REPRODUCTION
RESEARCH

High hydrostatic pressure: a new way to improve *in vitro* developmental competence of porcine matured oocytes after vitrification

Y Du[1,2], C S Pribenszky[3], M Molnár[3], X Zhang[4], H Yang[4], M Kuwayama[5], A M Pedersen[1], K Villemoes[1], L Bolund[2] and G Vajta[1]

[1]*Population Genetics and Embryology, Institute of Genetics and Biotechnology and* [2]*Institute of Human Genetics, University of Aarhus, DK-800 Aarhus, Denmark,* [3]*Clinic for Large Animals, Faculty of Veterinary Science, Szent István University, 2225 Üllő, Dóra major, Hungary,* [4]*Beijing Genomics Institute, Airport-Industrial zone B 6#, Beijing 101300, China and* [5]*Kato Ladies' Clinic, Nishishinjuku, Shinjuku, Tokyo 160-0023, Japan*

Correspondence should be addressed to Y Du at Faculty of Agricultural Sciences, Institute of Genetics and Biotechnology, University of Aarhus, Blichers Alle 20, Postbox 50, 8830 Tjele, Denmark; Email: duyt@genomics.org.cn

Abstract

The purpose of the present study was to improve cryotolerance using high hydrostatic pressure (HHP) pretreatment of porcine *in vitro* matured (IVM) oocytes, to facilitate their further developmental competence after parthenogenetic activation. A total of 1668 porcine IVM oocytes were used in our present study. The pressure tolerance and optimal duration of recovery after HHP treatment were determined. Oocytes were treated with either 20 or 40 MPa (200 and 400 times greater than atmospheric pressure) for 60 min, with an interval of 10, 70, and 130 min between pressure treatment and subsequent vitrification under each pressure parameter. Oocytes from all vitrification groups had much lower developmental competence than fresh oocytes ($P<0.01$) measured as cleavage and blastocyst rates. However, significantly higher blastocyst rates ($P<0.01$) were obtained in the groups of 20 MPa pressure, with either 70 (11.4±2.4%) or 130 (13.1±3.2%) min recovery, when compared with the vitrification control group without HHP treatment where no blastocysts were obtained. The influence of temperature at HHP treatment on further embryo development was also investigated. Treatments of 20 MPa with 70 min recovery were performed at 37 °C or 25 °C. Oocytes pressurized at 37 °C had a significantly higher blastocyst (14.1±1.4%) rate than those treated at 25 °C (5.3±1.1%; $P<0.01$). Our results demonstrate that HHP pretreatment could considerably improve the developmental competence of vitrified pig *in vitro* matured (IVM) oocytes. The HHP pretreatment will be tested as a means to improve survival and developmental competence at different developmental stages in different species including humans.

Reproduction (2008) **135** 13–17

Introduction

Oocyte cryopreservation has become one of the most challenging approaches to restore fertility of chemo- and radiation therapy treated women with compromised ovarian function (Falcone *et al*. 2004) and improve reproductive flexibility of *in vitro* assisted reproductive technologies in humans (Porcu & Venturoli 2006, Vajta & Kuwayama 2006, Vajta & Nagy 2006). In animals, endangered species and premium genetics from specific breeds could be rescued, while the expense and disease transmission during storage and transportation could be minimized (Vajta 2000). Still, some characteristics of oocytes are major obstacles for efficient cryopreservation (Woods *et al*. 2004). Cooling oocytes to low temperatures substantially disrupts cytoskeletal elements (Aman & Parks 1994), cortical granules (Pickering *et al*. 1990), and plasma membranes (Vincent & Johnson 1992), frequently leading to poor development and cell death. Despite all the existing and emerging demands, oocyte cryopreservation still remains unaccomplished as an established procedure.

