Short-term ischaemic storage of human uterine myometrium—basic studies towards uterine transplantation

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Introduction

The advances in assisted reproductive techniques during the past decades have made it possible to circumvent many causes of male and female infertility. However, uterine infertility is still largely untreatable. Minor uterine malformations can be corrected by surgery (Donnez and Nisolle, 1997; Deffarges et al., 2001) but absence of the uterus as in Rokitansky syndrome (Van Waart and Kruger, 2000; LeRoy, 2001) or after hysterectomy is untreatable. The option for a woman with absolute uterine infertility to become a mother today is either through adoption, where she will be the social mother, or through gestational surrogacy, where she will be both the genetic and social mother of the child. As we have discussed previously (Brännström et al., 2003), a method for transplantation of the uterus could offer a means for these women to become both social and genetic as well as gestational mothers. However, the concept of uterine transplantation poses a number of medical questions and it is important to point out that before any clinical trials should be performed, several aspects of the uterine transplantation method and the functionality of the transplanted organ must be assessed. Some of the issues that need to be addressed are the development and evaluation of surgical techniques for vascularization of the transplanted uterus, the effect of cold ischaemia and reperfusion on the transplant and the effects of immunosuppression on mother and child. For this purpose, we have developed a method of uterine transplantation in the mouse (Racho El-Akouri et al., 2002), and in a syngeneic model achieved pregnancies that produced normal offspring (Racho El-Akouri et al., 2003a). This model was also used to test the effect of prolonged cold ischaemic storage on the function of the murine uterus (Racho El-Akouri et al., 2003b). In that study, it was shown that 24 h of cold storage in a protective storage solution before transplantation did not impair the ability of the uterus to implant transferred embryos and to generate normal offspring.

Cold storage with a protective buffer is today the most common method used to preserve an organ intended for transplantation during the ischaemic time between procurement and reperfusion within the recipient. Lowering of tissue metabolism by hypothermia together with the protective properties of different preservation solutions slow down the development of ischaemic injuries, but the tolerable time window is still restricted. Extended cold ischaemia induces injuries that manifest themselves mainly at reperfusion since the treatment...
triggers an upregulation of pro-inflammatory factors (Dragun et al., 2001) and endogenous production of reactive oxygen (ROS) and nitrogen species (RNS) that can induce vascular and parenchymal injuries (Inauen et al., 1989; Parolari et al., 2002). There is a wide range in time of ischaemic tolerance between organs, which may reflect the differences in vascularization and parenchymal cell function. For example, the clinically practised time limit for cold storage ranges from 6 h for hearts up to 36 h for kidneys (Muhlbacher et al., 1999; Huddleston and Mendeloff, 2000).

A number of preservation solutions exist and they are all designed to minimize loss of cellular homeostasis, provide antioxidant defence at reperfusion and enable quick recovery from the ischaemic depletion of energy stores. These preservation solutions can be divided into two different categories based on whether their electrolyte (i.e. sodium and potassium) composition is similar to the intracellular or extracellular milieu. Intracellular-like preservation solutions were initially developed for cold storage of kidneys, based on the assumption that intra- and extracellular ion concentrations eventually equilibrate and that the lack of an ionic gradient would prevent cell swelling and preserve high-energy phosphates. The intracellular-like preservation solution Belzer University of Wisconsin (UW) provides good protection of solid abdominal organs (Roels et al., 1998; de Boer et al., 1999; Faenza et al., 2001) and is today the gold standard in kidney storage. Extracellular-like solutions are used especially for storage of thoracic transplants. Theoretically, an extracellular composition would maintain the reversed gradient of sodium and potassium induced by hypothermia and thus protect against cell swelling but will at reperfusion require large amounts of high-energy phosphates to reinstall a normal gradient. Despite this, intracellular solutions have been reported to be more efficient than intracellular ones in cold storage of hearts and lungs (Struber et al., 2001; Warnecke et al., 2002). Perfadex® (PER) is an extracellular-like preservation solution designed mainly for the storage of lung transplants (Rabanal et al., 2003; Steen et al., 1993), but also has proven efficiency in preserving contractility of rat vascular smooth muscle (Ingemanson et al., 1995) and porcine pancreatic function (Montgomery et al., 1993) after extended cold ischaemia.

