CASE REPORT

Mitochondrial fatty acid transport enzyme deficiency—implications for in vitro fertilization

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Objective: To describe the individualized clinical and embryologic care required by a patient with adult-onset carnitine palmitoyl transferase II deficiency during IVF treatment and in a subsequent pregnancy.
Design: A case study.
Setting(s): A private fertility unit and public obstetric hospital.
Patient(s): A patient with a rare mutation in the mitochondrial fatty acid transport enzyme, carnitine palmitoyl transferase II.
Intervention(s): The patient received (IVF) fertility treatment and pregnancy care.
Main Outcome Measure(s): Pregnancy and delivery of a healthy child.
Result(s): This patient conceived on her first cycle of IVF and went on to deliver a healthy child.
Conclusion(s): Patients with adult-onset carnitine palmitoyl transferase II deficiency can have successful pregnancy outcomes after IVF, provided clinical and embryologic care is optimized. (Fertil Steril® 2009;91:2732.e11–e14. ©2009 by American Society for Reproductive Medicine.)

Key Words: Mitochondria, carnitine palmitoyl transferase II, IVF, fatty acid transport, embryo culture, pregnancy

A 30-year-old female special needs teacher requested fertility treatment with her partner of 9 years, a merchant seaman. After several years of suffering from exercise-induced muscle cramping and weakness, she had been diagnosed at 14 years of age as having adult-onset carnitine palmitoyl transferase II (CPT II) deficiency based on carnitine palmitoyl transferase studies on both muscle biopsy and cultured fibroblasts (1). She underwent a normal menarche at age 14 years, followed by two menstrual cycles prior to commencing the combined oral contraceptive pill.

On presentation, she had been amenorrhoeic for a year after stopping the pill. Her blood pressure was 125/80 mmHg and her body mass index was 24.8 kg/m^2. A pituitary hormone profile and serum androgens were in the normal range. Baseline serum potassium and creatinine concentrations were mildly increased, consistent with mild chronic renal impairment associated with CPT II deficiency. Serum anti-mullerian hormone and follicle stimulating hormone (FSH) concentrations, together with ovarian volume and antral follicle counts, indicated preserved ovarian reserve. Hysterosalpingogram showed patent fallopian tubes and her partner's semen analysis was normal according to the World Health Organization reference ranges.

Prior to presenting for fertility treatment, she had been advised by her metabolic physician and geneticist (J.L.) of the low risk of having a child affected by CPT II deficiency. An obstetric physician (W.M.H.) had advised that there was no absolute contraindication to fertility treatment.

She was diagnosed as having anovulation because of hypothalamic dysfunction. Polycystic ovarian syndrome was excluded by a normal ultrasound scan and the absence of biochemical or symptomatic evidence of hyperandrogenism. Despite two clomiphene cycles in which ovulation was successfully induced and a subsequent intrauterine insemination cycle using 50 IU FSH (so that stored frozen sperm could be used while her partner was working at sea), a pregnancy did not result. The couple therefore proceeded to IVF and intracytoplasmic sperm injection (ICSI) because of the reduced quality of freeze-thawed sperm.

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This is an unfunded study and this work has not been presented at a meeting.
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To reduce the risk of ovarian hyperstimulation, a low dose of FSH 100 IU daily (Puregon, Organon Australia Pty Ltd) was used in a long down-regulated cycle (MLH). On day 12 of the cycle, serum estradiol was 2.9 ng/mL, and there were three follicles over 18 mm in diameter (10 follicles over 13 mm). An hCG 5000 IU trigger (Pregnyl, Organon) was given 36 hours prior to transvaginal oocyte retrieval (TVOR). Intravenous glucose (dextrose 10%) was commenced prior to sedation for the TVOR and a forced air warming blanket used as previously recommended by an anesthetist (D.N.). On day 15 of the IVF cycle, 11 oocytes were obtained at TVOR.

As CPT II is a nuclear encoded mitochondrial protein, ovarian cells, eggs, and embryos prior to the eight-cell stage have only maternally inherited transcripts, and proteins and can be expected to have a CPT II deficiency. Therefore, to reduce the chance of carbohydrate deficiency for oocytes and embryos, individualized media, containing elevated levels of glucose (increased from 0.5 to 3.15 mM for cumulus oocytes complexes) and pyruvate (increased from 0.35 to 0.5 mM for denuded oocytes and cleavage-stage embryos), were used for oocyte collection and manipulation as well as for embryo culture. Pyruvate provided an additional source of antioxidant to reduce oxidative stress. All cumulus oocyte complexes and embryos were treated individually to minimize carbohydrate depletion in the media. Embryos post-compaction were exposed to standard media, as by that stage of development the embryonic genome is activated and the affected metabolic pathways are reinstated by the paternally inherited genome.