After many years' efforts, some successes were achieved in oocyte cryopreservation with different species. Various strategies have been proposed to increase viability and developmental competence of cryopreserved oocytes, with the two main aspects of technical improvements and modifications of oocytes. The application of ultrarapid vitrification has considerably increased the cooling/warming rates and reduced the risk of chilling injuries (Vajta *et al*. 1998, Lomber *et al*. 1999, Dinnyes *et al*. 2000, Kuwayama *et al*. 2005). The original purpose for modifications of oocytes is to reduce their sensitivity to low temperature. Currently, most methods still rely on extrinsic chemical treatment (Fujihira *et al*. 2004, Shi *et al*. 2006), changing lipid composition (Zeron *et al*. 2002*a*, 2002*b*), or removing intercellular lipids either mechanically (Nagashima *et al*. 1994, Esaki *et al*. 2004, Du *et al*. 2007) or chemically (Men *et al*. 2006). Even though these methodological

ISSN 1470–1626 (paper) 1741–7899 (online)
DOI: 10.1530/REP-07-0362
Online version via www.reproduction-online.org

modifications have improved the cryotolerance of oocytes to some extent, the limited number of offspring produced justifies their possible negative effects on postimplantation development.

High hydrostatic pressure (HHP) was introduced in food processing many years ago to inactivate microorganisms as well as enzymes responsible for shortening the life of a product (Knorr 1993). However, studies have shown increased synthesis of heat shock proteins (HSPs) by HHP in several bacteria, including *Escherichia coli* (Welch *et al.* 1993), yeast (Domitrovic *et al.* 2006, Miura *et al.* 2006), and also mammalian cells (Kaarniranta *et al.* 1998). Shock proteins could be provoked by other types of stresses, such as heat and cold shock, as well. The type, amount, and proportion of proteins to be synthesized/activated in the stressed cells are dependent on the intensity and type of the sublethal shock itself, as well as on the stressed cell. The responses for different types of stresses can be similar, providing a possibility for 'cross-protection'. Molecular chaperones, induced by sublethal stress, could provide cellular protection, maintaining homeostasis and can even be applied in cryoprotective therapy.

It has been investigated whether sublethal HHP impulses could improve the cryotolerance of mouse embryos, *in vitro* produced (IVP) bovine embryos, and semen from bulls and boars (Pribenszky *et al.* 2005*a*, 2005*b*, 2006, Kuo *et al.* 2007, unpublished observations). Porcine oocyte cryopreservation has remained one of the biggest challenges due to the extreme sensitivity to chilling injury mostly caused by the high intracellular lipid content. Some species-specific reasons also hamper the successful application of resolutions working appropriately in several other species. The objective of the present study was to apply a new pretreatment system with HHP to porcine oocyte vitrification and investigate how HHP could influence developmental competence of vitrified oocytes after parthenogenetic activation (PA).

Results

The optimal parameters of HHP for improved survival and development of vitrified porcine IVM oocytes were investigated in the first experiment. As shown in Table 1, vitrified oocytes had severely reduced developmental competence when compared with fresh oocytes, regardless of HHP treatment. However, those oocytes that were pretreated with HHP (20 MPa, 60 min) and recovered for 70 or 130 min had a significantly improved *in vitro* development ($P<0.01$) when compared with the vitrification control group as evaluated by cleavage rates (38.4±6.8% for 70 min group, 41.7±6.5% for 130 min group versus 14.5±5.2% for vitrification control) and blastocyst rates (11.4±2.4% for 70 min group, 13.1±3.2% for 130 min group versus 0.0±0.0% for vitrification control). Comparable cell numbers were obtained in blastocysts produced from these two groups (37.5±3.4 for 70 min group, 36.6±3.7 for 130 min group), which were significantly less than observed in the activation control group (55.7±4.0) where fresh oocytes were activated.

In the second experiment, a possible temperature effect on HHP treatment was investigated. HHP parameters resulting in the best results in the first experiment (20 MPa 60 min, with 70 min recovery before vitrification) were applied. When HHP treatment was performed at 37 °C, a significantly higher blastocyst rate (14.1±1.4%) was obtained than that observed with HHP treatment at 25 °C (5.3±1.1%) and in the vitrification groups (Table 2).