The aim of the current study was to evaluate the time dependence of cold ischaemia-induced changes in human uterine tissue and to compare the effect of different preservation solutions. This was done by use of parameters indicating cold ischaemic injuries such as loss of cellular integrity, decline in high-energy phosphate pool and impaired antioxidant defence. Thus, the ability to contract spontaneously and in response to prostaglandin F2α (PGF2α) stimulation, histology by light and electron microscopy, and concentrations of glutathione, ATP and protein were analysed.

Materials and methods

Uterine tissue

Tissue samples were taken from uteri from pre-menopausal patients (n = 7, 38–50 years) hysterectomized due to benign diagnosis (three myomas, four bleeding disorders). The patients gave their informed consent prior to surgery and the study was approved by the local ethics committee.

Study design

Endometrial and myometrial tissue specimens were kept at 4°C for 6 or 24 h in Ringer acetate solution (RIN; Baxter, Deerfield, IL), UW solution (ViaSpan, DuPont Chemicals Ltd, Wilmington, DE) or PER solution (kindly provided by Vitrolife, Kungsbacka, Sweden). The specimens were assessed for changes in five parameters: (i) ability of muscle strips from the outer muscle layer of the fundus to generate spontaneous isometric contractions and respond to stimulation by PGF2α; (ii) morphology of myometrium and endometrium by light and electron microscopy; (iii) total glutathione (GSHtot) and oxidized glutathione (GSSG) in the myometrium; (iv) concentration of ATP in the myometrium; and (v) protein concentration in the myometrium.

Preservation solutions

The UW and PER solutions were chosen for comparison of the effect of intracellular- and extracellular-like preservation solutions. The specific compositions of each of the solutions are listed in Table I. Apart from the differences in sodium and potassium concentrations, they contain different colloids [UW, lactobionate, raffinose and hydroxyethyl starch (HES); PER, dextran 40]. UW also contains glutathione and allopurinol as antioxidants as well as adenosine as metabolic aid, while PER contains glucose as metabolic aid and no additional antioxidants. RIN was used as a control solution. RIN and UW solution were used without additives. PER was adjusted to pH 7.4 with Tris (1.0 mol/l) according to the manufacturer’s instructions.

Sample preparation and storage

Immediately after the uterus had been removed during surgery, it was brought to the laboratory and catheterized through the uterine arteries on both sides of the corpus. The vascular system of the uterus was flushed with sterile RIN at room temperature until the out-flowing fluid from the veins was clear. The uterus was then opened with a scalpel cut sagittally through the uterine wall, into the cavity. Tissue samples, each ∼5 × 10 × 30 mm, were cut from the outer and middle muscle layer of the fundus as well as from the tissue adjacent to and including the endometrium and then put in chilled RIN awaiting further divisions into smaller slices (1–2 mm thick). These slices were

### Table I. Compositions of storage solutions

<table>
<thead>
<tr>
<th>Component</th>
<th>RIN</th>
<th>UW</th>
<th>PER</th>
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<tbody>
<tr>
<td>K⁺</td>
<td>4</td>
<td>140</td>
<td>6</td>
</tr>
<tr>
<td>Na⁺</td>
<td>130</td>
<td>20</td>
<td>138</td>
</tr>
<tr>
<td>Mg²⁺</td>
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<td>5</td>
<td>0.8</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>110</td>
<td>142</td>
<td>4</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ac</td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H₂PO₄⁻ + HPO₄²⁻</td>
<td></td>
<td>25</td>
<td>0.8</td>
</tr>
<tr>
<td>HES</td>
<td>50</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Dextran 40</td>
<td></td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>100</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Raffinose</td>
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</tr>
<tr>
<td>Lactobionate</td>
<td></td>
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</tr>
<tr>
<td>Allopurinol</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Glutathione</td>
<td>3</td>
<td></td>
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</tr>
<tr>
<td>Osmolarity</td>
<td>270</td>
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<tr>
<td>pH</td>
<td>5.5</td>
<td>7.4</td>
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</table>

