The primary objective of this article is to review the most recent progress in oocyte cryopreservation using various slow-freezing and vitrification protocols. There is a significant improvement in the clinical outcomes with higher pregnancy rates following mature metaphase II oocyte cryopreservation. Moreover, some reassuring data were recently reported regarding the long-term safety of this technology. However, overall clinical pregnancy rates following oocyte cryopreservation remain less efficient than the more established practice of embryo cryopreservation. Oocyte cryopreservation is a viable alternative for fertility preservation for women at risk of premature ovarian failure due to diverse factors, such as gonadotoxic chemotherapy or radiotherapy. Currently, there are two distinct techniques for oocyte cryopreservation: slow freezing and vitrification. Oocyte vitrification is gaining popularity as the technique of choice; however, well-designed, randomized, controlled trials are still required to establish which method is the most efficient and safest for oocyte cryopreservation.

**KEYWORDS**: cryopreservation • fertility preservation • implantation • intracytoplasmic sperm injection • IVF • oocyte • slow-freezing • vitrification
membrane permeability between the two cell types. Lipid phase-transition temperature is defined as the temperature at which the cell membrane transitions from the liquid to the gel state. It has been shown that oocytes at different developmental stages are more vulnerable to thermal injury compared with zygotes, probably due to the high lipid phase-transition temperature of oocytes. This might explain the poor cryosurvival of oocytes [40].

It was also shown that the characteristics of oocyte membrane permeability change throughout development. Using the rodent model, it was found that, after maturation, the oocyte membrane becomes less water permeable [41] and difficult to dehydrate, increasing the odds of intracellular ice formation. The change in water permeability may be reversed within 3 h of sperm penetration into the oocyte [42]. This decrease in water permeability may explain why the same freezing protocol resulted in poor survival rates when MII oocytes were frozen, but high rates of survival when similar protocols were applied to immature [43] and bipronuclear (2PN)-stage oocytes [44]. Moreover, oocytes’ surface area-to-volume ratio is lower than that for 2PN oocytes and cleavage-stage embryos [45], making sufficient dehydration even more difficult [46].

The zona pellucida, the glycoprotein layer surrounding the plasma membrane of an oocyte, can also be affected during the cryopreservation procedure, by fractures [47] and/or hardening [48,49]. However, whether or not oocyte cryopreservation can lead to zona hardening is still controversial. Some investigators did not observe a change in cortical granules [50,51]. Moreover, the issue of zona hardening is not an actual problem in current IVF after the adoption of intracytoplasmic sperm injection (ICSI) technology.

Although Gook et al. have reported a similar fertilization rate of cryopreserved oocytes using IVF and ICSI, an increased cleavage rate up to the hatching blastocysts stage was documented following ICSI [52]. Furthermore, Kazem et al. reported that both fertilization and cleavage rates increased significantly upon using ICSI [53]. In addition, similar fertilization rates in fresh and cryopreserved—thawed oocytes inseminated with ICSI were reported by Tucker et al., who also announced that three pregnancies all resulted in early miscarriages [14]. Shortly after, the first live birth derived from oocytes cryopreserved using propanediol (PrOH) and inseminated by ICSI was published by Porcu et al. [5]. Subsequently, all investigators adopted ICSI to inseminate the cryopreserved—thawed oocytes.

The oocyte is rich in mitochondria and subcellular organelles, adding to their structural complexity. Such a complex subcellular matrix is particularly susceptible to temperature [54] and osmotic changes [55,56]. Likewise, the meiotic spindle structure can be disturbed during the freeze—thaw steps. Failure of spindle assembly can result in aneuploidy. The meiotic spindle is sensitive to cryoprotectant agents (CPAs) [57,58] and temperature changes [59–61]; thus, cryopreservation may induce depolymerization of the microtubules [62]. However, clinical observations have shown that after a period of 1–3 h of post-thaw incubation, the meiotic spindle generally reforms in the majority of oocytes [63,64]. Human studies have also shown comparable rates of aneuploidy between cryopreserved and fresh oocytes [65].

The oocyte developmental stage is another factor that affects cryopreservation outcomes. The initial poor cryosurvival rates of cryopreserved—thawed MII oocytes led the researchers to consider GV oocyte cryopreservation as an alternative. At the GV stage, the oocyte is smaller in size, has less developed zona, and the entire DNA material is enclosed within a nuclear membrane rather than arranged on a meiotic spindle. These criteria make the GV oocyte a better candidate for cryopreservation over the MII stage. Nevertheless, human in vitro maturation (IVM) systems are still not optimal.