Discussion

Cryopreservation of mammalian oocytes instead of embryos would offer considerable advantages in several situations. Unfortunately, in most species oocytes are more sensitive to cryoinjuries than embryos. Several strategies have been developed to overcome this problem, including application of new cryopreservation methods to decrease injuries or promote regeneration after the procedure. Vitrification with high cooling and warming rates may decrease chilling injury. Proper combinations of the appropriate permeable and non-permeable cryoprotectants as well as optimization of equilibration and rehydration parameters may significantly improve survival rates and further developmental competence (Vajta & Nagy 2006). Another approach is to modify oocytes before cryopreservation to increase the cryotolerance, e.g., by removing the chilling-sensitive lipid droplets (Nagashima

Table 1 *In vitro* development of vitrified-warmed porcine *in vitro* matured oocytes after being pretreated with high hydrostatic pressure under various parameters.

Pressure magnitude (MPa)	Recovery duration (min)	No. of treated oocytes	Cleavage rate (%, mean±S.E.M.)	Blastocyst rate (%, mean±S.E.M.)	Cell no.
20	10	166	11.2±3.5^{b}	1.9±0.8	–
	70	190	38.4±6.8^{c}	11.4±2.4	37.5±3.4^{b}
	130	201	41.7±6.5^{c}	13.1±3.2	36.6±3.7^{b}
40	10	77	16.2±3.2^{b}	5.9±3.1	–
	70	78	26.3±4.5^{b}	5.2±3.1	–
	130	93	25.1±4.1^{b}	5.3±0.9	–
Vitrification control	–	142	14.5±5.2^{b}	0.0±0.0	–
Activation control	–	161	86.9±2.9^{a}	81.5±9.1	55.7±4.0^{a}

Different superscripts within the same column denote significant difference ($P<0.05$).

Table 2 *In vitro* development of vitrified-warmed porcine *in vitro* matured oocytes pretreated with high hydrostatic pressure (HHP; 20 MPa for 60 min, recovered for 70 min) under different temperatures.

Group	No. of treated oocytes	Cleavage rate (%, mean±S.E.M.)	Blastocyst rate (%, mean±S.E.M.)
37 °C HHP	127	45.1±8.4[a]	14.1±1.4[a]
25 °C HHP	126	33.5±10.8[a]	5.3±1.1[b]
Vitrification control	100	21.8±15.6[b]	1.3±1.3[b]
25 °C vitrification control	98	4.4±2.9[c]	0.8±0.8[b]
PA CK	109	89.3±5.2[d]	80.0±2.1[c]

Different superscripts within the same column denote significant difference ($P<0.05$). PA CK, activation control.

et al. 1994, Esaki *et al*. 2004) or protecting the cellular architecture by cytoskeleton relaxants (Fujihira *et al*. 2004). However, all these approaches have resulted in only limited success in porcine oocyte cryopreservation.

This paper describes a new approach, HHP pretreatment, to protect porcine oocytes from cryoinjuries. The application of a sublethal HHP treatment to improve the stress tolerance of oocytes or embryos has been investigated only recently. Increased cryosurvival was achieved by this strategy with mouse and bovine embryos, and also with bull and boar semen (Pribenszky *et al*. 2005*a*, 2005*b*, 2006, Kuo *et al*. 2007, unpublished observations). The level and duration of the required pressure differs considerably between species, gamete, and developmental stage. For example, mouse and bovine IVP embryos tolerate a higher pressure stress (60–80 MPa up to 60 min duration) than porcine IVM oocytes where a HHP treatment of 60 MPa lasting for 30 min seems to induce irreversible damages (unpublished observations). We have found that a one- or two-hour recovery period between HHP treatment and vitrification was crucial for improving the viability and further developmental competence of porcine oocytes. The recovery period may be required for the synthesis of special molecules that protect the embryos from further damages.

The influence of treatment temperature was also investigated. A low treatment temperature (25 °C) has been adopted and functioned well in mouse and bovine embryos or semen for HHP, according to previous studies. However, no great improvement was achieved in the present study when porcine oocytes were pretreated with HHP at 25 °C before vitrification, even though seemingly more blastocysts were obtained than in the control groups. When the temperature was elevated to 37 °C, a significantly higher blastocyst rate was obtained than in the group with 25 °C HHP pretreatment. Furthermore, oocytes from the 25 °C vitrification control group have a much lower developmental competence than seen in the 37 °C vitrification control group, demonstrating the detrimental effect of low temperature on porcine oocytes.