RIN – Ringer Acetate, UW – University of Wisconsin preservation solution, PER – Perfadex, HES – Hydroxy ethyl starch
then distributed to different 40 ml polythene containers for storage in RIN, UW or PER (20 ml), or fixed for analysis immediately after the preparation. The warm (22°C) ischaemic time during transportation, flushing and dissection was on average 40 min for all specimens. The cold ischaemic time that included further dissection and preparation of the specimen for contractility measurements was on average 100 min. The procedure is illustrated in Figure 1.

**Contractility**

The ability of myometrial strips from the perimetrium of the fundus to generate spontaneous contractions and respond to added PGF2α was examined on (i) control samples (two strips per patient); (ii) samples preserved at 4°C for 6 h in either RIN, UW or PER (one strip per treatment and patient); and (iii) samples preserved at 4°C for 24 h in RIN, UW or PER (one strip per treatment and patient). Tissue strips of ~8 mm length and 1–2 mm² cross-sectional area were dissected from the samples taken from the perimetrium. The dissection was performed in chilled HEPES buffer (122 mmol/l NaCl, 4.7 mmol/l KCl, 1.9 mmol/l KH₂PO₄, 1.19 mmol/l MgCl₂, 5.0 mmol/l HEPES, 2.5 mmol/l CaCl₂, pH 7.37) under a stereomicroscope, using micro-forceps and a scalpel. Care was taken that the main muscle fibre direction was parallel to the long axis of the preparation. Two thin (6-0) silk ligatures were tied around the ends of each specimen, ~5 mm apart, and the preparation was mounted in an organ chamber between a metal hook and a force transducer, as previously described (Ekerhovd et al., 1997). The organ chamber contained 10 ml of HEPES buffer with 11.5 mmol/l D-glucose maintained at 37°C and was continuously gassed with 100% oxygen. A passive tension force of 5 mN was applied and the preparation was allowed to accommodate until spontaneous contractions were stable or for 120 min. Subsequent doses of PGF₂α (final concentration: 1, 10 and 100 ng/ml, PGF₂α trissalt; Sigma Chemicals, St Louis, MO) were added at intervals of 40 min with intermediate changes of buffer. Isometric contractions were recorded for 10 min before the first addition of PGF₂α and 10 min after addition of each PGF₂α concentration, on a PC through a Grass polygraph (model 7D; Grass-Telefactor, West Warwick, RI) using LabVIEW® software (National Instruments, Austin, TX).

Contractions were analysed in terms of start of spontaneous contractions (minutes after set-up), the quality of the shape and rhythm of the curve as well as the area under the curve (AUC) for the 10 min measured, both before and after stimulation. The response to administered PGF₂α was expressed as the log-ratio of the AUC after dose (AUC) dose and AUC for the spontaneous contractions (AUC) spont of the same specimen.

**Histology**

Samples from the myometrium and endometrium were fixed—at baseline time as described above or after storage at 4°C for 6 or 24 h in RIN, UW and PER—in 4% formaldehyde in cacodylate buffer pH 7.4 (Kidney biopsy solution, Bie & Berntsen A-S, Rodovre, Denmark) for 24 h. The samples were then dehydrated and embedded in paraffin. Three non-consecutive sections (3–6 µm thick) of tissue from each preparation were cut in a microtome and mounted on glass slides, stained with haematoxylin and eosin and evaluated by light microscopy. For electron microscopy, endometrial and myometrial samples were fixed in 3% glutaraldehyde in 0.1 mol/l sodium cacodylate buffer, postfixed in 1% osmium tetroxide, dehydrated and embedded in epoxy resin. Three non-consecutive ultrathin sections contrasted with uranyl acetate and lead citrate were studied in a Philips 400 electron microscope.