Furthermore, it was shown that the metabolic coupling of the oocyte and surrounding cumulus cells is lost in cryopreserved—thawed GV oocytes [66]. The integrity of cumulus cells and their gap junctions with GV oocyte is vital for normal maturation and development [66]. Thus, to date, there are very few pregnancies reported using GV oocyte cryopreservation [10,67–69]. Moreover, it was found that cryopreserved—thawed human GV oocytes had significantly higher spindle and chromosomal abnormalities after IVM compared with their fresh counterparts [62,70]. Yet more clinical trials are still needed to truly evaluate the efficiency of cryopreserving GV versus MII oocytes [71].

The importance of cumulus cells during cryopreserving MII oocytes is still a matter of debate. Some studies showed a better cryosurvival after denuding the human MII oocyte of its cumulus cells [50,72], while others reported contradictory results [73].

Finally, the oocyte is a short-lived cell that should be fertilized at a precise time, otherwise it undergoes degeneration [74]. Given such a unique structure, oocytes are very sensitive to all kinds of insults, particularly the freeze—thaw trauma.

In summary, an oocyte has many unique characteristics that mandate the development of an oocyte-tailored cryopreservation protocol. This, in part, explains the relatively long evolutionary path of oocyte cryopreservation techniques from the 1950s to the present day with the achievement of a clinically meaningful outcome.

Extrinsic factors

Oocyte cryopreservation outcomes are also affected by extrinsic factors related to the cryopreservation process itself, such as CPAs, cryodevices and cooling/thawing rates. Oocyte cryopreservation is often performed using either a slow cooling rate via the slow freeze/rapid-thaw procedure or, alternatively, by the vitrification technology.

During the cryopreservation—thawing process, the oocyte is vulnerable to chilling injury, intra- or extra-cellular ice formation and/or uncontrolled dehydration. These injuries can be controlled to an extent by using the appropriate CPAs and cooling/thawing rates. However, CPAs themselves can induce some chemical and osmotic injuries that vary with different types and concentrations of CPAs, as well as with different temperatures of exposure (reviewed in [75]).

Many CPAs are currently in use. The most popular slow-freezing protocols use a combination of PrOH and sucrose [76], whereas a combination of sucrose, ethylene glycol and/or dimethyl
Oocyte cryopreservation: a technical & clinical update

Sulphoxide is the commonly used oocyte vitrification protocol [77]. The effect of various CPAs on oocyte cryopreservation was previously described in detail (reviewed in [78]) and is beyond the scope of the current review. Nevertheless, to date, there is no consensus on a universal cryopreservation protocol.

Similarly, the cryodevice or carrier used for cryopreservation can indirectly affect outcomes by changing cooling and warming rates. Traditional cryopreservation tools, including the cryovial and the 0.25-ml insemination straw, have relatively thick walls that allow limited cooling and warming rates (approximately 2500°C/min) [75] and are usually used for slow-freezing protocols. In contrast, vitrification carriers usually have very thin or no walls (open systems), allowing the loading of microquantities of sample and attain very high cooling and warming rates.

Many carriers have been suggested as vitrification vehicles, including: open-pulled straw [7], cryoloop [39], electron microscopy grid [79], cryotop [32–35,77] and the McGill cryoleaf [80]. The aforementioned carriers are considered open carrier systems, where there is direct contact between the sample and liquid nitrogen. The risk of cross-contamination during storage in liquid nitrogen have been raised [81,82]. In the past few years, new vitrification closed systems, such as the cryotip [83,84] and high-security vitrification straw, were developed [85]. Although the walls of these new devices are very thin, cooling and warming rates are expected to be lower than those achieved with the open-device systems.

However, the need to use ultrarapid cooling devices to achieve good embryo and oocyte vitrification outcomes has recently been challenged [86,87]. Yet more clinical trials are needed to evaluate the efficiency of closed systems, such as conventional 0.25-ml plastic straws, cryotips and high-security vitrification straws for embryo and oocyte vitrification. Furthermore, the recent US FDA and European Tissue Directive regulations are expected to greatly impact upon many of the techniques that are routinely used in IVF laboratories, including cryodevices, and thus, the use of closed, sealed systems may be more plausible and acceptable than open cryodevices.

