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Case Report

Successful Pregnancy Outcome Using Frozen Oocytes and Sperm: A Case Study from Jordan

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Abstract

Background: This case report is significant as it documents the first successful pregnancy in Jordan through the use of cryopreserved oocytes and sperm. The novelty lies in the successful implementation of advanced cryopreservation and assisted reproductive technologies (ART) in a clinical setting within a diverse cultural context.

Case Presentation: A 45-year-old woman, who had previously undergone oocyte cryopreservation at the age of 36 due to potential fertility challenges and medical reasons, and her husband, who also cryopreserved his semen due to logistical issues, were involved in this case. The couple, after experiencing fertility challenges, decided to utilize their cryopreserved gametes. The oocytes were thawed and fertilized using intracytoplasmic sperm injection (ICSI) with the husband's thawed sperm. Out of six oocytes, five were successfully fertilized, and two embryos were transferred on the second day of development. The patient achieved a successful pregnancy, confirmed by positive beta-HCG levels, and delivered a healthy baby.

Conclusions: This case underscores the importance of meticulous laboratory techniques and controlled thawing methods in ensuring the viability and functionality of cryopreserved gametes. It also highlights the potential of cryopreservation as a viable option for fertility preservation and assisted reproduction, particularly in cases involving advanced maternal age and medical indications. The success achieved in this case demonstrates the potential for interdisciplinary collaboration in enhancing fertility care.

Keywords: ICSI (Intracytoplasmic Sperm Injection); Embryo Transfer; Oocyte Frozen; Sperm Frozen; Oocyte Survival; Embryo Grading; Fertilization; Fertility

Introduction

ICSI: Intracytoplasmic Sperm Injection; ART: Assisted Reproductive Technology; Lh: Luteinizing Hormone; Fsh: Follicle-stimulating Hormone; Beta-HCG: Beta-Human Chorionic Gonadotropin; AMH: Anti-Müllerian Hormone

Background

Within the realm of Assisted Reproductive Technology (ART), ongoing progress in laboratory methodologies is reshaping the field. Notably, cryopreservation represents a significant advancement.

While the freezing of sperm and embryos has been commonplace for some time, the successful preservation and subsequent utilization of cryopreserved oocytes have presented considerable challenges, resulting in a relatively small number of live births worldwide.

The field of Assisted Reproductive Technology (ART) has experienced a significant rise in attention towards oocyte cryopreservation, particularly due to its potential in preserving fertility before chemotherapy, addressing premature ovarian failure, and facilitating the postponement of reproductive function. Success rates in oocyte cryopreservation vary depending on factors such as the

woman's age, oocyte quality, cryopreservation technique, and clinic expertise. Generally, younger women tend to have higher success rates due to better-quality oocytes.

After vitrification and thawing, oocytes demonstrate a high survival rate of 90% to 97%, indicating effective preservation. Fertilization rates range from 71% to 79%, showing successful fertilization potential. Implantation rates, reflecting embryo attachment to the uterine lining, vary from 17% to 41%, suggesting good prospects for pregnancy. Clinical pregnancy rates per vitrified and thawed oocyte range from 4.5% to 12%, indicating the likelihood of achieving pregnancy. Overall, these findings underscore the efficacy of cryopreserved oocytes in assisted reproduction, offering hope to individuals seeking fertility treatment or preservation [1]. We report the first pregnancy from frozen oocyte and frozen sperm in Jordan.

Case Report

In 2015, at the age of 36 and unmarried, the patient chose to freeze her oocytes due to several factors. Her small ovarian size and low anti-Müllerian hormone (AMH) level indicated potential fertility challenges. With advancing age and being unmarried, she recognized the importance of preserving her fertility options before facing additional obstacles. The patient underwent oocyte cryopreservation using the agonist protocol, resulting in the successful freezing of six mature oocytes out of nine-retrieved. A year later, the patient married her husband, who lived in Britain, presenting new challenges due to geographical distance and visa issues. Despite this, they experienced a spontaneous pregnancy in 2017, leading to the birth of their daughter. However, when they tried to expand their family four years later, age-related fertility issues and visa concerns resurfaced. After unsuccessful attempts to conceive, they consulted their doctor, leading to the decision to thaw the frozen eggs as part of their fertility treatment plan.Logistical challenges arose due to the husband's visa issues, prompting him to freeze his semen sample in advance. This proactive step ensured the availability of viable sperm for fertilization despite his absence during the egg thawing procedure. The thawed oocytes were fertilized using the thawed sperm through intracytoplasmic sperm injection (ICSI), resulting in the successful fertilization of five out of six oocytes.