**Glutathione assay**

Determination of total and reduced glutathione in myometrial samples was done by the use of a modified method developed by Vandeputte.
et al. (1994). The method utilizes the monomerization of 5,5-dithiobis (2-nitrobenzoic acid) (DTNB) by reduced glutathione (GSH) in the presence of NADPH. The resulting monomer has a peak absorption at 412 nm. Samples from myometrium, preserved as described above and frozen at −70°C, were ground with a pestle in a mortar on dry ice. The resulting frozen powder was thawed by addition of 5.0 mol/l phosphate buffer containing 1.5 mmol/l EDTA and 0.25 mmol/l CHAPS (3-(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate), acidified with concentrated HCl to pH 5.2. The samples were sonicated on ice three times for 15 s to lyse the cells and centrifuged for 5 min at 10 000 g to remove cell debris. A portion of the supernatant was collected for protein measurement before sulphosalicylic acid (SSA; 6.5% in dH2O) was added to the rest of the supernatant to precipitate proteins. The samples were left on ice for 10 min and then centrifuged for 15 min, 10 000 g at 4°C. The resulting supernatant was collected in two vials for analysis of GSHtot (reduced and oxidized glutathione) and GSSG (oxidized glutathione).

For determination of GSHtot, 60 µl of standard [0.250–8.0 µmol/l GSH (Roche Diagnostics, Mannheim, Germany) in 10 mmol/l HCl + 1% SSA] or diluted sample (1:10 in 10 mmol/l HCl + 1% SSA) and 160 µl of assay buffer (0.41 mmol/l NADPH and 1.2 mmol/l DTNB in phosphate buffer with 1.5 mmol/l EDTA) was pipetted in a well of a microtitre plate. The plate was left for 5 min in room temperature, glutathione reductase (GR; 25 µl, 20 IU/ml, Sigma Aldrich, St Louis, MO) was added to each well and the plate was read kinetically in a spectrophotometer (UV-max spectrophotometer, Molecular Device, Sunnyvale, CA) at 405 nm for 2 min with one reading every 10 s. All samples were analysed in duplicate and the concentration of GSHtot was expressed as nmol equivalent GSH/mg protein.

For determination of GSSG, 2-vinyl pyridine (5 µl/100 µl of sample) was added to each sample to bind all free sulphydryl groups (i.e. all reduced GSH), mixed thoroughly and shaken for 30 min before analysis. The samples were assayed as described above and the results were expressed as nmol equivalent GSH/mg protein. The ratio of oxidized and reduced glutathione ([GSSG]/[GSH]) was also calculated.

**ATP assay**

Determination of ATP concentration was performed by the use of the luciferin–luciferase enzyme luminescence method described by Lyman and De Vincenzo (1967). Frozen samples from myometrium were ground with a pestle in a mortar on dry ice and metabolites were extracted with trichloroacetic acid (TCA). Approximately 1.0 ml of 0.1 mol/l TCA was added per 100 mg of frozen and ground tissue. The samples were homogenized and centrifuged at 10 000 g for 15 min at 4°C and the supernatant was collected for ATP analysis. The pellet was later used for measurement of protein content (see below). The supernatant portion of the sample was then mixed six times with diethyl ether to remove TCA and then left under a stream of air until all diethyl ether had evaporated. The ATP measurements were done in a luminometer (GENios, Tcecn, Maennedorf, Switzerland, maximum dynamic range integration time, gain 150), using an ATP-kit (CLS II, Roche Diagnostics, Mannheim, Germany) according to the manufacturer’s instructions. A 50 µl aliquot of ATP standard and samples was pipetted into the wells of a white 96-well microtitre plate with an opaque bottom (NUNC, Weisbaden, Germany). The reaction solution (50 µl) containing luciferin and luciferase was then added to each well and the plate was read immediately. Both standard and samples were analysed in triplicate. The results were expressed as nmol/mg protein.

