Journal of Assisted Reproduction and Genetics (2019) 36:15–17
https://doi.org/10.1007/s10815-018-1378-z

COMMENTARY

A subspecialty of the assisted reproductive technologies: cryogenic inventory maintenance

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Received: 15 November 2018 / Accepted: 18 November 2018 / Published online: 15 December 2018

Cryopreservation of human embryos and gametes is a subspecialty of the assisted reproductive technologies just as assisted fertilization (ICSI) and preimplantation genetic testing are. While attention has been focused on mastering the most efficacious methods for cryopreserving and warming embryos, the same intensity of focus and energy may not have been universally invested in strategies for *storage*.

As custodians and guardians of their patients' eggs and embryos, the professional community of embryologists was profoundly dismayed by the news describing catastrophic losses of cryopreserved egg and embryo inventories at two well-established laboratories earlier this year [1, 2]. Shock and disappointment likely led to immediate critical appraisal of their own storage dewars and the monitoring and backup systems. "Could this happen at our lab?" was the question that many centers asked of themselves and were asked by their patients seeking reassurance about their own stored samples.

While we aspire to maximal vigilance over our cryogenic inventories, Dr. Schiewe and his colleagues, in their point-by-point consideration of best practices for cryopreservation storage, exercise candor in observing, using blunt and even painful terms, that there may be "complacency in quality control (QC) practices" and "forgetfulness." In their timely article, they have thoughtfully taken measure of the gap between what may represent current practice and what is best practice.

Cryopreservation of embryos was the first procedure to complement in vitro fertilization (IVF) [3]. As we mark the 40th anniversary of Louise Brown's birth [4] and the momentous integration of IVF into reproductive medicine, we can rightfully marvel at the portfolio of techniques that comprise the array of assisted reproductive technologies.

Advances in ART represented greater opportunity for patients to succeed in their treatments, even those with the most severe infertility diagnoses. A virtual technological cascade ensued. Refinements in culture medium design allowed extended culture to blastocyst [5]; higher blastocyst formation rates led to the opportunity for trophectoderm biopsy and genetic testing using state of the art molecular platforms for chromosome screening [6]; vitrification yielded the higher post-warming survival rates [7], allowing more frequent blastocyst biopsy for chromosome copy number screening and cryo-all cycles in which embryo transfer is delayed to a more physiologic uterine environment in a non-hyperstimulated cycle [8]. More advantageous culture conditions were also realized from revisions in incubator design and low oxygen atmosphere [9]. Single embryo transfer, conferring greater safety of IVF for both patients and offspring, also emerged as a desired dividend of the continual evolving of ART and the increasing implantation rates [10]. The array of these technical advances catalyzed, for many laboratories, a burgeoning of their cryogenic inventories.

Cryopreservation is no longer an *adjunctive* procedure used to address a patient's "extra" embryos after fresh transfer in the treatment cycle; it is now *central* to treatment, enabling preimplantation genetic diagnosis after blastocyst biopsy, or, *is* the treatment, with the freezing of *all* embryos for deferred transfer to a more favorable uterine environment. In addition, many laboratories have been adding more vitrified eggs to their tanks, given the ASRM's practice guideline indicating that egg freezing was no longer "experimental" [11]. This collective paradigmatic shift has resulted in embryologists' effectively presiding over two operations: the ART laboratory and a biorepository of their patients' cryopreserved samples.

Schiewe et al. highlight the points that comprise a total quality management (TQM) program for maintenance of a cryogenic inventory that must be written, reviewed, and updated at regular intervals and be accompanied by documentation as evidence of adherence. It begins before any liquid nitrogen comes roaring through a dispersion nozzle.

For the long-term satisfaction of patients, a clear, well-written consent for cryopreservation that will ensure

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communication with the owner(s) regarding the disposition of the embryos or cells is an essential foundation. In addition, a strong consent may preclude the phenomenon with which many laboratories and clinics must grapple: "abandonment" of embryos, and encourage patients to address the ramifications of freezing their embryos with foresight and maximal information.

For the embryologists, a structured training program in the safe and appropriate handling of cryopreservative reagents and solutions; the cryodevices, dewars, and liquid nitrogen should be included. Education about personal protection by face masks and gloves and through environmental measures such as adequate ventilation must be provided.

With respect to the embryos or gametes, a strong labeling system employing a *minimum* of two unique identifiers is an absolute requirement. An unerring method to establish the identity and ownership of the cryopreserved samples must be at the foundation of a quality program and will inspire and validate confidence by all parties, i.e., patients, embryologists, clinicians, and clinic administrators.

The physical integrity of the storage dewars must be routinely assessed, through vigilance for signs of external damage, or unusually high consumption rates of liquid nitrogen that reflect a failing vacuum. The automatic filling systems and their mechanical components must be regularly examined to stave off failure and the false security of an assumed backup for liquid nitrogen (LN_2) replenishment.

Monitoring systems for temperature or LN_2 levels inside tanks must be routinely appraised for their effectiveness in detecting departures from ideal conditions and notifying an embryologist(s), using a redundant system such as a telephone number "tree."

An audit of all the tanks and their contents on a regular basis to confirm the accuracy of the electronic inventory and/or the written log of samples ensures that patients and the clinic are correctly advised on the number and origin (cryopreservation date) of each patient's samples. This exercise complements the effort to keep patients apprised of their stored samples and in uninterrupted communication with the clinic to provide any advice on disposition.

A quality plan encourages the laboratory to assess how storage tanks are housed within the clinic. With so many therapeutic plans driving cryopreservation, the resultant expanding number of tanks may provide challenges in identifying adequate space in which they can securely reside. With square footage in many laboratories already at a premium, it is not unusual for tanks to be stored *outside* the laboratory. A dedicated cryo storage facility where all tanks are located, with a monitoring/alarm system and manifold-delivery of LN_2, is an ideal model, but it is not unusual that some laboratories are, instead, pressed to store tanks in any available space in the clinic, placing them in "obscure low traffic locations" as Schiewe et al. noted.

A TQM program will address that with the sheer expansion of the demands of an ART laboratory—the significant volume of tasks and procedures that must be fulfilled in a day—appropriate allocation of time must be devoted by the embryology team or trained, authorized support staff to provide maintenance and oversight of the tanks and the cryo inventory according to a written, structured schedule.

Advances in ART, with increasing preimplantation genetic testing, cryo-all cycles, more supernumerary embryos requiring freezing from SET, and more frequent oocyte freezing, have contributed to the emergence of cryobiology as a subspecialty within the ART laboratory. Schiewe et al. have presented a template for a quality management program for cryopreservation. Each section is an interrogative point for the adequacy of how a laboratory achieves effective and responsive oversight of multiple facets of its cryogenic inventory. For those laboratories evaluating how well their cryopreservation programs achieve quality as reflected by meticulous monitoring, maintenance, and scrupulous record keeping, their observations and recommendations are strong guidance.

In practicing effective methods to safeguard them, we acknowledge that the embryos and gametes within our cryo storage tanks represent the aspirations for family of our patients and the collective efforts of dedicated teams of professionals to help them realize those aspirations.

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