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ASSISTED REPRODUCTION TECHNOLOGIES

mTOR inhibitor sirolimus negatively impacts in vitro fertilization outcomes

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Introduction

The use of a gestational carrier and assisted reproductive technology (ART) can allow women who cannot safely carry a pregnancy to have biologic children. A woman's inability to safely carry a pregnancy is often due to a chronic medical condition. As an increasing number of women with chronic medical conditions are exploring the reproductive option of in vitro fertilization (IVF) with use of a gestational carrier, we continue to expand our knowledge regarding the impact of different medical conditions and treatments on fertility and ovarian biology.

Here we present a case of a patient on immunosuppression following a heart transplant as a child. Specifically, she was on mycophenolic acid (Myfortic, Novartis Pharmaceuticals Corporation) and sirolimus (Rapamune, Pfizer) for immunosuppression. Sirolimus is an inhibitor of mTOR (mammalian target of rapamycin) and is commonly used in patients following solid tissue transplant for prevention of rejection. mTOR is a serine-threonine kinase that is part of a multi-protein complex and pathway widely recognized for its role in cellular metabolism, proliferation, and differentiation [1]. In mice, mTOR has been shown to play a role in spindle function during mitosis and meiosis and actin-dependent division during meiotic maturation [2]. Studies have also demonstrated its role in promoting follicle growth, maintenance of oocyte genomic integrity, completion of meiosis, embryonic development, and fertility in mice [3–5]. In humans, the role of mTOR in follicle growth, oocyte development, and fertility has not been well established.

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Case presentation

A 32-year-old G0 woman presented to the University of California, San Francisco Center for Reproductive Health (UCSF CRH) in February 2017 desiring IVF with the use of a gestational carrier. The patient's history was notable for a heart transplant 22 years prior, after developing non-ischemic myocarditis, dilated cardiomyopathy, and congestive heart failure as a child.

She was started on cyclosporine for immunosuppression following her successful heart transplant in 1995. She remained on cyclosporine for 20 years, before developing stage 3 chronic kidney disease. In 2015, secondary to the development of chronic kidney disease, she was transitioned from cyclosporine 75 mg twice daily, sirolimus 1.5 mg daily, and prednisone 5 mg daily to mycophenolic acid 720 mg twice daily, sirolimus 1 mg daily, and prednisone 5 mg daily.

She was followed with annual cardiac catheterization and at the time of presentation was noted to have an International Society for Heart and Lung Transplantation cardiac allograft vasculopathy grade 1, which is consistent with interstitial and/or perivascular infiltrate with up to one focus of myocyte damage [6]. Graft function was noted to be normal. Functional status was excellent and classified as New York Heart Association class 2. Her kidney function at the time of presentation was stable, with a creatinine ranging from 2.1 to 2.4 mg/dL.

She underwent menarche at age 14 and had no prior pregnancy history or attempts. She had a history of irregular menstrual cycles, but at the time of presentation had been on oral contraceptive pills for 4 years. After discontinuation of oral contraceptive pills, her ovarian reserve was evaluated and her antral follicle count was 14 and AMH 1.3 ng/mL.

A multidisciplinary approach was used in the treatment of this patient and after evaluation by reproductive endocrinology, anesthesiology, and her primary nephrologist and cardiologist, the patient was felt to be a reasonable candidate to undergo controlled ovarian hyperstimulation and egg retrieval.

The patient's primary medical providers, in conjunction with maternal fetal medicine, all agreed that use of a gestational surrogate was the safest option for this patient, following controlled ovarian hyperstimulation and IVF.

The patient's family history was notable for a paternal half-brother with a history of myocarditis as a child and a father with heart disease in his 6th decade of life. In this setting, she was referred to medical genetics and she underwent genetic screening with a comprehensive cardiomyopathy and inherited arrhythmia panel. The panel screened for 113 gene mutations associated with cardiomyopathy. The patient tested positive for two variants of uncertain significance. One was a mutation in the *TNNT2* gene, which codes for the cardiac troponin T protein and has been associated with dilated cardiomyopathy [7–9]. The second was in the *VCL* gene, which codes for vinculin, a cytoskeletal protein associated with cell-cell and cell-matrix junctions and has also been associated with dilated cardiomyopathy [10]. Subsequently, her father and affected half-brother also tested positive for both of these variants of undetermined significance. After extensive counseling and review of the findings by both medical genetics and her primary cardiologist, the *TNNT2* gene variant was felt to more likely contribute to her history of dilated cardiomyopathy. The patient elected to undergo preimplantation genetic testing for monogenic gene defects (PGT-M) for the *TNNT2* gene variant, in addition to preimplantation genetic screening for aneuploidy (PGT-A). The patient did not elect to also screen for the *VCL* gene variant due to concerns that screening for two gene variants would significantly diminish her probability of having an unaffected embryo.