**Protein concentrations**

The protein concentrations in the supernatant from the glutathione assay as well as total protein content of tissue specimens used for ATP analysis were measured by the use of a BCA kit (Pierce, Rockford, IL) according to the manufacturer’s instructions. For measurement of total protein content of the samples from the TCA extraction of ATP described above, the pellet was dissolved in 0.1 mmol/l NaOH (~40 times the volume of the pellet), centrifuged at 10 000 g for 10 min and the supernatant was used for analysis. The protein concentration in the pellet was expressed in µg/mg tissue wet weight and in mg/ml in the supernatant from the glutathione analysis.

**Statistics**

Concerning contractility, the difference between log(AUC) dose and log(AUC) spont was related to log dose using an orthogonal linear regression within each specimen. The difference in dose–response between groups was evaluated using Wilcoxon test of the slope and intercept parameters from the regression models. The line corresponding to the median slope and the intercept was used to characterize the estimated dose–response for each group graphically.

GSHtot and [GSSG]/[GSH] as well as [ATP] and [protein] of the different cold ischaemic groups were compared by the use of Kruskal–Wallis analysis of variance (ANOVA) by ranks test and, where significant differences were found, multiple comparison between groups was done by Wilcoxon matched pairs test. A P-value of <0.05 was considered significant.

**Results**

**Contractility**

Cold storage for 6 or 24 h in any of the solutions decreased the ability of the myometrial specimens to start contractions spontaneously in comparison with control. However, all specimens stored in UW and PER for 6 h contracted either spontaneously or after stimulation with a low (1 ng/ml) concentration of PGF2α in contrast to those stored in RIN where only one specimen produced contractions. Specimens stored in UW for 6 h produced curves and responded to PGF2α in a similar manner to controls, while after 24 h storage the quality of the contractions and responses were clearly altered (Figure 2). The PER–preserved specimens showed a contraction pattern that was less regular and rhythmic compared with control and specimens stored in UW for 6 and 24 h (Figure 2). The response to different concentrations of PGF2α was also analysed by comparison of the slope and intercept of an orthogonal regression fitted to the log-ratio of the AUC after and before dose. The analysis showed no significant differences in slope of the orthogonal regression between controls and the different experimental groups, while storage in PER for 24 h, but none of the other groups, gave rise to a significant difference for the intercept of the orthogonal regression (Figure 3).

**Histology**

On evaluation by light microscopy, no apparent histological changes were seen in the myometrium or the endometrium after cold ischaemic preservation in any of the solutions for 6 or 24 h (Figure 4). Using electron microscopy, degenerative changes such as hydropic degeneration and slight nuclear changes (coarse chromatin) were observed in tissue stored for
protein for control specimens. The \([\text{GSH}_{\text{tot}}]\) was significantly higher in specimens preserved in UW for 6 and 24 h compared with control as well as RIN and PER (Figure 6). \([\text{GSH}_{\text{tot}}]\) in PER- or RIN-preserved myometrium did not differ from control. The concentration of GSSG ranged from 0.36 to 1.48 nmol equivalent GSH/mg protein in control specimens (results not shown). Comparison of the ratio of oxidized and reduced glutathione \((\text{GSSG}/\text{[GSH]})\) showed that specimens preserved in UW for 6 or 24 h contained a significantly larger proportion of GSSG compared with control as well as with specimens preserved in PER for 6 h (Figure 6).

**ATP**

Concentrations of ATP in tissue homogenates from myometrium showed a large variation, with values ranging from 0.35 to 11.29 nmol/mg protein for the control samples. ATP levels were significantly higher after both 6 and 24 h cold storage in UW and PER compared with the control samples. RIN-preserved specimens did not differ from controls even though a slight decrease in median level was seen after 24 h storage. A significantly higher concentration of ATP was found in specimens preserved in UW for 24 h compared with those preserved in PER for 24 h (Figure 6).