The retrieved oocytes were cryopreserved using the rapid vitrification method. Freezing was started 1-2 hours after retrieval, prior to freezing, cumulus denudation was preformed using hyaluronidase and the maturity of oocytes was confirmed. Kitazato media was used for oocyte vitrification. Kitazato media is composed of Hydroxypropyl cellulose (HPC) that fends off contamination, Trehalose that replaces sucrose for safer osmosis, protecting cellular membranes better, DMSO with ethylene glycol that ensures minimal toxicity and excellent post-warming results, plus, gentamicin that extends media sterility, making solution handling safer.

The vitrification process of oocytes involved several meticulous steps, each carefully timed and measured to ensure optimal results. Initially, oocyte equilibration began with the placement of specific volumes of BS, VS1, and VS2 solutions onto a Repro Plate, with 20μL for BS and 300μL each for VS1 and VS2. The oocytes were then transferred with minimal medium volume to the bottom of BS and left for 3 minutes. During this time, ES solution was gently added to the TOP of BS and left for another 3 minutes, followed by the addition of another ES solution for another 3 minutes. Subsequently, another ES solution was added and left for 6 to 9 minutes. After equilibration, the oocytes were aspirated in VS1 solution within 0.5 minutes and stirred five times around the oocyte. This process was repeated three times, changing positions within VS1. The outer solution was completely displaced until the remaining ES visually disappeared. Within the same time, the remaining VS1 was blown out, fresh VS2 was aspirated, and the oocytes were transferred to VS2 solution, where they were stirred twice to ensure complete displacement of the outer solution and dehydration. Finally, the oocytes were aspirated and placed on a Cryotop sheet, where excess solution was removed using a pipette. This meticulous process ensured the preservation of oocytes through vitrification for potential future use in assisted reproductive technologies [2]. The patient's endometrium was prepared for embryo transfer through a protocol involving estrogen and progesterone administration. In February 2024, the doctor stated a protocol with the patient for endometrium preparation. On the second day of her cycle, she took estrofen (estrogen) two tb daily for 6 days, then 3 tb daily till the end of the cycle. Progesterone was added from day twelve orally and vaginally. The patient had itching from the suppository hence she was shifted to subcutaneous progesterone. Her frozen eggs were thawed in day 14 (Figure 1). A viability assessment conducted via inverted microscopy revealed the presence of six oocytes that successfully endured the thawing procedure. Oocytes exhibiting an intact thick zona pellucida, clear perivitelline space, and discernible polar body were classified as normal and subsequently subjected to culture in a medium maintained at 37°C with 6% CO2 for a duration of 2 hours before injection. Following this incubation period, the oocytes were injected with a prepared sample of the husband's sperm that had undergone freezing and thawing.

After the ICSI treatment (Figure 2. a), the oocytes were cultured in cleavage media within the embryoscope until a Pronucleus check was performed 16–18 hours later (Figure 2. b). This meticulous monitoring process exemplifies the significance of real-time observation in assisted reproductive technology. The embryoscope, cou-

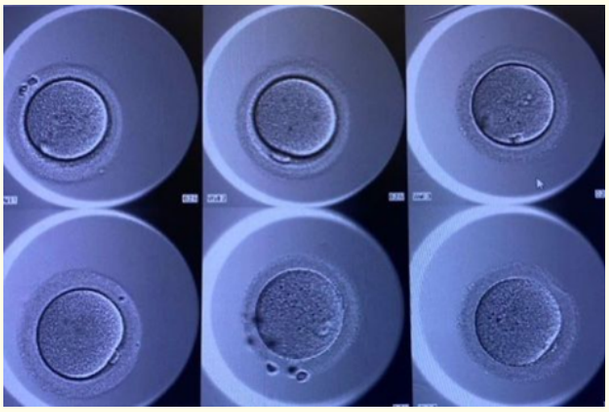

Figure 1: The thawed oocytes.