First IVF cycle

Ovarian stimulation was carried out using a GnRH antagonist protocol. The patient underwent priming with oral contraceptive pills for 14 days, followed by initiation of stimulation on cycle day 2 with 225 IU rFSH (Gonal-F, Serono) and 150 IU HMG (Menopur, Ferring). After 12 days of ovarian stimulation, transvaginal ultrasound showed a total of 9 follicles, with 8 follicles ranging from 15 to 19 mm in size. The estradiol concentration on the day of trigger shot was 1269 pg/mL. 10,000 IU of hCG was administered as the trigger shot and 4 oocytes were retrieved with transvaginal follicle aspiration 36 h later.

At the time of egg retrieval, all follicles noted during the ovarian stimulation were present. There was no indication of premature ovulation. During the retrieval, adequate hCG exposure was verified by a positive urine pregnancy test. The retrieval was uncomplicated; however, egg yield was less than expected, as multiple follicles did not yield any oocytes or granulosa cells.

Recovered oocytes were placed in 0.6 mL Global (Life GLOBAL, LGGG-050) supplemented with 10% Life Global Protein Supplement (LGPS-050) overlaid with 0.3 mL of Lifeguard Oil (Life GLOBAL, #LGUA-500). Oocytes were then incubated at 37 °C (7% CO_2, 5% O_2) and assessed for maturity following cumulus cell stripping. The MII oocytes were injected with a single sperm 40–42 h following hCG administration. Zygotes were identified 16–18 h post-insemination and group-cultured (up to 3) in 50 μL of culture medium Global supplemented with 10% LGPS-050 overlaid with 10 mL of Lifeguard Oil. Morphology of each embryo was assessed using standardized institutional scoring criteria.

Only 2 of the 4 collected oocytes were at the MII stage and these were noted to have large perivitelline spaces and one with possible fragmented polar body (Fig. 1). The maturity of the additional two oocytes was GV and parthenogenesis. Frozen sperm sample with the following analysis was used: volume 1.1 mL, sperm concentration 62.0 M/mL, 33% motility, and total motile count of 22 million. ICSI was performed with successful fertilization (2PN) of one of the two eggs. One embryo, grade 5BB, underwent trophectoderm biopsy on day 6 for PGT and was frozen from this cycle. The single embryo returned euploid and did not carry the *TNNT2* gene variant.

Second IVF cycle

After the first IVF cycle, it was hypothesized that the poor egg yield and maturity may be secondary to use of sirolimus, given its impact on the mTOR signaling pathway. Under the guidance of the patient's cardiologist, sirolimus and mycophenolate sodium were stopped and she was switched to tacrolimus and prednisone alone for immunosuppression. Tacrolimus, unlike sirolimus, does not impact the mTOR signaling pathway. Rather, tacrolimus binds to the intracellular protein FKBP-12 and inhibits calcineurin phosphate activity, ultimately preventing production of IL-2 and related cytokines [11].

Eight weeks following discontinuation of sirolimus, the patient again underwent ovarian stimulation using the same protocol as in the first cycle (a GnRH antagonist protocol). The patient underwent priming with transdermal estrogen for 10 days starting in the luteal phase, followed by initiation of simulation on cycle day 2 with 225 IU rFSH (Gonal-F, Serono) and 150 IU HMG (Menopur, Ferring). After 8 days of ovarian stimulation, transvaginal ultrasound showed a total of 19 follicles, with 12 follicles ranging from 14 to 21 mm in size. The estradiol concentration on the day of trigger was 2241 pg/mL. 10,000 IU of hCG was administered as the trigger shot and 15 oocytes were retrieved with transvaginal follicle aspiration 36 h later.

The retrieval was uncomplicated and 10 of the 15 oocytes collected matured to the MII stage. ICSI was performed using a frozen semen sample from the same semen collection used in cycle 1. Six of the oocytes were successfully fertilized (2PN). Culture conditions were identical to those described

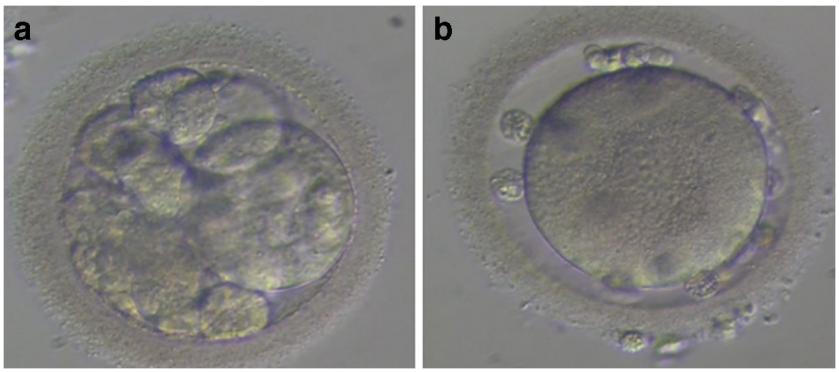

Fig. 1 Images of oocytes obtained from cycle 1. **a** Oocyte with significant fragmentation and **b** oocyte with large perivitelline space, multiple microvesicles, and possible fragmented polar body

in cycle 1 above. Four of the 6 embryos grew to day 5 in culture and underwent trophectoderm biopsy for PGT. The grades of the four frozen embryos were 5BB, 5AB, 5BB, and 5BB. Two of the four embryos returned euploid, one of which was a carrier for the *TNNT2* gene variant and one of which was not.