**Protein**

Protein concentration in myometrium ranged from 32.0 to 53.4 \(\mu\)g/mg tissue wet weight for control specimens. No significant difference was seen between groups (Figure 6).

**Discussion**

Women with absolute uterine infertility who wish to become genetic mothers instead of adopting a child can in some countries utilize IVF surrogacy as a means to build a family. Even though ethically complex (Shuster, 1992; Mann, 1998; Brinsden, 2001), this is an accepted method in some countries (Beski et al., 2000; van den Akker, 2000; Soderstrom-Anttila et al., 2002), but in many nations and religious communities IVF surrogacy

![Figure 2. Contraction curves from myometrial strips after preservation for 6 or 24 h in different preservation solutions (RIN, Ringer acetate; UW, University of Wisconsin preservation solution; PER, Perfadex). Representative example of spontaneous and stimulated contractions of myometrial strips from one patient. The arrow designates addition of PGF\(_{2\alpha}\) (10 ng/ml); the vertical scale bar represents 5 mN; the horizontal scale bar represents 10 min.](https://academic.oup.com/humrep/article-abstract/20/10/2736/603260/0)

![Figure 3. Lines corresponding to median parameters of individual orthogonal linear regression models of the log-ratio of area under the curve (AUC) for 10 min duration after PGF\(_{2\alpha}\) addition and AUC for 10 min of spontaneous contractions in myometrial strips from fresh samples (control) and those preserved in Ringer acetate (RIN), University of Wisconsin solution (UW) or Perfadex (PER) for 6 (left panel) or 24 h. No significant difference was found concerning the slope of the regression by the Wilcoxon test. The intercept for PER 24 h differed significantly from control \((P < 0.05)\) as indicated by *.](https://academic.oup.com/humrep/article-abstract/20/10/2736/603260/0)
is illegal or considered unethical (Cohen and Jones, 2001; Serour and Dickens, 2001). In these communities, uterine transplantation could offer an alternative to adoption for women who lack a functioning uterus and wish to carry their own pregnancy. The concept of uterine transplantation as infertility treatment is not new, and during the 1960s and 1970s several studies in different animal models were performed (Eraslan et al., 1966; Bland, 1970; Scott et al., 1971). These studies mainly concerned the surgical technique for revascularization of the uterus, and the lack of efficient immunosuppression and procedures for IVF at that time probably contributed to the fact that these studies did not continue towards clinical application in the human. With the development of IVF as well as efficient immunosuppressive regimens over the past decades, the idea of transplantation of the uterus has been actualized (Ortega-Moreno and Caballero-Gomez, 1992; Lee et al., 1995; del Priore et al., 2001; Motoc et al., 2003), and in 2000 the first attempt to transplant a human uterus was performed (Fageeh et al., 2002). The 26-year-old recipient had lost her uterus due to post-partum haemorrhage and the donor was a 46-year-old woman who underwent hysterectomy due to ovarian cysts. Standard immunosuppression was used to prevent rejection but the uterus had to be removed after 3 months, due to necrosis of the organ. The authors considered this to be caused by thrombosis of the uterine arteries due to inadequate support of the uterus and torsion of the anastomosed vessels, rather than by rejection. In our view, this first human uterine transplantation was not backed up by enough animal studies concerned with crucial aspects of the transplantation procedure to warrant a continuation of human trials at this stage. Thus, we think that it is essential to investigate and

Figure 4. Light microscopy. Representative examples of control specimens (a) and specimens preserved for 24 h in Ringer acetate (RIN) (b), University of Wisconsin solution (UW) (c) and Perfadex (PER) (d). The upper part of each figure shows endometrial glands and stroma, and the lower part shows the muscular layer. There are no light microscopic differences between the specimens. All samples were taken from the same uterus. The scale bar represents 36 µm.

