Overnight maturation of a metaphase I oocyte retrieved from a natural cycle using human tubular fluid: a case report

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Introduction

Since the first successful pregnancy and live birth was achieved through natural cycle in vitro fertilization (IVF) in 1978 [1], there have been many technological advancements and refinements of protocols and techniques through the years [2]. Ovarian hyperstimulation was favored over unstimulated cycles, due to the increased number of embryos available for transfer and hence increased success rate. However, stimulated IVF is also associated with side effects such as ovarian hyperstimulation syndrome [3] and it is costly [4].

For patients who have failed to conceive after repeated stimulated IVF cycles, natural cycle IVF may be a cost-saving alternative [5, 6] which allows patients to avoid further exposure to the side effects associated with stimulated IVF cycles [7, 8]. However, one of the drawbacks of pure natural cycle IVF is its higher cancellations rates (28.9 % per cycle) and lower pregnancy rate (0 to 12.5 % per cycle) [5]. The main reasons for cancellation are premature ovulation before oocyte retrieval, failure to retrieve oocytes and retrieval of immature oocytes. The stage of maturation of the oocyte cannot be determined before retrieval as well. When an immature oocyte is retrieved, it is usually discarded and the cycle is cancelled.

To maximize the utilization of the immature oocytes collected from unstimulated cycles, our centre evaluated our protocol and started incubating the immature oocytes overnight in human tubular fluid (HTF) (Irvine Scientific, USA) instead of discarding them. Standard IVF culture media has been shown to be as efficacious as specialized IVM media in ‘rescuing’ immature oocytes retrieved from standard stimulated IVF cycles [9]. When fully matured, the oocytes are fertilized, allowed to develop into embryos in culture and eventually transferred. We report the first case of a successful implantation and pregnancy achieved from this ‘rescue’ protocol in a natural IVF cycle.

Case report

Materials and methods

A 34 year old nulligravid woman with a history of severe endometriosis and her 35 year old husband were referred to Kangar Kerbau Women’s & Children’s hospital (KKWCH) in August 2009 for in-vitro fertilization (IVF) treatment due to the presence of tubal factor infertility and severe endometriosis. She has no known medical or drug allergy history of note and had a regular menstrual cycle. Her surgical history included a laparoscopic ovarian cystectomy in 2007 and laparoscopy, hysteroscopy and hydrotubation in May 2009. Intraoperative findings include Grade V endometriosis, right and left endometriotic cysts, with severe adhesions resulting in a frozen pelvis and bilateral tubal blockage.

Her hormone profile on day 2 of her last menstrual period (LMP) was as follow: FSH 9 U/L, LH 6 U/L and Prolactin 170 mU/L. The sonohysterography performed did not detect any structural uterine problems.

The couple underwent 3 cycles of conventional IVF and 1 natural thaw cycle. The conventional long protocol was used in the first and second cycle and the short protocol was used in

Capsule IVM could be achieved using human tubular fluid.

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the third. All 3 conventional IVF cycles did not result in a clinical pregnancy.

In view of recurrent IVF failure, natural cycle IVF was offered. However, the patient's first natural cycle was cancelled due to premature ovulation.

The following cycle started in March 2012. Ultrasound monitoring started on day 9 of her LMP. Endometrial thickness was 6 mm, follicles of mean diameter 13 and 3 mm were seen in the right ovary and 5, 3 and 3 mm were seen in the left ovary. Urine LH was negative, hormone profile as follows: serum LH 6.94 IU/L, P4 0.44 nmol/L, E2 579 pmol/L. On day 11, sonography revealed a dominant follicle with a mean diameter of 19 mm. Four other follicles were seen in the right and left ovaries but they were all below 8 mm in diameter. Endometrial thickness was 8 mm, urine LH was positive hence a decision was made to retrieve the oocyte on the same day. This was performed about 2 h after the ultrasound assessment.

The retrieval was performed in the operating theatre, under IV conscious sedation (75 mg Fentanyl and 90 mg Propofol). Transvaginal ultrasound-guided oocyte collection was performed with a single-lumen 17-gauge aspiration needle with an aspiration pressure of 100 mmHg. Only the dominant follicle was aspirated. The aspirate was collected in a 14 mL culture tube and the flushing medium consists of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (Irvine Scientific, USA) and 10 % Human Serum Albumin (HSA).