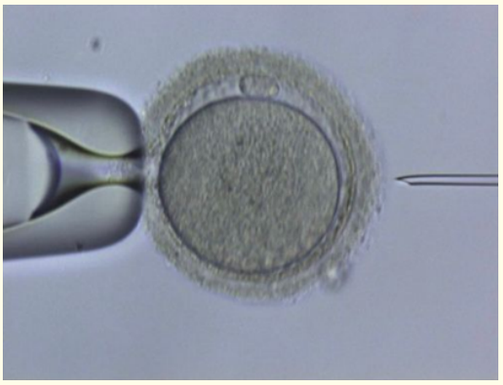

Figure 2a: ICSI treatment, which is the intracytomplasic sperm injection.

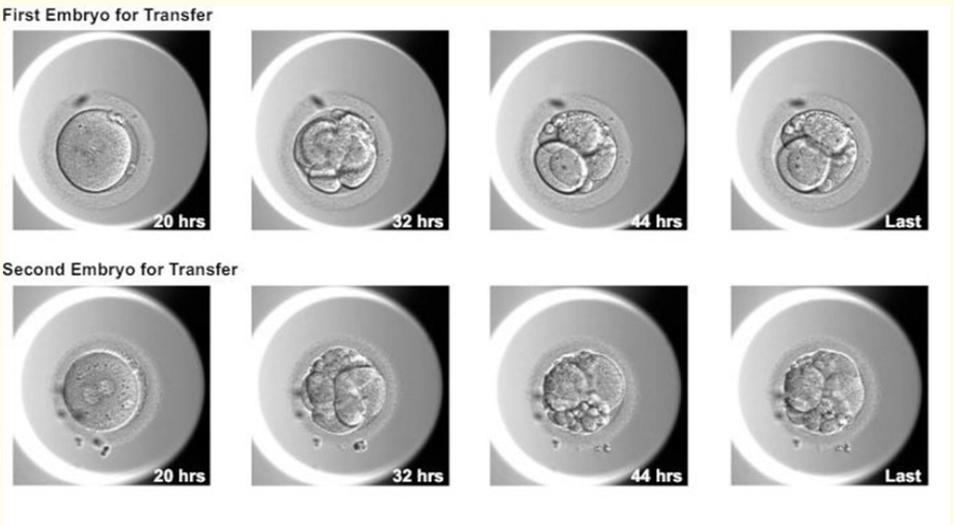

Figure 2b: The development of the two embryos that we transferred (from day zero until the day of transfer which is day 2).

pled with Vitrolife's incubation system, offers a breakthrough in this field, providing continuous time-lapse imaging and maintaining precise environmental conditions, including temperature, CO2, and O2 levels within the normal range. By seamlessly integrating these technologies, it enhances not only the monitoring and development of embryos but also the selection of viable embryos for transfer, ultimately improving the success rates of assisted reproductive procedures. This version maintains the original content while smoothly connecting the specific step of oocyte culture to the broader advancements enabled by the embryoscope and Vitrolife's incubation system (Figure 3).

Two embryos, graded as 5 B1- and 5 B2- (two embryos each with 5 cells) according to established criteria, were selected for transfer on day two of development using a cook catheter widely utilized device known for its efficacy and safety in such procedures. Subsequent pregnancy testing yielded positive results, confirming the successful implantation of at least one of the transferred embryos. Beta-HCG was done on 14th march and it was 172 miu/ml, the test was repeated on 16th March and was 532 miu/ml. Overall, the patient's proactive decision to freeze her oocytes enabled her to overcome fertility challenges and achieve successful pregnancy through assisted reproductive techniques.

Figure a, contains detailed information about the morphological grade and dynamic score of embryos.