Figure 5. Electron microscopy showing glandular epithelial cells of the uterine endometrium. (a) Control tissue showing normal cells. The organelles are well preserved and the nuclei show a normal chromatin pattern. (b) Tissue stored in RIN for 24 h. The glandular epithelial cells display a general hydropic degeneration in the cytoplasm and the nuclear chromatin is coarse. Tissue stored (c) in UW solution for 24 h and (d) PER for 24 h. There are no major morphological changes compared with the control sample (a). S = stromal compartment. Magnification 5500×.
optimize the procedures of the surgical techniques, types and doses of immunosuppression as well as tolerability and effects of cold ischaemia and reperfusion in studies involving experimental animals and in vitro studies of human tissue before further clinical attempts should be performed.

In the present study, human uterine tissue was subjected to cold ischaemia during different times using different storage solutions. Both hypoxia and hypothermia contribute to the large number of consecutive and intertwined changes causing cold ischaemia/reperfusion injuries. Ischaemia causes a rapid decline in ATP concentrations resulting in an inability of cells to maintain homeostasis. Hypothermia delays the deprivation of ATP but also contributes to the loss of cellular homeostasis due to changes in membrane integrity and cytoskeletal stability (Stefanovich et al., 1995; Breton and Brown, 1998). Moreover, during oxygen deprivation, ATP is degraded and converted to hypoxanthine, which at reperfusion and the re-entry of oxygen can react with xanthine oxidase to generate ROS. If the antioxidant capacity of the cell is insufficient, the oxidative stress at reperfusion will induce several mechanisms that will lead to apoptotic cell death (McCord et al., 1985).

In the present study, the ability to generate spontaneous contractions and respond to PGF$_{2\alpha}$ stimulation were chosen as parameters to measure cold ischaemia-induced injury, since contraction is a main function of the myometrium and changes in this ability would indicate an important functional change. The experimental set-up used has been thoroughly tested in experiments involving human myometrium (Wiqvist et al., 1983), oviductal tissue (Ekerhovd et al., 1997) and cervical tissue (Ekerhovd et al., 1998). In this in vitro system, there is a high supply of oxygen, with pH and temperature being at physiologically relevant levels. Thus, this situation of transition of the specimen from cold ischaemia to the in vitro system resembles in some aspects the situation of reperfusion after cold storage. PGF$_{2\alpha}$ was chosen as a stimulator of contractility, since the contractile response of human myometrium to this specific prostaglandin is independent of hormonal status (Wiqvist et al., 1983). In the present study, the ability to generate spontaneous contractions after cold ischaemic storage seemed to improve after 24 h storage, but a qualitative analysis of the rhythmical and shape of contraction curves revealed a clearly disturbed pattern after 24 h storage compared with control and after 6 h storage (Figure 2). These changes were worse in RIN-preserved specimens compared with the others. A likely explanation for this is that RIN, in contrast to UW and PER, lacks factors that favour cellular homeostasis such as physiological pH and osmolarity, as well as antioxidants and metabolic precursors. The regularity of contractions was better preserved by UW than PER, and analysis of the AUC shows a significant difference in the intercept of the orthogonal regression.
of the log-ratio of AUC for PER after 24 h storage, reflecting a larger difference between spontaneous and \(\text{PGF}_2\alpha\)-stimulated contractions, as compared with control. These differences between UW- and PER-preserved myometrium could possibly be ascribed to the different ionic composition of the solutions, since intra- and extracellular ionic concentrations are important components in the excitation of muscle cells. In contrast, the dose dependence of the \(\text{PGF}_2\alpha\) response, represented by the slope of the orthogonal regression of the log-ratio of AUC, did not differ significantly between the storage solutions. In comparison with the results of our previous study of uterine contractions after cold ischaemia of the murine uterus (Raicho El-Akouri et al., 2003b), the results of the present study indicate that the human myometrium is less resistant to cold ischaemia than the mouse uterus. It should be noted though that even though the murine specimens were approximately of the same size as the human myometrial strips used in the present study, the previous study on the mouse (Raicho El-Akouri et al., 2003b) included the whole top portion of the uterus with both circular and longitudinal muscle layers. This might have an impact on the regularity of contractions in this \textit{in vitro} setting, and the difference may show up in the human myometrial specimens.