In summary, different CPAs and carriers are used for oocyte cryopreservation. The development of open-system carriers for oocyte vitrification contributed significantly to the increasingly promising results obtained with oocyte vitrification.

Clinical application of frozen oocytes

The number of reproductive-aged women diagnosed with cancer each year is increasing worldwide. With the recent improvements in chemo- and radio-therapy protocols, these patients are expected to survive their cancers; however, this may be at the expense of their reproductive potential. Oocyte cryopreservation offers these women the chance to preserve their fertility so that they can have their own genetic children. In addition, noncancer patients, such as those with autoimmune disease, recipients of bone marrow or stem cell transplantation, or those undergoing oopherectomy for benign disease or prophylactically, can also very much benefit from oocyte cryopreservation technology [88].

Oocyte cryopreservation can also be very valuable to women at risk of premature ovarian failure due to genetic problems, such as Turner’s syndrome [89].

In 2007, Yang et al. reported on the first successful application of MII oocyte cryopreservation as a fertility preservation tool. A healthy male was born from frozen–thawed oocytes belonging to a Hodgkin’s lymphoma patient after embryo transfer (ET) to a surrogate [90]. Recently, Porcu et al. reported the first pregnancy in an ovariec-tomized cancer patient using her own slowly frozen—rapidly thawed MII oocytes that resulted in the delivery of two healthy females [91].

However, in most cancer patients, the immediate start of chemo- and radio-therapy is mandatory, leaving no time for ovarian stimulation protocols to retrieve mature oocytes. Yet, ovarian tissue cryopreservation and/or the retrieval of immature oocytes during a natural cycle are possible alternatives in these cases. To date, only five live births and two ongoing pregnancies were reported worldwide using autotransplanted cryopreserved—thawed ovarian tissue [92].

Our systematic review on reproductive outcomes after ovarian tissue transplantation points to the relative current inefficacy of transplantation of cryopreserved—thawed ovarian tissue compared with fresh grafts [93]. This is, in part, due to tissue ischemia and vascular insufficiency of the transplanted ovarian tissue [94].

Given the current limitations of ovarian tissue cryopreservation, it is very important to improve on the other available option, which is retrieval and cryopreservation of immature oocytes followed by IVM of frozen/thawed oocytes [95–99]. Alternatively, human GV oocytes can be successfully cryopreserved after being matured in vitro [100]. However, the number of retrieved immature oocytes is usually small [101] and the IVM technique is still in its infancy.

Furthermore, embryo freezing and disposal of excess cryopreserved embryos can often present an ethical dilemma to patients for a variety of reasons, including religion. Cryopreservation of unfertilized oocytes is, therefore, an appealing option for those patients. Oocyte freezing is the only legal option in some countries, such as Italy, in which the law completely prohibits embryo cryopreservation, except in cases where fresh ET is impossible [102]. This technology could also rescue oocytes in cases of ovarian hyperstimulation syndrome and the inability to find sperm, especially in cases of surgically retrieved spermatozoa.

Another application for oocyte cryopreservation could be for women who desire to postpone childbearing for the purpose of education, career or other reasons. Cryopreservation at a young age may ensure a chance of a future pregnancy. This could also be applied to women with a family history of early menopause, as they could have an increased risk of diminished ovarian reserve at a young age. However, there are not yet sufficient data to recommend oocyte cryopreservation to circumvent reproductive aging in healthy women [88]. Oocyte cryopreservation could also overcome the current limitations of oocyte donation, including donor availability, cost, the need to synchronize donor and recipient cycles, travel requirements and the inability to quarantine oocytes.
In summary, there are four major indications for oocyte cryopreservation; first, it can be applied as a fertility preservation strategy for those at risk of premature ovarian failure; second, it can be made available to those undergoing treatment with assisted reproductive technologies and do not consider embryo freezing as an option for ethical or other reasons; and third, those who would like to preserve their future reproductive potential because they do not have a partner, or for other personal, social or medical reasons. Finally, oocyte cryopreservation facilitates the oocyte donation process.

Clinical outcome of oocyte cryopreservation

Over the past 20 years, over 600 children worldwide were born from cryopreserved–thawed oocytes [103,104]. The American Society for Reproductive Medicine acknowledged the improved oocyte survival, fertilization and pregnancy rates from frozen–thawed oocytes in IVF [105]. They also noted that, to date, there does not appear to be an increase in chromosomal abnormalities, birth defects or developmental deficits in the children born from cryopreserved oocytes. Yet, they recommended that, pending further research, oocyte cryopreservation should be used only on an investigational basis and under the guidance of an institutional review board [105].