PA instead of *in vitro* fertilization (IVF) was performed to investigate the developmental competence of vitrified oocytes for two main reasons. First, even though IVF embryos (in contrast to PA ones) may have the potential for full term development after transfer to foster mothers, their *in vitro* developmental competence is considerably lower and less consistent (Du *et al*. 2005). In our present study where vitrified oocytes were investigated, very few blastocysts were obtained in the control groups, while around 11–14% blastocyst rates were achieved after HHP treatment. Based on these data and also on our preliminary (unpublished) experiments, a similar study using IVF with vitrified oocytes would not be able to demonstrate significant benefits of HHP treatment. Secondly, PA could be used as a model of somatic cell nuclear transfer, which will be our next goal using vitrified oocytes (with HHP pretreatment) after enucleation as recipients. Additionally, some factors that may influence the outcome of IVF, including possible structural changes of the zona pellucida that may decrease sperm penetration, do not play a role in the outcome of PA, therefore the PA system may give more information regarding the integrity and developmental competence of the oocyte itself.

According to previous studies, sublethal HHP treatment could induce production of specific proteins (e.g. HSPs) that play essential roles in improving stress tolerance, protein folding, signal transduction in various bacteria, and mammalian cells (Csermely *et al*. 1998), and may further cross-protect the cells in other stress conditions. HSPs play essential roles not only in a stress situation but also for normal development in mammalian embryos. Esfandiari *et al*. (2007) demonstrated that inhibition of HSP function by antibodies causes a significant reduction in blastocyst development and an increase in cell death. Even though oocytes are regarded as inactive cells at the level of transcription, the mechanism of posttranscriptional stabilization of HSP70 mRNA may partially explain the improved developmental capacity of vitrified oocytes with HHP pretreatment. This posttranscriptional regulation of HSP70 has been observed in human chondrocyte-like cells, which responded to continuous HHP by elevating mRNA and protein levels of HSP70, without activating heat shock transcription factor 1 (HSF1) of the HSP70 gene (Kaarniranta *et al*. 1998).

In conclusion, HHP pretreatment was found to be efficient in increasing the *in vitro* development of vitrified porcine oocytes after PA. Further investigations regarding gene transcription and protein synthesis are required to understand the precise mechanism of this effect. The described approach may eventually help to increase the

efficiency of cryopreservation of oocytes in pigs and other mammalian species.

Materials and Methods

Unless otherwise indicated, all chemicals were purchased from Sigma Chemical Co.

Oocyte collection and IVM

Cumulus–oocyte complexes (COCs) aspirated from 2 to 6 mm follicles from slaughterhouse-derived sow ovaries were matured in groups of 50 in 400 µl bicarbonate-buffered TCM-199 supplemented with 10% (v/v) cattle serum, 10% (v/v) pig follicular fluid, 200 mM glutamine, 10 IU/ml eCG and 5 IU/ml hCG (Suigonan Intervet; Skovlunde, Denmark) at 38.5 °C in the Submarine Incubation System (SIS; Vajta *et al*. 1997) in 5% CO_2 in humidified air for 41–44 h.

COC treatment with HHP

Expanded COCs in HEPES-buffered TCM-199 (TCMH) were loaded into 0.5 ml plastic straws with a 2 ml syringe. Straws were then placed into the pressure chamber of the pressurizing device (Cryo-Innovation Inc., Budapest, Hungary), containing water as pressure medium. The chamber was made of stainless steel with an inner diameter of 2 cm and was connected to a pressure gauge. Pressure was generated by a moving piston inside the pressure chamber at the speed of 1 min/10 MPa. When the required pressure was reached it was maintained continuously for the specified period. The rate of decompression to atmospheric pressure was 1 min/10 MPa. After HHP treatments, COCs were released from straws and incubated in the original IVM medium until the start of the next procedure.