Cell death caused by cold ischaemia alone occurs mainly by necrosis (Shah et al., 1997; Salahudeen et al., 2001), and the first visible signs of damage at the light microscopic level is seen as loss of intercellular contacts, hydropic changes, irregular condensation of chromatin and cell swelling (Momii and Koga, 1990; Eberl et al., 1999; Uhlmann et al., 2002). In the present study though, no major histological changes could be seen by light microscopy after 6 and 24 h of cold storage in any of the solutions. However, by electron microscopy, it was obvious that after 24 h storage the RIN-preserved specimens had degenerative changes that were not present in tissue stored in UW or PER. This observation is consistent with the results from the contractility tests, where RIN-preserved specimens showed a lack of spontaneous contractility and little or no response to \(\text{PGF}_2\alpha\) and it also correlates with the results from the previous study on the murine uterus where disturbances in contractile ability in \textit{vivo}, as well as severe non-functionality after transplantation preceded the occurrence of histological changes at the light microscopic level after cold ischaemia alone (Raicho El-Akouri et al., 2003b).

In the transplantation setting, the significance of glutathione as a ROS scavenger and the use of glutathione-related enzymes as a ROS scavenger and the use of glutathione-related enzymes as biochemical markers for cold ischaemia/reperfusion injury have been reported in several experimental transplantation models (Armeni et al., 2000; Santori et al., 2000). In the present study, the significantly higher concentration of total glutathione in UW-preserved myometrium was expected, since glutathione is a component of UW. UW-preserved myometrial specimens also contained a significantly greater proportion of GSSG than both control and samples preserved in PER for 6 h. The cellular uptake of reduced glutathione from the interstitial space has been reported to be limited (Meister, 1988), and it is likely that at least a portion of the excess of total GSH in the UW-preserved samples derives from the interstitial space and is oxidized in the course of time. However, about a third of the excess of total GSH in UW-preserved samples exists in its reduced form and might still be useful in protection against ROS at reperfusion.

The ATP levels were significantly higher after storage in UW and PER compared with control. This is contradictory to both theoretical assumptions and experimental findings where, for example, isolated myometrial strips lost 70% of the initial ATP concentration after <5 h of cold storage in HEPES buffer (Wedenberg et al., 1991, 1995). The rise of ATP levels in the present study can partly be explained by the fact that the control values constitute the common baseline after 40 min of warm ischaemia, followed by 100 min of cold ischaemia and that the rise in [ATP] is a recovery from a lower level than would be present immediately after disruption of circulation. This suggests that the myometrium, given the protective environment of UW or PER, can utilize glycolysis and/or the oxygen dissolved in the storage solution to maintain a higher production than consumption of ATP when the metabolic rate is suppressed by hypothermia.

It has been shown previously that extended cold storage leads to a reduction of protein synthesis and that the degree of reduction differs between organs (Fisher et al., 1993; Lindell et al., 1994; Vreugdenhil et al., 1999; Bull et al., 2000). In the present study, protein concentrations did not change with cold ischaemic storage time in any of the solutions used.

To confirm a safe time frame for cold storage of the whole human uterus, more elaborate studies concerning the patency of both vasculature and endometrium—after cold storage as well as after reperfusion—are needed. However, taken together, the results from the present study indicate that human myometrial tissue can tolerate cold ischaemia for at least 6 h if stored in a protective solution. This information is useful in further research studies on the issue of transplantation of the whole uterus after hysterectomy or congenital malformation, but can of course also be of use in a situation where transplantation of uterine myometrial tissue could be used to strengthen a structural defect after myomectomy.

References


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