Discussion

The successful pregnancy resulting from the thawing of frozen oocytes and intracytoplasmic sperm injection (ICSI) with previous-

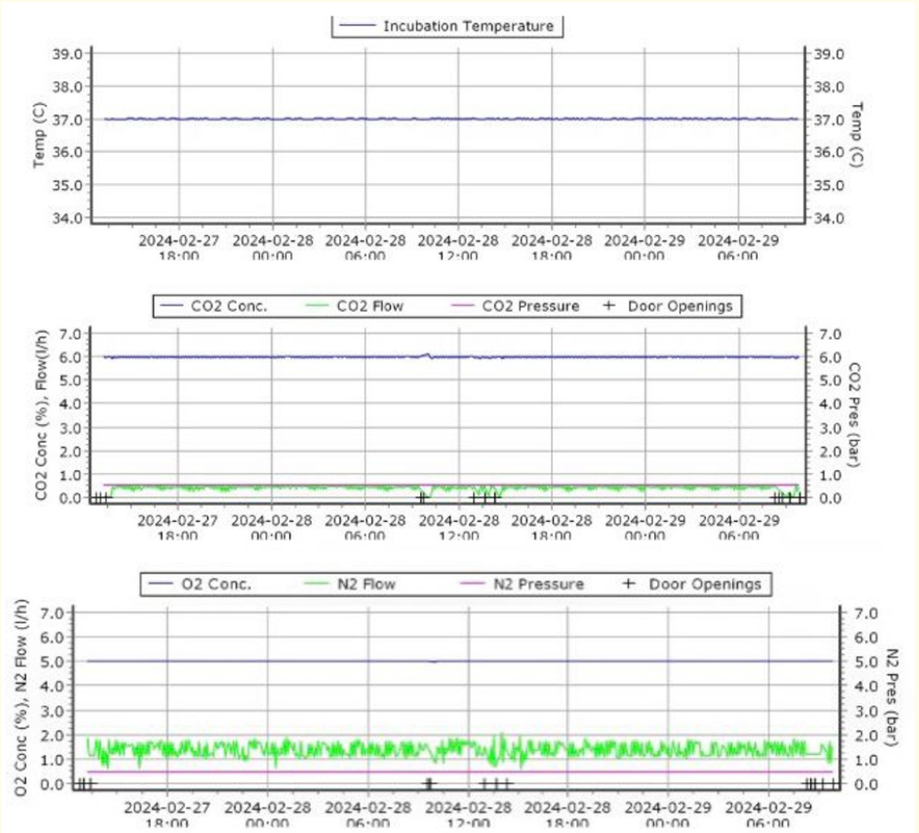

Figure 3: The embryo scope, in tandem with Vitrolife's incubation system, revolutions assisted reproductive technology. Continuous time-lapse imaging and precise environmental control optimize embryo monitoring and development, facilitating better embryo select.

Well	Embryo ID	Embryo Description	Morph. Grade	Dynamic Score	Decision
1	AA1	arrested	Arrested	NA	Avoid
2	AA2	arrested	arrested	NA	Avoid
3	AA3	arrested	arrested	NA	Avoid
4	AA4	5b1-	5b1-	1	Transfer
5	AA5	5b2-	5b2-	1	Transfer
6	AA6	arrested	arrested	NA	Avoid

Figure a: Contains a description of embryos, morphology, and dynamic score using embryo scope.

ly frozen sperm is indeed a rare and noteworthy occurrence, especially in the context of Jordan and the broader Middle East region.

The success of this case can be attributed to several factors. First, the use of advanced cryopreservation techniques, such as vitrification for the oocytes and slow freezing for the sperm, likely contributed to the preservation of gamete integrity. Second, the application of intracytoplasmic sperm injection (ICSI) ensured successful fertilization despite the use of frozen-thawed sperm. Finally, the careful monitoring and management of the patient's cycle and the embryo transfer process were critical in achieving a healthy pregnancy.

Cobo., *et al*. (2016) demonstrated that oocyte vitrification is a safe and effective technique for fertility preservation, with comparable outcomes to fresh oocyte [1] Wu., *et al*. (2022) analyzed the outcomes of ICSI using frozen sperm and found no significant differences in clinical pregnancy rates compared to ICSI using fresh sperm [2].