Recent refinements of slow-freezing protocols have played a role in the improvements of the clinical outcomes reported with oocyte cryopreservation. Fabbri et al. have shown that increasing the concentration of sucrose from 0.1 to 0.3 M significantly increased the survival rate of frozen–thawed oocytes from 34 to 82% [20]. This positive effect of using a higher sucrose concentration further demonstrated achieving survival rates of 74–76% [26,106]. However, clinical pregnancy and implantation rates remained low [26,27].

Similarly, intracellular injection of nonpermeable CPA, such as trehalose, was reported to have a favorable effect on human oocytes against freeze stresses [107]. Replacing sodium with choline is considered another enhancement that can be applied to slow-freezing protocols. It was first implemented in mice [56,108] but was later shown to improve human oocyte post-thaw survival and pregnancy rates [22,109,110].

Recent studies reported on improved clinical outcomes of cryopreserved–thawed oocytes (Table 1). In 2006, Borini et al. reported their 7-year cumulative experience with oocyte slow freezing. Using 1.5 M PrOH and 0.1 M sucrose, 918 oocytes from 67 patients were slowly frozen–rapidly thawed, resulting in a 43.4% survival rate and a 51.6% fertilization rate. Clinical pregnancy and implantation rates reported were 19.2 and 12.3%, respectively [111].

In the same year, a large study was published by Levi-Setti et al., where a total of 2900 oocytes from 286 patients in 303 freezing cycles were slowly frozen using 1.5 M PrOH and 0.3 M sucrose. In 159 cycles, oocytes were rapidly thawed with a 69.9% survival and a 67.5% fertilization rate. However, the clinical pregnancy (12.4%) and implantation (5.7%) rates reported were low [27].

Recently, Bianchi et al. reported their experience with 403 slowly frozen–rapidly thawed oocytes, using a modified slow-freezing protocol. A 1.5 M PrOH and 0.2 M sucrose solution was used for freezing, while a decreasing concentration of PrOH and 0.3 M sucrose was used for thawing. Their protocol resulted in a 75.9% survival rate and a 76.2% fertilization rate. After ET, a clinical pregnancy rate of 21.3% was reported and 13.5% of the transferred embryos resulted in a successful implantation [112].

In the same year, Borini et al. published their overall experience with 5448 slowly frozen oocytes. After thawing 3238 oocytes from 510 patients, 68.1 and 76.1% of oocytes survived and were successfully fertilized, respectively. In 590 ET cycles, they achieved clinical pregnancy and implantation rates of 14.9 and 8.1%, respectively [113].

Application of vitrification technology to MII oocyte cryopreservation was another milestone in improving post-thaw survival and clinical outcomes. Using cryotops, Antironi et al. vitrified a total of 463 oocytes collected from 120 volunteers. After warming 330 oocytes, 99.4% survived and 92.9% were successfully fertilized. In 120 ET cycles, clinical pregnancy and implantation rates of 32.5 and 13.2% were reported, respectively [34]. More recently, the same group reported their 3-year experience with 770 vitrified–warmed oocytes in 295 ICSI cycles. In total, 99% of oocytes survived, of which, 91.5% were fertilized and resulted in a 27.8% clinical pregnancy rate and 12.8% implantation rate. A total of 50 healthy babies were delivered, and 18 pregnancies were still ongoing at the time of the report. Similar results were shown by Yoon et al. when 364 oocytes were vitrified using slush nitrogen. On warming, 85.1% of oocytes survived, of which, 77.4% were fertilized. In 30 ET cycles, 120 embryos were transferred; 14.2% were implanted successfully and resulted in a 43.3% clinical pregnancy rate [35].

Likewise, Cobo et al. vitrified 693 oocytes in an oocyte donation program and reported survival and fertilization rates of 96.1 and 73.1%, respectively. A total of 117 embryos were transferred in 57 ETs, of which, 38.5% implanted and resulted in a 63.2% clinical pregnancy rate. However, it is important to note that the clinical pregnancy and implantation rates were defined as the number of gestational sacs and not as the number of fetal hearts per number of ETs and embryos transferred, respectively [36]. Moreover, successful pregnancies [115] and live births [116] were reported after transfer of vitrified–warmed embryos derived from vitrified–warmed oocytes, emphasizing the improvements and well performance of the vitrification cryotechnology.