One oocyte was retrieved from the dominant follicle. The oocyte was denuded in an IVF 4 well dish (Nunc, Thermo Scientific) containing Cumulase (Origio, Denmark), HEPES and 10 % HSA. At time of denuding, the oocyte was found to be immature at metaphase I and no polar body was seen. Following the laboratory protocol, it was cultured in Human Tubal fluid (HTF) (Irvine Scientific, USA) at an atmosphere of 6 % CO₂ within the IVF 4 well dish. After 20 h in culture, the oocyte was found to be matured, at metaphase II with a fragmented polar body. Fresh sperm was obtained and intracytoplasmic sperm injection (ICSI) was performed using a micro pipette (The Pipette Company, Adelaide, Australia) and micromanipulator (InjectMan NI 2 Micromanipulator, Eppendorf, Canada). At the same time, luteal support was provided in the form of vaginal utrogestan 200 mg three times daily starting on the day of ICSI.

Results

The oocyte was confirmed to be fertilized 16 to 18 h after ICSI with visualization of 2 pronuclei. Forty eight hours after ICSI, a 4-cell grade 4 embryo was produced (KKWCH's IVF centre embryo scoring system takes into account cell stage, blastomere size and shape, uniformity, appearance of cytoplasm and degree of fragmentation; on a

grading scale of 1 to 5, 1 is poor, 3 onwards is usable and 5 is excellent). Transfer of the day 2 embryo was done on day 3 of oocyte retrieval. Successful implantation occurred. 2 weeks after embryo transfer (ET), the serum β -hCG was 1020.5 IU/L. A transvaginal ultrasound done at 4 weeks after ET showed an intrauterine pregnancy with a crown rump length of 5 mm corresponding to a gestation of 6.2 weeks with positive cardiac activity. At the time of submission of the report, the patient had delivered a healthy baby girl via caesarean section at 34 weeks due to placenta previa.

Discussion

Natural cycle IVF has been proposed as an alternative treatment option for women with normal ovulatory function after poor response or repeated IVF failures. However, the low pregnancy rate (0 to 12.5 % per cycle) is largely hampered by high cancellation rates (28.9 % per cycle), and low numbers of embryo transferred in each cycle. Cancellation is caused by a variety of reasons, mainly premature LH surge and ovulation, failure of oocyte retrieval, and no embryos available for transfer [5]. It is a combination of all these factors that make natural cycle IVF a 'last resort' option of treatment as the repeated cycle cancellations and failures can be frustrating for the patient. In the case described above, the decision to retrieve the oocyte 'early' after detection of LH surge may have lowered the chances of premature ovulation occurring but increases the risk of aspirating an immature oocyte. With overnight maturation, cancellation due to this reason may be potentially averted.

The process of folliculogenesis and oocyte maturation is a complex one that is not fully understood. One of the major challenges of maturing oocytes in vitro is the re-creation of the follicular environment in which the oocyte matures before ovulation. This is crucial in the production of a healthy and developmentally competent oocyte, which would then influence the rates of success in fertilization, implantation and clinical pregnancy. The varying degrees of susceptibility to errors in meiosis under different culture environments [10] and the influence of the composition of in vitro culture medium on the metabolism of immature oocytes [11, 12] further support the above statements.

While a report has shown that human tubal fluid (HTF) is suboptimal in terms of maturation rates, fertilization rate and embryo quality as compared to TCM 199 (an in vitro maturation medium) in women with PCOS undergoing unstimulated cycles [13], the sample size is small and the results only applicable to a subgroup of patients. It has been shown that specific IVM media is no better than commercial IVF culture media (Quinn's Advantage Cleavage Media) in rescuing immature oocytes (at germinal vesical or metaphase I stage) retrieved from standard gonadotrophin stimulated IVF cycles

[9]. Given that HTF is already used in culture, allowing the oocytes to continue maturing in HTF seems to be an inexpensive way to maximize the use of immature oocytes retrieved as well as to avoid cancellation of the cycle.

In conclusion, this report demonstrates that in a natural cycle IVF where there is no proper set up for IVM, HTF (a standard culture media) may be an option as a rescue maturation medium when immature oocytes are retrieved. More studies should be carried out in investigating the efficacy of the ad hoc use of HTF and other conventional IVF culture medium as a maturation medium in natural cycle IVF.

Conflict of interest The authors declare that they have no conflict of interest.

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