Oocyte denudation and vitrification

The cumulus investment of the COCs was removed by repeated pipetting in 1 mg/ml hyaluronidase in TCMH. From this point (except where otherwise indicated), all manipulations were performed on a heated stage adjusted to 39 °C. Cryopreservation was carried out by vitrification with a Cryotop device and factory-prepared vitrification and warming solutions (Kitazato Supply Co., Fujinomiya, Japan) as described previously (Kuwayama *et al*. 2005). Oocytes were transferred into equilibration solution (ES) consisting of 7.5% ethylene glycol (EG) and 7.5% dimethylsulfoxide (DMSO) in TCMH and 20% synthetic serum substitute (SSS; Cat. No. 99193, Irvine Scientific, Santa Ana, CA, USA) at 39 °C for 5–15 min. After an initial shrinkage, oocytes regained their original volume. Ten to twenty oocytes were transferred into a 20 µl drop of vitrification solution (VS) consisting of 15% EG, 15% DMSO, and 0.5 M sucrose dissolved in TCMH and 20% SSS. After incubation for 20–30 s, oocytes were loaded on Cryotop and plunged into liquid nitrogen. The process from VS exposure to plunging was completed within 1 min.

Vitrified oocytes were warmed by immersing Cryotop directly into 39 °C thawing solution (1.0 M sucrose dissolved in TCMH and 20% SSS) for 1 min and then transferred to dilution solution (0.5 M sucrose in TCMH and 20% SSS) for 3 min. Subsequently, oocytes were incubated twice for 5 min in the washing solutions (TCMH and 20% SSS).

PA and embryo culture

After warming, oocytes were equilibrated shortly in activation medium drops (0.3 M mannitol, 0.1 mM $MgSO_4$, 0.1 mM $CaCl_2$, and 0.01% polyvinyl alcohol) for 5 s. Under a 1.25 kV/cm alternative current, oocytes were aligned to the wire of a fusion chamber (Microslide 0.5 mm fusion chamber, model 450; BTX, SanDiego, CA, USA). Then a single direct current pulse was applied to the oocytes for electrical activation. After washing thrice in drops of TCMH supplemented with 10% calf serum, activated oocytes were incubated in culture medium (PZM-3 medium supplemented with 4 mg/ml BSA; Yoshioka *et al*. 2002), 5 µg/ml cytochalasin B, and 10 µg/ml cycloheximide at 38.5 °C in 5% CO_2, 5% O_2, and 90% N_2 with maximum humidity. After 4 h treatment, embryos were washed and then cultured in culture medium.

Embryo evaluation

Cleavage rates were evaluated on day 2. Blastocyst rates were recorded after 8 days of culture. To determine total cell numbers, day 8 blastocysts were fixed and mounted on a glass microscopic slide in glycerol containing 20 µg/ml Hoechst 33342 fluorochrome. After staining for 24 h, embryos were observed under a Diaphot 200 inverted microscope with epifluorescence attachment and UV-2A filter (Nikon, Tokyo, Japan).

Experimental design

Experiment 1

Based on our previous experience in HHP tolerance of porcine IVM oocytes, 20 and 40 MPa pressure lasting for 60 min were applied in the first experiment, with recovery times of 10, 70, and 130 min between pressurization and vitrification. Two control groups were used, one untreated vitrification control and one direct activation control. At least three repeat experiments were performed for each group.

Experiment 2

Parameters resulting in the highest cleavage and blastocyst rate in experiment 1 were applied in experiment 2. HHP was performed at either 37 or 25 °C to investigate possible influence of treatment temperature. Three control groups were set up. Oocytes loaded in straws without HHP treatment were kept either at 37 or 25 °C and used as vitrification control (37 °C vitrification group, 25 °C vitrification group). Oocytes with neither HHP nor vitrification were parthenogenetically activated directly as activation controls. The experiments were performed with three replicates.

Statistical analysis

Data were analyzed by a generalized linear model of SAS 9.1 (SAS Institute Inc., Cary, NC, USA). A probability of $P<0.05$ was considered to be statistically significant.