A very interesting study performed genomic hybridization on the first polar body of 323 MII human oocytes from egg donors, out of which only 111 oocytes turned out to be euploid. Upon selective vitrification of those euploid oocytes, 96% survived and 46% developed into expanded blastocysts and were transferred into 16 embryo recipients. The birth rate with a maximum of two embryos transferred was 75%, and the implantation rate was 61% [39]. These excellent results could open a new avenue for improving oocyte cryopreservation outcomes. However, more studies are needed to validate the promising strategy of oocyte selection through genomic hybridization. Nevertheless, it is fair and important to emphasize the fact that most of the aforementioned vitrification studies used oocytes retrieved from young donors [34,39,117].
### Table 1. Summary of recent clinical studies that investigated slow freezing or vitrification of oocytes.

<table>
<thead>
<tr>
<th>Study (year)</th>
<th>Technique</th>
<th>Frozen oocytes (n)</th>
<th>Patients (n)</th>
<th>Thawed oocytes (n)</th>
<th>Thaw cycles (n)</th>
<th>Survival rate (%)</th>
<th>Fertilization rate (%)</th>
<th>Cleavage rate (%)</th>
<th>Embryo transfers (n)</th>
<th>Clinical pregnancy rate/embryo transfers (%)</th>
<th>Implantation rate (%)</th>
<th>Miscarriage rate (%)</th>
<th>Live births (n)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Borini et al. (2006)</td>
<td>Slow freezing</td>
<td>918</td>
<td>67</td>
<td>705</td>
<td>101</td>
<td>43.4</td>
<td>51.6</td>
<td>86</td>
<td>73</td>
<td>19.2</td>
<td>12.3</td>
<td>28.6</td>
<td>12</td>
<td>[111]</td>
</tr>
<tr>
<td>Levi Setti et al. (2006)</td>
<td>Slow freezing</td>
<td>2900</td>
<td>286</td>
<td>1087</td>
<td>159</td>
<td>69.9</td>
<td>67.5</td>
<td>53.5</td>
<td>145</td>
<td>12.4</td>
<td>5.7</td>
<td>33.3</td>
<td>13</td>
<td>[27]</td>
</tr>
<tr>
<td>Bianchi et al. (2007)</td>
<td>Slow freezing</td>
<td>1083</td>
<td>141</td>
<td>403</td>
<td>90</td>
<td>75.9</td>
<td>76.2</td>
<td>93.8</td>
<td>80</td>
<td>21.3</td>
<td>13.5</td>
<td>11.8</td>
<td>4</td>
<td>[112]</td>
</tr>
<tr>
<td>Borini et al. (2007)</td>
<td>Slow freezing</td>
<td>5448</td>
<td>510</td>
<td>3238</td>
<td>660</td>
<td>68.1</td>
<td>76.1</td>
<td>91.8</td>
<td>590</td>
<td>14.9</td>
<td>8.1</td>
<td>21.6</td>
<td>60</td>
<td>[113]</td>
</tr>
<tr>
<td>Antironi et al. (2007)</td>
<td>Vitrification</td>
<td>463</td>
<td>120</td>
<td>330</td>
<td>120</td>
<td>99.4</td>
<td>92.9</td>
<td>96.7</td>
<td>120</td>
<td>32.5</td>
<td>13.2</td>
<td>20.5</td>
<td>3</td>
<td>[34]</td>
</tr>
<tr>
<td>Yoon et al. (2007)</td>
<td>Vitrification</td>
<td>426</td>
<td>28</td>
<td>364</td>
<td>NA</td>
<td>85.1</td>
<td>77.4</td>
<td>94.3</td>
<td>30</td>
<td>43.3</td>
<td>14.2</td>
<td>15.4</td>
<td>5</td>
<td>[35]</td>
</tr>
<tr>
<td>Antironi et al. (2008)</td>
<td>Vitrification</td>
<td>NA</td>
<td>NA</td>
<td>770</td>
<td>NA</td>
<td>99</td>
<td>91.5</td>
<td>NA</td>
<td>NA</td>
<td>27.8</td>
<td>12.8</td>
<td>NA</td>
<td>50</td>
<td>[114]</td>
</tr>
<tr>
<td>Cobo et al. (2008)</td>
<td>Vitrification</td>
<td>693</td>
<td>47</td>
<td>693</td>
<td>NA</td>
<td>96.1</td>
<td>73.1</td>
<td>NA</td>
<td>57</td>
<td>63.2*</td>
<td>38.5*</td>
<td>16.6</td>
<td>28</td>
<td>[36]</td>
</tr>
<tr>
<td>Sher et al. (2008)</td>
<td>Vitrification</td>
<td>111</td>
<td>16</td>
<td>78</td>
<td>NA</td>
<td>96</td>
<td>NA</td>
<td>76</td>
<td>16</td>
<td>75</td>
<td>61</td>
<td>6.3</td>
<td>12</td>
<td>[39]</td>
</tr>
</tbody>
</table>

