Cryopreservation of intact human ovary with its vascular pedicle—or cryopreservation of hemiovaries?

Sir,
We read with interest the manuscript of Bedaiwy et al. (2006) entitled ‘Cryopreservation of intact human ovary with its vascular pedicle’, but feel there are some important details that the authors need to address for a clearer understanding of their work.

First, we do not agree with the title, which suggests that ‘intact human ovary has been cryopreserved with its vascular pedicle’. Indeed, in both the title and the manuscript, the authors defined the ovaries as intact ovaries for all the steps of the cryopreservation protocol. However, after perfusion with cryoprotective agent prior to freezing, the ovaries were bisected and therefore, from this moment on, they should be called hemiovaries. Hence, the first aim of their study was not achieved as they described a protocol that allows preservation of a hemiovary, not a whole ovary, and so the title should be ‘Cryopreservation of human hemiovaries’. The present title causes confusion and falsely implies that the ovary was cryopreserved with its vascular pedicle with a view to future transplantation by vascular anastomosis, which is simply not true! Moreover, in each case, one ovary was cryopreserved in the form of cortical strips and not as a whole ovary. We would also like to have more information on the methodology used to bisect the ovary, mainly regarding the vascular pedicle, as the ovary only has one ovarian artery.

Second, we would like to know the size of the two intact human ovaries that were used in the study. Indeed, even though they came from older patients (44 and 46 years of age), and were cut into two parts, it seems hardly likely that they could fit into cryovials of 1.27 cm diameter. It also means that if this protocol were used for younger patients, whose ovaries have a mean size of 2.5–3 x 2 x 2 cm (Motta and Balboni, 1994), the ovaries would have to be cut into more than two pieces. The cryovial used in Bedaiwy’s study had a volume capacity of 5 ml but, according to Kupesic et al. (2003), the mean human ovarian volume is 10 ml in women <30 years of age, 8.30 ml from 31 to 35 years and 6.85 ml from 36 to 40 years. Therefore, regardless of the age of patients, an intact human ovary could never fit into a 5-ml cryovial. Thus, cryopreservation of intact human ovaries cannot be performed with a standard programmable Planer freezer, as used by Bedaiwy et al. (2004, 2006), because cryovials with a diameter greater than 1.27 cm do not fit into its cryochamber.

Third, we do not understand why Bedaiwy used fetal calf serum (FCS) in the freezing and thawing medium as, for transplantation purposes, animal-free media must be used to avoid the risk of contamination.

Fourth, as mentioned by the authors, their work was presented at the ESHRE annual meeting in Berlin, 2004 (Bedaiwy et al., 2004). We, however, note discrepancies in the described protocols between the paper submitted to ESHRE and the present paper, although they apparently concern the same patients aged 44 and 46 y. Indeed, in the 44-year-old patient, one ovary was cryopreserved as an intact ovary without bisection and the other as cortical pieces, whereas in the 46-year-old, both ovaries were frozen in the form of cortical strips (Bedaiwy et al., 2004).

Fifth, the authors described a thawing protocol where the ovaries were perfused in order to remove the cryoprotectant, but how could adequate perfusion be performed if the vascular network, as well as the rest of the ovary, were cut into two parts? Indeed, areas of the hemiovary previously served by a vessel that has been bisected cannot be perfused, and thus the cryoprotectant cannot be removed by perfusion. In these areas, the only way of removing the cryoprotectant would be by diffusion from the deepest parts to the surface. However, 20 min seems insufficient to achieve complete diffusion through a thick tissue fragment like a hemiovary. Moreover, the composition of the thawing medium differed between the ESHRE abstract and the present paper.

Sixth, in the discussion, the authors compared their results on follicular viability (using 1.5 M DMSO) with those of Martinez-Madrid et al. (2004) (using 10% DMSO), stating that ‘this could probably mean that ovarian tissue could tolerate adequately varying concentrations of DMSO with comparable post-thaw survival’, as follicular viability in both studies was similar. We do not understand what they mean by varying concentrations, as 1.5 M DMSO is almost the same as 10% DMSO (1.4 M).

In effect, Bedaiwy’s study described a protocol for cryopreservation of hemiovaries and, moreover, in older patients. The clinical interest of this study is questionable since vascular anastomosis cannot be performed with a hemiovary, and thus the goal of avoiding ischemic damage after transplantation cannot be achieved. We were very surprised that these comments were not made before publication.