Informed consent was obtained from the patient to investigate the properties of the granulosa and cumulus cells collected as byproducts of their IVF cycle. With informed consent the granulosa and cumulus cells surrounding the oocytes were trimmed and assessed for REDOX state using a fluorescent dye (Redox Sensor Red, Molecular Probes, Eugene, OR). The levels of REDOX imaged in these cumulus masses were significantly higher than those found in the normal population, indicating that elevated intracellular levels of reactive oxygen species were present in these complexes. Additionally, granulosa cell morphology was grossly altered compared to those usually observed in follicular fluid (Fig. 1). Granulosa from the CPT II-deficient patient were consistently observed to have a less well-defined matrix structure, with larger cells seen within the sheet of granulosa.

Ten of the oocytes were in metaphase II of meiosis and were inseminated using ICSI. Nine oocytes fertilized normally and seven developed to seven- to eight-cell embryos on day 3, whereas two embryos showed delayed cleavage rates. After 2 further days of culture, embryo transfer of a single blastocyst (Grade 4AA) (2) was performed. Four additional blastocysts were of a suitable quality for freezing on day 5 (n = 2) and day 6 (n = 2).

Serum β-hCG (297 IU/L) was recorded 16 days post-TVOR, and a viable singleton pregnancy was observed at an 8-week scan. The patient was referred to a high-risk pregnancy team for obstetric management. A plan to manage energy requirements in pregnancy and labor was formulated.

The antenatal course was unremarkable until 20 weeks gestation, when she was admitted to hospital afebrile but with acute-onset right-sided abdominal pain requiring morphine. Rhabdomyolysis was diagnosed by an elevation of serum creatinine kinase (CK) (1,777 U/L) and myoglobinuria. An ultrasound scan revealed an enlarged right ovary (6.45 × 3.5 × 5.28 cm), demonstrating adequate blood flow. The differential diagnosis included transient ovarian torsion, ovarian bleeding or non-ovarian related pain. She was commenced on a 10% dextrose and a sodium bicarbonate infusion. A PCA pump was started for analgesia. With this conservative management her rhabdomyolysis resolved and she was discharged 5 days after admission with ongoing regular follow-up.

At 38 weeks gestation, gestational hypertension or possible mild preeclampsia was diagnosed when she became hypertensive (repeated blood pressure of 130/90 mmHg) with borderline proteinuria (spot urine protein creatinine ratio 29). A decision was made to induce labor, and 2 mg prostaglandin E_2 gel (Dinoprostone, Pfizer Australia Inc., West Ryde, NSW) was inserted per vaginam. An intravenous 10% dextrose infusion was instituted. Good hydration and normothermia was maintained, with regular measurement of temperature and assessment of serum CK, lactate hydrogenase, and electrolytes. Six hours later, labor was established and epidural analgesia was instituted. The membranes then ruptured spontaneously revealing clear liquor with full dilatation being achieved after 2.5 hours. As labor was progressing rapidly, she was permitted to push for a limited period of time, with a view to an instrumental delivery if the second stage of labor were prolonged. A healthy female infant weighing 3,290 g was delivered spontaneously after less than 30 minutes of active pushing, with Apgar scores of 9 and 9 at 1 and 5 minutes, respectively. Apart from a mildly elevated CK (peaking at 496 U/L on day 1 postdelivery), she recovered well and was discharged with her baby 3 days postpartum, having successfully established breastfeeding. Hypertension had settled prior to discharge and she was well 6 weeks postnatally.

DISCUSSION

Carnitine palmitoyl transferase II (CPT II) is an enzyme located on the inner mitochondrial membrane that is responsible for the transport of long chain fatty acids into mitochondria to provide a substrate for beta oxidation and ATP production (3). The adult (myopathic) form of CPT II deficiency is an autosomal recessive disorder resulting from "mild" (missense) mutations in the CPT2 gene located on chromosome 11p32 leading to a reduction of normal CPT II enzyme activity. Although >50 disease causing mutations have been identified to date in CPT2, two mutations, p.S113L and Q413fs, account for ~80% of mutant alleles in individuals with the myopathic form of CPT II (4). Despite