*Number of sacs/embryo transfers.

†Number of sacs/number of embryos transferred.

NA: Not available.
In their systematic review update of data published from January 1986 to May 2008, Cil et al. reported a live-birth rate per oocyte thawed of 1.7% for slow-frozen oocytes and 5% for vitrified oocytes. They also noted that clinical pregnancy and implantation rates with vitrification (33.4 and 15%, respectively) appear to be higher than those for slow freezing (15 and 8.1%, respectively), an effect that could be attributed to the higher number of embryos transferred in the vitrification group compared with the slow-freezing group (2.7 vs 2.2), and the older mean age of women that utilized slowly frozen–thawed oocytes (34.5 vs 32.2). In addition, they evaluated the time trend for the success of both techniques and found that pre-2006, slow freezing resulted in clinical pregnancy and implantation rates of 22.8 and 10.9%, respectively, compared with 42.3 and 17.6%, respectively, in the vitrification group. Since 2006, clinical pregnancy rates of 11.1 and 31.5%, and implantation rates of 6.4 and 14.3% for slow-freezing and vitrification techniques, respectively, have been reported. Despite the fact that both freezing techniques showed a downward trend, results were still in favor of vitrification. Their final conclusion was that oocyte vitrification is promising but larger prospective studies are indeed mandatory [103].

The controversy regarding the better oocyte cryopreservation technique remains unsolved. A relatively recent, prospective, randomized, controlled clinical trial was performed by Fioravanti et al. They analyzed 28 slow freeze–thaw and 35 vitrification–warming cycles. Cleavage rates, day-3 embryo quality and biochemical pregnancy rates were significantly higher in the vitrification group compared with the slow freezing one. Although statistically nonsignificant, survival, fertilization and clinical pregnancy rates were numerically in favor of vitrification [118].

A more recent, randomized, prospective study published by Garcia et al. showed no significant differences between both techniques. Embryos resulting from 134 oocytes slowly frozen using 1.5 M PrOH and 0.2 M sucrose, and 84 oocytes vitrified on cryotops using ethylene glycol, dimethyl sulphoxide and sucrose were transferred to 30 patients. Survival rate of frozen–thawed oocytes (88%) was not different from that of the vitrified group (92%). A total of 62 and 68% of frozen–thawed and vitrified–warmed oocytes, respectively, were successfully fertilized. A clinical pregnancy rate of 25% was reported in the slow-freezing group and was not different from that of the vitrification group (31%) [119]. However, the number of patients is insufficient to come to a solid conclusion. Well-designed, large, randomized, controlled trials are required to consolidate and validate these results.

Recently, the Italian National Register collected 4 years’ worth of data on 46,517 thawed eggs between 2004 and 2007, 7695 transferred embryos and 923 pregnancies after oocyte cryopreservation. This is probably the largest database on oocyte cryopreservation. More details regarding this database are to follow [MM Bedaiwy et al., Pers. Comm.].

Discussion

The ultrastructure of cryopreserved–thawed oocytes is another valuable tool that is also used for evaluation of various oocyte cryopreservation protocols. The polarized light and transmission electron microscopes can be used for this assessment. The polarized light microscopy (Poloscope™) was newly introduced for the noninvasive spindle evaluation in living oocytes [120]. The Poloscope is informative regarding presence, position and birefringence of the meiotic spindle. An interesting study was performed by Rienzi et al. using a 1.5 M PrOH and 0.1 M sucrose to freeze oocytes that were then examined post-thaw using the Poloscope. They showed that immediately after thawing, the meiotic spindle in all slowly frozen MII oocytes completely disappeared, but totally reformed after 3 h of incubation at 37 °C. The functional capacity of thawed oocytes with newly reformed spindles was questioned [63]. Similar results were also reported by Bianchi et al. [24].

