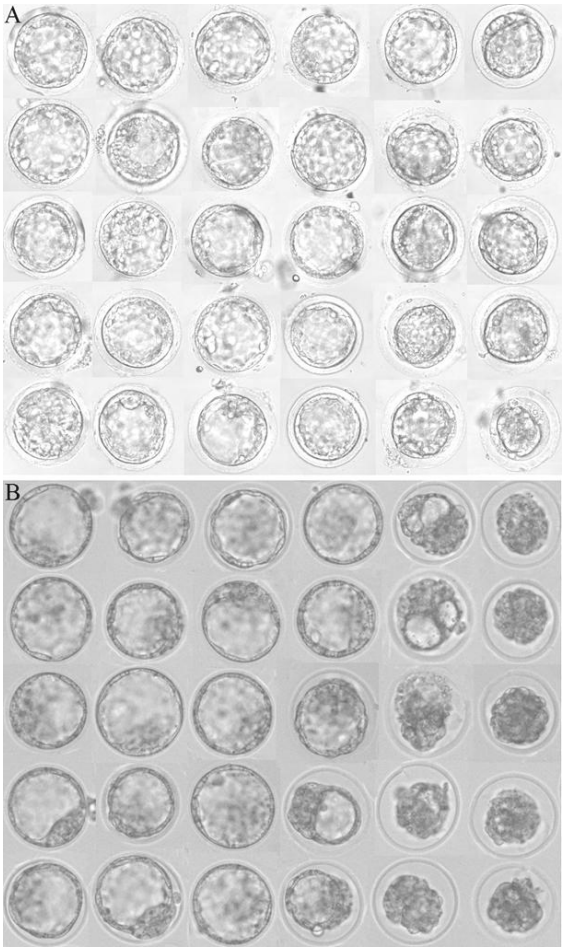

Figure 3. Blastocysts morphology (magnification x 200) 1 hour after exposition to pressure fluctuation: A) mouse blastocysts, B) rabbit blastocysts.

Table 1. Blastocysts after exposition to the pressure fluctuations: M – mouse blastocysts exposed to the pressure fluctuations, R – rabbit blastocysts exposed to the pressure fluctuations, MC – mouse blastocysts not exposed to the pressure fluctuations, RC – rabbit blastocysts not exposed to the pressure fluctuations.

	M (n=30)		**R (n=30)**		**MC (n=30)**		**RC (n=30)**	
Time after blastocyst exposure to pressure fluctuation (min)	5	60	5	60	5	60	5	60
Collapsed blastocysts (No.)	4	8	-	1	-	-	-	-
Collapsed blastocysts (%)	15*	27*	-	3	-	-	-	-

*p<0.05

DISCUSSION

The results of the present study indicate that rabbit blastocysts suffered less damage, in terms of morphological changes than the mouse blastocysts when exposed to steep pressure fluctuations.

The role and the function of the mucin coat stay unclear. The study conducted by Murakami and Imai proved that the presence of the mucin coat is essential to embryo implantation [4]. However, the exact mechanism in which the mucin coat facilitate the embryo development is obscure. There is a hypothesis that the mucin layer may slow the rate of blastocyst expansion, resulting in more cells in the inner cell mass and in this way increase the chances for embryo development [4]. The other hypothesis states that the mucin coat prevents the rabbit embryo from the exposure to the pathogenic viruses [9]. A possible hypothesis may be that the mucin layer may protect the blastocyst from direct exposure to a deleterious uterine environment, for example pressure fluctuations generated by the contractile activity of the rabbit uterus. According to the study conducted by the Herczeg and Torok the intrauterine pressure fluctuations of non-pregnant rabbit could reach amplitude 59.7 ± 1.9 mmHg and frequency 35.4 ± 4.6 peaks per 30 minutes [10].

In the literature, there is some evidence for the cell damage caused by local pressure fluctuations [8, 11, 12]. Bilek and Gaver investigated the surface-tension-induced lung epithelial cell damage in a model of airway reopening. They concluded that the steep pressure gradient was the most likely cause of the observed cellular damage. According to the Bilek at al. the magnitude of the pressure gradient was directly correlated to the epithelial cell layer damage [13]. Similarly, Wong et all, in a study of cell damage caused by bubble motion in a circular-lumen endothelialized model of a microvessel, demonstrated that the steep pressure gradient at the bubble front would be the most likely cause of cell damage [14]. Kay et al. provided also compelling evidence that the magnitude of the pressure gradient on the cell is the factor that induces membrane damage and the propensity for cell membrane disruption decreases with decreasing pressure amplitude [15]. Furthermore, it is certain that positive pressure does not actually cause damage but the steep increase in pressure, followed by negative pressure, does cause damage since biological structures can only be damaged by shear or extension and not by positive pressure (compression). A generalized consideration is that the degree of cell damage will depend upon the amplitude. Thus, low amplitude pressure fluctuations will only disturb cell functions (reversibly), while higher amplitudes pressure fluctuations can destroy cells and their functions.

The present study has certain limitation. It would be ideally to compare the reaction to the pressure fluctuation between rabbit blastocysts with and without mucin coat. However, to obtain the blastocysts without mucin coat, it requires isolation of the antral follicles from rabbit ovary, fertilization and mature in-vitro conditions to blastocyst stage. Unfortunately, the authors had no access to such technology at the time of conducting experiment.

The results of the present study indicated that the mucin sheaf provided better protection of rabbit embryos against the local pressure fluctuation than the zona pellucid alone of the mouse embryos. As far as we know, it is the first report demonstrating the protective property of the mucin coat against pressure fluctuation.

CONCLUSION

Mucin coat has a unique property of protecting embryo from a deleterious effect of the rapid pressure fluctuations.

Conflicts of interest

The authors have declared no conflicts of interest.

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