The unaffected embryo from this second IVF cycle was transferred into a surrogate and resulted in an ongoing clinical pregnancy.

Discussion

There have been numerous prior studies in mice demonstrating the importance of mTOR in spindle function, follicle growth, maintenance of oocyte genomic integrity, completion of meiosis, and embryonic development [1–4]. There is paucity of data regarding the role of mTOR in humans and its relation to fertility. In women, there is very limited understanding of the impact of sirolimus use on ovarian function. There is a single publication reporting a female who developed amenorrhea while on sirolimus after a renal transplant and two women diagnosed with infertility after treatment with sirolimus [12]. Our case, of a woman undergoing ovarian stimulation, provides strong evidence that mTOR does play an important role in oocyte maturation and female fertility, as has been demonstrated in mice. Further, to our knowledge, this is the first report of a demonstrated impact of sirolimus use during ART technology in women.

In men, sirolimus has been associated with altered sex hormone levels, impaired spermatogenesis, and reduced fertility [13–15]. As in this case, following discontinuation of sirolimus, improved fertility has been demonstrated in male patients [13].

As previously shown by Yu et al., inhibition of mTOR results in compromised granulosa proliferation and reduced follicle growth in mice [5]. mTOR links with other proteins to form at least two different multi-protein complexes, mTORC1 and mTORC2. In both of these complexes, mTOR functions as a serine/threonine protein kinase, which promotes protein synthesis at the ribosome. A lack of mTOR phosphorylation of p70 S6 kinase (p70S6K) results in reduced 40S ribosomal protein S6 and eIF4B, two proteins previously shown to be important in the induction of several follicular differentiation markers by FSH [5, 16]. We therefore suspect that the use of sirolimus by our patient in her first IVF cycle led to decreased activity of a similar pathway and ultimately resulted in poor granulosa cell development and or function. This may be supported by the low peak E2 of 1269 pg/mL with 8 mature follicles (158.6 pg/mL) measuring 15 mm or greater in size. The peak E2 in the second cycles was 2241 with 12 mature follicles (186.8 pg/mL per follicle), a slight improvement. Further, the phosphorylation of p70S6 by mTOR is enhanced during the M-phase and therefore inhibition of mTOR may have also contributed to the cycles outcome through incomplete reentry into meiosis and contributed to the poor egg yield and quality observed [5].

The results of the second stimulation were significantly improved, with 10 of the 15 oocytes collected maturing to the MII stage. Six of the 10 MII oocytes were then fertilized (2PN), which is less than our clinic's average rate of 75–80%. While the semen analysis was slightly abnormal with respect to volume and total motile count, it was not significantly abnormal to explain lower fertilization with ICSI. One could hypothesize that perhaps the patient had not fully cleared the sirolimus, with a residual amount negatively impacting fertilization. However, the half-life elimination of sirolimus ranges 46–78 h and it is primarily cleared through the liver. A minimal 2.2% of the drug and its metabolites are excreted through the renal system and thus our patient's chronic kidney disease was unlikely to significantly affect the elimination half-life. Based on this information, sirolimus should be completely cleared from the body within 10–14 days of discontinuing use. We can therefore be reasonably assured that the patient had fully cleared the sirolimus during the 8 weeks between her two stimulations and this is unlikely to have impacted her fertilization rate.

Aside from the change in immunosuppressant agents, the only other change between the two cycles was priming with E2 rather than oral contraceptive pills. While such change

may have a small impact on total oocyte yield, there is no prior data to suggest a significant difference in oocyte quality with these two different methods of priming and therefore this is unlikely to have contributed to the different cycle outcomes [17].

The use of sirolimus during ovarian stimulation is rare; however, its use in solid organ transplant patients is not. Although we present only a single case report, this case does suggest that, when safe, one should consider transitioning a patient on sirolimus to a different immunosuppressive agent, not involved in the mTOR pathway, prior to proceeding with ART. There is also a paucity of data looking at natural conception while on sirolimus and the potential impact of sirolimus on fertility. As such, there is a need for further research focused on understanding the impact of mTOR inhibitors on fertility and oocyte health in women. This is especially true given that quality of life after undergoing transplant is an important patient care issue, which often includes being able to have genetically related children.

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