There have been documented cases of successful pregnancies resulting from the combination of frozen oocytes and frozen sperm using intracytoplasmic sperm injection (ICSI) [3,4]. However, this is the first case reported in Jordan and the Middle East, probably suggesting that the expertise and technology in vitrification, thawing, ICSI, and incubation until success that is used in our lab is comparable to the above.

Despite this success, there are several limitations to consider. As a single case report, these results may not be generalizable to all patients undergoing similar treatment. Factors such as the patient's age, the quality of the frozen gametes, and the specific laboratory protocols used can significantly impact outcomes. Additionally, while this case demonstrates the technical feasibility of using frozen oocytes and sperm, larger studies are needed to fully assess the efficacy and safety of this approach.

The implications of this case extend beyond the individual patient. For cancer patients and others facing fertility-threatening medical treatments, oocyte and sperm cryopreservation offer a means of preserving reproductive potential. Additionally, for couples struggling with infertility, the use of frozen gametes can expand their options for building a family. In regions like the Middle East, where there may be cultural or religious objections to embryo freezing, gamete cryopreservation can provide an ethically acceptable alternative.

This case also highlights the importance of advancing reproductive medicine in the Middle East. By achieving this milestone, we demonstrate the capabilities of our center and pave the way for further innovation and excellence in the region. We hope that this success will raise awareness about the potential of fertility preservation and inspire further research and collaboration in the field.

Conclusion

In conclusion, this case report represents a significant breakthrough in the use of frozen oocytes and sperm for achieving a successful pregnancy. While further research is needed to fully explore this approach, the results underscore the immense promise of gamete cryopreservation for preserving fertility and building families. As the first such case reported from Jordan and the Middle East, it underscores the importance of advancing reproductive medicine in our region and highlights the potential for future innovation and success.

Ethics Approval and Consent to Participate

Not applicable.

Consent for Publication

The patient provided consent for the publication of their data, as well as all the participants in this study.

Availability of Data and Materials

The data and materials used in this study are available upon request.

Competing Interests

- We have no conflicts of interest to disclose.
- All authors declare that they have no conflicts of interest.

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Authors' Contributions

We, the authors of the manuscript titled "Successful Pregnancy Outcome Using Frozen Oocytes and Sperm: A Case Study from Jordan," declare our individual contributions.

- **Dr. Sulieman Ghunaim (First Author, Corresponding Author):** Provided extensive guidance, conceptualization, and oversight. Contributed to writing of the case report, and wrote the discussion section.
- **Rama Karadsheh (Researcher, Writer, Editor):** Contributed to writing the introduction, case report, and conclusion. Gathered case files and information and completed journal-requested edits. Submitted the article.
- **Carla Shannakian (Researcher, Writer, Editor):** Contributed to writing the introduction, case report, and conclusion. Assisted in gathering case files and completed journal-requested edits.
- **Sereen Manna (Writer, Editor):** Contributed to writing the case report. Conducted grammar and other edits.
- All authors reviewed the final manuscript and approved its submission.

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Bibliography

1. Cobo A., *et al*. "Oocyte vitrification as an efficient option for elective fertility preservation". *Fertility and Sterility* 105.3 (2016): 755-764.e8.

2. Wu S., *et al*. "Comparison of pregnancy and neonatal outcomes of intracytoplasmic sperm injection performed with frozen versus fresh testicular sperm". *Translational Andrology and Urology* (2021).

3. R Azambuja., *et al*. "Successful birth after injection of frozen human oocytes with frozen epididymal spermatozoa". *Reproductive BioMedicine* 11.4 (2005): 449-451.

4. Andrea Borini., *et al*. "Cumulative pregnancy rates resulting from the use of fresh and frozen oocytes: 7 years' experience". *Reproductive BioMedicine* 12.4 (2006): 481-486.

5. Petropanagos A., *et al*. "Social egg freezing: risk, benefits and other considerations". *CMAJ: Canadian Medical Association journal = Journal De l'Association Medicale Canadienne* 187.9 (2015): 666-669.

6. "Vitrification and thawing media". Kitazato IVF (2023).