On the other hand, Larman et al. compared meiotic spindle stability in sibling MII oocytes cryopreserved using vitrification versus slow-freezing methodology. When vitrification and warming steps were carried out at 37 °C, they detected the meiotic spindle by the Polscope in all oocytes (ten out of ten) immediately after warming. This contrasted sharply with slowly frozen oocytes in which all steps were carried out at room temperature. Here, none of the MII oocytes exhibited spindles immediately upon thawing. What was even more interesting was that the spindle retardances measured pre- and post-vitrification was not different, suggesting that the technique did not adversely affect spindle assembly. However, the number of investigated oocytes in this study was too small for a definitive judgment [121].

Similarly, a study by a group in China compared survival, fertilization, embryo development and meiotic spindle configuration in slowly frozen and vitrified human MII oocytes [122]. Vitrified–warmed oocytes expressed significantly better postwarming survival, fertilization, cleavage, blastulation, spindle assembly and chromosome alignment than their slowly frozen counterparts [122]. However, it is important to note that the cleavage rate (54.4%) of embryos derived from slowly frozen oocytes, reported by Cao et al. [122], is much lower than that reported by other authors using the same slow-freezing protocol [25,26]. By contrast, a recent study, published by Cobo et al., compared the immediate post-thaw survival and meiotic spindle assembly in slowly frozen and vitrified oocytes after thawing. Cryopreserved–thawed oocytes were fixed after 3 h of a 37°C incubation post-thaw. Vitrified oocytes resulted in better survival, but both cryopreservation techniques had similar rates of normal meiotic spindle configuration ranging from 74 to 89% [84]. Thus, whether oocyte vitrification better preserves meiotic spindle assembly in comparison to slow freezing is still a matter of controversy.

Another helpful tool for evaluating oocyte cryopreservation techniques is the electron microscopic examination of cellular structures and organelles of cryopreserved–thawed oocytes. Using transmission electron microscopy, Nottola et al. investigated the efficiency of different slow freezing [49,123] and vitrification [124] protocols in preserving the ultrastructure of cryopreserved human MII oocytes. Oocytes that were slowly frozen using an ethylene–glycol protocol showed many ultrastructural alterations when compared with fresh counterparts, such as disorganized mitochondria smooth endoplasmic reticulum.
aggregates, less microvilli and cortical granules and a higher number of vacuoles and secondary lysosomes [49]. However, when the same research group used PrOH as the CPA, the frozen–thawed oocytes presented with less ultrastructural disorganizations, but still had fewer cortical granules and abundant vacuolization compared with fresh controls [123]. On the other hand, vitrified warmed oocytes showed sparse microvacuolization and disorganized mitochondria smooth endoplasmic reticulum aggregates. However, they still presented few cortical granules and nonhomogenous microvilli [124].

The safety of any technique used in humans is a very important issue. Fortunately, data regarding safety of oocyte cryopreservation are beginning to accumulate. A total of 105 babies born from slowly frozen oocytes were followed by Borini et al. Only two babies were found to have congenital anomalies, namely choanal atresia and Rubinstein–Taybi syndrome. However, the fathers of these two affected babies suffered from severe male-factor infertility [125].

It is important to mention that the high concentrations of CPAs used in oocyte vitrification have raised concerns regarding the safety of the technique. However, some reassuring data regarding birthweights and congenital anomalies of children born from embryos derived from vitrified–warmed oocytes were recently reported [80,126].

Chavez-Badiola et al. performed a mean follow-up of 18.4 months, for the psychomotor development of 52 children born from vitrified–warmed oocytes using the cryotop. Only one child was reported with a congenital anomaly, which was interventricular septal defect. Moreover, all the 52 followed-up children had normal psychomotor development, as was shown by the Gesell’s scale [126].

Furthermore, Chian et al. reported the obstetric and perinatal outcome of a total of 200 infants born from vitrified oocytes on the McGill cryoleaf and cryotop. The study was performed in three countries: Canada, Colombia and Mexico. Congenital anomalies in the form of biliary atresia, club foot, skin hammerioma and ventricular septal defect were reported in five children. Yet, the mean birthweight and incidence of congenital anomalies were comparable to those born after spontaneous or assisted pregnancies [80].