Acknowledgements

The authors thank Ruth Kristensen and Janne Adamsen for their excellent technical assistance, and Yali Hou for the kind help in data analysis. The work was supported by grants from the Danish Research Council System ('Comparative Genomics Focusing on Man and Pig', 'Production of genetically modified pigs as human disease models', and 'Metabolic Programming in Fetal Life') and the University of Aarhus. The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

References

Aman RR & Parks JE 1994 Effects of cooling and rewarming on the meiotic spindle and chromosomes of *in vitro*-matured bovine oocytes. *Biology of Reproduction* **50** 103–110.

Csermely P, Schnaider T, Soti C, Prohaszka Z & Nardai G 1998 The 90-kDa molecular chaperone family: structure, function, and clinical applications. A comprehensive review. *Pharmacology and Therapeutics* **79** 129–168.

Dinnyes A, Dai Y, Jiang S & Yang X 2000 High developmental rates of vitrified bovine oocytes following parthenogenetic activation, *in vitro* fertilization, and somatic cell nuclear transfer. *Biology of Reproduction* **63** 513–518.

Domitrovic T, Fernandes CM, Boy-Marcotte E & Kurtenbach E 2006 High hydrostatic pressure activates gene expression through Msn2/4 stress transcription factors which are involved in the acquired tolerance by mild pressure precondition in *Saccharomyces cerevisiae*. *FEBS Letters* **580** 6033–6038.

Du Y, Kragh PM, Zhang X, Purup S, Yang H, Bolund L & Vajta G 2005 High overall *in vitro* efficiency of porcine handmade cloning (HMC) combining partial zona digestion and oocyte trisection with sequential culture. *Cloning and Stem Cells* **7** 199–205.

Du Y, Zhang Y, Li J, Kragh PM, Kuwayama M, Ieda S, Zhang X, Schmidt M, Bogh IB, Purup S, *et al.* 2007 Simplified cryopreservation of porcine cloned blastocysts. *Cryobiology* **54** 181–187.

Esaki R, Ueda H, Kurome M, Hirakawa K, Tomii R, Yoshioka H, Ushijima H, Kuwayama M & Nagashima H 2004 Cryopreservation of porcine embryos derived from *in vitro*-matured oocytes. *Biology of Reproduction* **71** 432–437.

Esfandiari N, Falcone T, Goldberg JM, Agarwal A & Sharma RK 2007 Heat-shock proteins modulate the incidence of apoptosis and oxidative stress in preimplantation mouse embryos. *Fertility and Sterility* **87** 1214–1217.

Falcone T, Attaran M, Bedaiwy MA & Goldberg JM 2004 Ovarian function preservation in the cancer patient. *Fertility and Sterility* **81** 243–257.

Fujihira T, Kishida R & Fukui Y 2004 Developmental capacity of vitrified immature porcine oocytes following ICSI: effects of cytochalasin B and cryoprotectants. *Cryobiology* **49** 286–290.

Kaarniranta K, Elo M, Sironen R, Lammi MJ, Goldring MB, Eriksson JE, Sistonen L & Helminen HJ 1998 Hsp70 accumulation in chondrocytic cells exposed to high continuous hydrostatic pressure coincides with mRNA stabilization rather than transcriptional activation. *PNAS* **95** 2319–2324.

Knorr D 1993 Effects of high-hydrostatic pressure processes on food safety and quality. *Food Technology* **47** 5.

Kuo YH, Pribenszky C & Huang SY 2007 Higher litter size is achieved by the insemination of high hydrostatic pressure-treated frozen–thawed boar semen. *Proceedings of the 6th International Conference on Boar Semen Preservation*, Alliston, Ontario, Canada [in press].

Kuwayama M, Vajta G, Kato O & Leibo SP 2005 Highly efficient vitrification method for cryopreservation of human oocytes. *Reproductive BioMedicine Online* **11** 300–308.

Lomber SG, Payne BR & Horel JA 1999 The cryoloop: an adaptable reversible cooling deactivation method for behavioral or electrophysiological assessment of neural function. *Journal of Neuroscience Methods* **86** 179–194.

Men H, Agca Y, Riley LK & Critser JK 2006 Improved survival of vitrified porcine embryos after partial delipation through chemically stimulated lipolysis and inhibition of apoptosis. *Theriogenology* **66** 2008–2016.