We would finally like to point out that cryopreservation of a whole ovary, using the vascular pedicle for perfusion of cryoprotectant and with a view to transplantation by vascular anastomosis, has been successfully carried out in nine patients in our group so far according to our previously described protocol (Martinez-Madrid et al., 2004; Donnez et al., 2006).
Letters to the editor

Reply: Cryopreservation of intact human ovary with its vascular pedicle—or cryopreservation of hemi ovaries

Sir,

We would like to thank Professor Donnez and associates for their interest in our manuscript entitled ‘Cryopreservation of intact human ovary with its vascular pedicle’. Regarding the questions they raised with respect to our work, our responses are as follows.

First, we need to re-emphasize that this work is purely experimental and the result of the observation that ischemia is responsible for most of the graft failures. This work is simply an evolution of our sheep data published previously. We first described the technique of cryopreserving a whole ovary with its vascular pedicle in the sheep animal model, and we demonstrated the potential feasibility as evidenced by vascular patency and hormonal function (Jeremias et al., 2002; Bedaiwy et al., 2003). More recently, Imhof et al. (2006) described pregnancy and delivery using an orthotopic approach of whole ovary vascular anastomosis (Imhof et al., 2006). Our present paper was intended to take it to the next experimental level with human ovaries (Bedaiwy et al., 2006). We did not intend to proceed to transplantation but simply to see if we can carry out the steps we reported.

As noted in our manuscript, the main and the most critical step in the entire process—the perfusion of the cryoprotectant—was performed via the ovarian vessels to ensure adequate distribution of the cryoprotectant via the intraovarian vascular network. Bisecting the ovary to fit in the largest available cryovial thereafter would not change the fact that the perfusion process was performed on an intact ovary and the entire ovary cryopreserved. Should a cryovial with an adequate size be available, the rest of the process could have been performed with the ovary intact as we did in our initial experiment in sheep ovaries (Bedaiwy et al., 2003). A specially designed cryovial and cryochamber to accommodate an entire ovary with its vascular pedicle is being developed by industry.

Second, both ovaries fit after bisection in the cryovial described in our study. We agree with their concern regarding the fact that fitting ovaries with larger sizes in this particular cryovial is challenging. In this limited series, we purposely chose older women whom we knew would have small ovaries. The data they quoted in their letter state that there is a progressive decline in ovarian volume from 10 ml in women less than 30 years of age to 6.8 ml in women from 36 to 40 years of age. Our two patients were 44 and 46 years of age and easily fit into the 5 ml cryovial. We agree that in clinical practice when we are dealing with young women we can never use the present cryovials. We simply chose ovaries that we knew were at the end of their reproductive life with small ovaries. As for the preparation of the ovarian cortical strips, we used the protocol described by Gosden and associates (Gosden et al., 1994).

Third, we used fetal calf serum for experimentation purposes only. Indeed animal free media would be the only way to proceed if autotransplantation is planned. This study was again just an experiment to assess feasibility.

Fourth, this paper was presented, in part, as an abstract in the ESHRE meeting of 2004. Only one patient from that abstract published in 2004 was included in this report and the other patient and all the controls in the current manuscript were from subsequent work. The patient in common between the abstract and the current manuscript is the 44 years old patient who underwent laparoscopic hysterectomy and bilateral salpingo-oophrectomy. The other patient from the 2004 ESHRE abstract, who underwent cryopreservation of both ovaries as ovarian cortical strips, was not included in the current manuscript because; (i) both ovaries were cryopreserved as ovarian cortical strips and we did not do a whole ovary freezing on her and (ii) we did not run some of the in vitro assessment strategies, namely, the CD 34, PAS and Masson’s trichome stains on the tissues obtained from this patient.

Fifth, as demonstrated in the elaborate in vitro experiments we did, adequate and comparable tissue survival was achieved in both study arms using the described protocol of thawing. Cannulation of the largest branch of the ovarian vessels in the section that did have the pedicle connected was performed to wash out the cryoprotectants. We also would like to emphasize that we did not use sucrose and PBS as a thawing medium in this study and it was quoted in the 2004 ESHRE abstract by mistake. We used only leibovitz L-15 medium supplemented with 10% FCS to wash out the cryoprotectant following the protocol we used earlier (Bedaiwy et al., 2003).

Sixth, regarding our statement that ovarian tissue could tolerate adequately varying concentrations of DMSO with comparable post-thaw survival, we agree that the difference in

References


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doi:10.1093/humrep/dem047
Advance Access publication March 30, 2007

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