Conclusion
Oocyte cryopreservation is still an experimental technique with an expanding spectrum of indications. Contrary to the initial frustration that lasted for almost a decade after the first report by Chen in 1986 [6], the results of oocyte cryopreservation are now more efficacious and reproducible. Both the mean survival and birth rates per thawed oocyte have doubled over the past decade. Both slow freezing and vitrification are being practiced worldwide without conclusive evidence that one technique is better than the other. Well-designed, randomized, controlled trials are needed to evaluate whether one technique is truly more effective than the other. Despite the initial concern regarding the meiotic spindle integrity and the risk of aneuploidy, recent data supported the repolymerization potential of the human meiotic spindle and the nullification of the aneuploidy risk. With the continuous refinement of the available cryopreservation protocols, survival and implantation rates should be further improved. Moreover, physicians should be aware of the experimental nature of this procedure and that these protocols should be used under the guidance of an institutional review board.

Despite the experimental nature of oocyte cryopreservation, the similarity with other innovative procedures in reproductive medicine, such as ICSI and embryo cryopreservation, is striking. The natural evolution of this technology is similar to other developments in IVF and is progressively becoming more acceptable by patients, physicians and ethicists. It is hoped that oocyte cryopreservation will soon become incorporated as an integral part of almost all IVF programs.

Expert commentary
Oocyte cryopreservation can overcome the religious, ethical and social issues faced with embryo cryopreservation. Besides, it may be a very valuable tool for fertility preservation for women with no male partners and who refuse the use of donor sperm. Although the recent remarkable improvements in clinical outcomes of oocyte cryopreservation, embryo cryopreservation remains the only evidence-based fertility preservation method. To date, oocyte cryopreservation is only used within an experimental context and under an institutional review board protocol. Oocyte vitrification seems to be a simple, cheap and more efficient technique compared with slow freezing. However, randomized, controlled clinical trials are indeed required to prove the efficiency and safety of this promising fertility preservation tool.

Five-year view
Oocyte cryopreservation may be an integral part of all IVF programs. It is anticipated that vitrification will be the most applied oocyte cryopreservation technique. It is simpler, less expensive and, so far, more effective than slow freeze–rapid thaw techniques. Rather than the various haphazardly designed oocyte vitrification protocols, a universal protocol will be designed based on oocyte biology and cryosensitivity. More attention will be paid to vitrification carriers and how to implement a closed, sterile system to lessen the risk of cross-contamination during liquid nitrogen storage.

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Key issues

- Oocyte cryopreservation is a promising fertility preservation option.
- Remarkable progress has been achieved in the area of oocyte cryopreservation.
- Vitrification seems to be an efficient and safe oocyte cryopreservation technique.
- Although limited, current data regarding safety of oocyte cryopreservation is reassuring.
- Well-designed, randomized, controlled trials are mandatory to compare the effectiveness and long-term safety of the slow-freezing and vitrification techniques for oocyte cryopreservation.

References

Papers of special note have been highlighted as:

• of interest
** of considerable interest


7. First pregnancy reported after slow-freezing human mature oocytes.


11. First pregnancy reported after cryopreservation of human immature oocytes.


17. First study to show that increasing concentration of sucrose could improve survival of frozen–thawed human oocytes.


26. Borini A, Sciaino R, Bianchi V, Sereni E, Flamigni C, Coticchio G. Clinical outcome of oocyte cryopreservation after...


• Presents the clinical outcome of 1078 frozen–thawed human oocytes.


• Reports the clinical outcome of 364 human oocytes, vitrified using slush nitrogen.


• Shows that only approximately a third of investigated human mature oocytes turned out to be euploid, and that oocyte vitrification could be very much improved if only euploid oocytes were selectively vitrified.


Review

AbdelHafez, Desai, Ali, Sayed, Abu-Alhassan & Bedaiwy


**Very interesting review on the details of vitrification technology.**


**Detailed review on different aspects of oocyte cryopreservation.**


**First live birth (gestational carrier) from frozen–thawed oocyte harvested from a cancer patient.**


**First live birth from frozen–thawed autologous oocytes in a cancer patient.**


117 Shows that vitrified–warmed oocytes have the potential to mature and develop in rates similar to fresh oocytes.


123 Shows that on performing oocyte vitrification and warming steps at 37°C, meiotic spindles were evident immediately postwarming, and even their retardance values were the same before and after vitrification.


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