Miura T, Minegishi H, Usami R & Abe F 2006 Systematic analysis of HSP gene expression and effects on cell growth and survival at high hydrostatic pressure in *Saccharomyces cerevisiae*. *Extremophiles* **10** 279–284.

Nagashima H, Kashiwazaki N, Ashman RJ, Grupen CG, Seamark RF & Nottle MB 1994 Removal of cytoplasmic lipid enhances the tolerance of porcine embryos to chilling. *Biology of Reproduction* **51** 618–622.

Pickering SJ, Braude PR, Johnson MH, Cant A & Currie J 1990 Transient cooling to room temperature can cause irreversible disruption of the meiotic spindle in the human oocyte. *Fertility and Sterility* **54** 102–108.

Porcu E & Venturoli S 2006 Progress with oocyte cryopreservation. *Current Opinion in Obstetrics and Gynecology* **18** 273–279.

Pribenszky C, Molnar M, Cseh S & Solti L 2005*a* Improving post-thaw survival of cryopreserved mouse blastocysts by hydrostatic pressure challenge. *Animal Reproduction Science* **87** 143–150.

Pribenszky C, Molnar M, Ulrich P & Keiko L 2005*b* Improving the post thaw survival of cryopreserved IVP bovine blastocysts by hydrostatic pressure challenge. *Reproduction in Domestic Animals* **40** 338.

Pribenszky C, Molnar M, Horvath A, Harnos A & Szenci O 2006 109 Hydrostatic pressure induced increase in post-thaw motility of frozen boar spermatozoa. *Reproduction, Fertility, and Development* **18** 162–163.

Shi WQ, Zhu SE, Zhang D, Wang WH, Tang GL, Hou YP & Tian SJ 2006 Improved development by Taxol pretreatment after vitrification of *in vitro* matured porcine oocytes. *Reproduction* **131** 795–804.

Vajta G 2000 Vitrification of the oocytes and embryos of domestic animals. *Animal Reproduction Science* **60-61** 357–364.

Vajta G & Kuwayama M 2006 Improving cryopreservation systems. *Theriogenology* **65** 236–244.

Vajta G & Nagy ZP 2006 Are programmable freezers still needed in the embryo laboratory? Review on vitrification *Reproductive BioMedicine Online* **12** 779–796.

Vajta G, Holm P, Greve T & Callesen H 1997 The submarine incubation system, a new tool for *in vitro* embryo culture. *Theriogenology* **48** 6.

Vajta G, Holm P, Kuwayama M, Booth PJ, Jacobsen H, Greve T & Callesen H 1998 Open pulled straw (OPS) vitrification: a new way to reduce cryoinjuries of bovine ova and embryos. *Molecular Reproduction and Development* **51** 53–58.

Vincent C & Johnson MH 1992 Cooling, cryoprotectants, and the cytoskeleton of the mammalian oocyte. *Oxford Reviews of Reproductive Biology* **14** 73–100.

Welch TJ, Farewell A, Neidhardt FC & Bartlett DH 1993 Stress response of *Escherichia coli* to elevated hydrostatic pressure. *Journal of Bacteriology* **175** 7170–7177.

Woods EJ, Benson JD, Agca Y & Critser JK 2004 Fundamental cryobiology of reproductive cells and tissues. *Cryobiology* **48** 146–156.

Yoshioka K, Suzuki C, Tanaka A, Anas IM & Iwamura S 2002 Birth of piglets derived from porcine zygotes cultured in a chemically defined medium. *Biology of Reproduction* **66** 112–119.

Zeron Y, Sklan D & Arav A 2002*a* Effect of polyunsaturated fatty acid supplementation on biophysical parameters and chilling sensitivity of ewe oocytes. *Molecular Reproduction and Development* **61** 271–278.

Zeron Y, Tomczak M, Crowe J & Arav A 2002*b* The effect of liposomes on thermotropic membrane phase transitions of bovine spermatozoa and oocytes: implications for reducing chilling sensitivity. *Cryobiology* **45** 143–152.

Received 3 August 2007
First decision 7 September 2007
Accepted 3 October 2007