Possible deleterious impact of putative gastrointestinal infections in the time period of oocyte recruitment on fertilization after IMSI: Two case reports of consecutive IVF attempts

Dietmar Spitzer · Maximilian Schuff · Martin Zintz ·
Maximilian Murtinger · Astrid Stecher ·
Pierre Vanderzwalmen · Mathias Zech ·
Barbara Wirleitner · Nicolas Herbert Zech

Received: 21 December 2011 / Accepted: 22 February 2012 / Published online: 13 March 2012
© Springer Science+Business Media, LLC 2012

Introduction

Diarrhea is often caused by viral or bacterial infections. Noteworthy, the influences of infections, on oocyte and blastocyst development are not well understood. On the one hand there might be a physical stress situation during the infection or poisoning by endotoxins. On the other hand cytokines or products of cytokine activation, like prostaglandin $F2\alpha$ ($PGF2\alpha$), are accumulated in inflammations and can cause processes, which have a negative impact on blastocyst outcome [6,8,9]. Additionally, hyperthermia an accessory symptom of inflammation is also known to have a detrimental impact on pregnancy outcome [5].

We here describe two cases, where a poor fertilization rate and no blastocyst formation were observed in repeated consecutive IVF attempt, while their other attempts revealed fertilization rates and blastocyst development in the normal range for their corresponding age groups. The first patient experienced diarrhea for 1 day, shortly after starting down-regulation with triptorelin and 15 days before initiating

follicle stimulation with human menopausal gonatropin (hMG). The second patient also contracted diarrhea and singular emesis 3 days after starting follicle stimulation with hMG. We might suppose gastrointestinal infections as a plausible causative factor for the poor oocyte and fertilization outcome.

Case report I

A 36-year-old gravida I, para 0, was referred with her partner to our clinic for the treatment of male infertility in October 2009. The patient had her menarche at the age of 14 and a cycle length of 29 to 32 days. Hormonal profiles measured in October 2009, showed normal values. Sperm parameters of her husband revealed an OAT-syndrome. Therefore IMSI was recommended. The patient underwent three consecutive IVF cycles in our infertility clinic during the last 2 years. Stimulation was performed using a GnRH long protocol. Down-regulation was always performed with triptorelin (Decapeptyl 0.1 mg 1×1 s.c/d) followed by 12, 11 and 12 days, respectively, of hMG administration (3×75 IU ampoules/day) beginning shortly after menstruation. In all three cycles human Chorionic Gonatropin (hCG) was administered when the cohort of follicles reached a diameter of 19 mm. Oocytes were collected 34–35 h after administration of 10,000 IU of hCG. In the first IVF attempt on November 26th, 2009, 16 metaphase II oocytes were collected, of which 15 were fertilized as evaluated 16 h after IMSI. On day three all 16 injected MII oocytes were divided and 5 d3 top-embryos were counted. On day five, seven embryos developed to the blastocyst stage and two top-

Capsule Possible influence of diarrhea on fertilization outcome.

D. Spitzer · M. Schuff · M. Zintz · M. Murtinger · A. Stecher ·
P. Vanderzwalmen · M. Zech · B. Wirleitner · N. H. Zech (✉)
IVF Centers Prof. Zech,
Salzburg, Austria
e-mail: n.zech@ivf.at

N. H. Zech
Department for Obstetrics and Gynecology, Unit of Gynecological
Endocrinology and Reproductive Medicine, University of Graz,
Graz, Austria

blastocysts were transferred back into the uterus. The patient was substituted with progesterone in oil (50 mg/day IM) and estradiol valerate (6 mg/day orally) until the negative pregnancy test 16 days later.

The second IVF attempt was initiated on February 12th, 2010 and stimulation began on March 2nd, 2010. On the day of the oocyte pick-up (March 15th, 2010), a total of 18 oocytes could be collected of which 12 were in metaphase II. All mature oocytes were injected using the IMSI technique and on day one, only three were in the 2PN stage. On day three, 5 embryos were divided, and one d3 top-embryo was counted. None of the fertilized embryos developed to the blastocyst stage on day 5. After re-analysis, the patient remembered that she had diarrhea for 1 day without perceiving fever or having emesis on the third day after starting down-regulation. The patient works in a nursing home and she reported that a couple of the residents had similar symptoms, some additionally with fever and emesis in the same time period. It was speculated that there was an infection with the Norovirus.

Thereafter, the patient decided to attempt a third IVF cycle starting with down-regulation on April 29th, 2010 using the same protocol as in the previous cycles. Thereby 21 oocytes were collected on May 21st, 2010, of which 19 were in metaphase II and injected with sperm using IMSI. Twelve fertilized oocytes were in 2PN stage on day one. On day three, 16 embryos were divided and 7 d3 top-embryos were counted. Seven embryos developed to the blastocyst stage until d5. After transfer of two blastocysts on day five, an intact pregnancy was observed by positive heartbeat of one embryo 6 weeks after surgery.

Notably, the sperm quality (according to [10]) was similar in all three IVF cycles. In all IVF attempts metaphase II oocytes could be fertilized with class one spermatozoa.

Case report II

Whilst writing this article, we recently observed a similar case. A 40-year-old-woman, gravida 0, para 0 and her 44-year-old partner came to our clinic in February 2011. An IVF attempt was started on July 3rd, 2011 with down-regulation using the GnRH long protocol. After 12 days of follicle stimulation with hMG, 12 oocytes could be retrieved of which 8 oocytes could be fertilized and two early blastocysts were transferred on day 5. Urine pregnancy test was positive but unfortunately the patient experienced an early abortion. A second IVF cycle was started using the same protocol 2 month later. However, this time follicle growth was delayed and hMG had to be given for 16 days until initiation of final oocyte maturation. Five oocytes could be retrieved of which two were in metaphase II, two were in metaphase I and one was a GV. One of the two metaphase I

oocytes was in the 2PN stage on day 1 and was cryo-conserved due to a thin endometrial lining. All other oocytes failed to fertilize. After re-analyzing the poor outcome, the patient reported that she experienced a gastro-intestinal infection for 2 days with diarrhea and singular emesis 3 days after starting follicle stimulation with hMG.

Discussion

Although it is still hypothetical that the experienced diarrhea and emesis originated from gastrointestinal infections and the low fertilization rate and blastocyst outcome were the result of these events, it might be the most likely explanation. Impaired oocyte development and reduced oocyte competency could be due to inflammation induced local heat stress and/or the occurrence of inflammatory based immune response such as pro-inflammatory cytokines which affects cell apoptosis, like TNF- α or assist to factors negatively influencing oocyte maturation in manifold ways [8].

In the literature, there are hints that local heat stress experienced not only in the late phase before ovulation but also during the early recruiting state can affect oocyte potential. It has been reported from animals that in periods of heat stress the production of embryos by superovulation is often reduced and associated with extenuated superovulatory response, lower fertilization rates as well as reduced embryo qualities (reviewed in [4]). Thereby, impairments in oocyte development and maturation initiated by a slight increase in the temperature are thought to play crucial roles.

It has been assumed that heat stress affects follicular development, thus compromising oocyte competence for embryonic development after fertilization. Hyperthermia was reported to disrupt the competence of mouse follicle-enclosed oocytes at various stages of folliculogenesis, thus negatively impacting on the developmental competence of developing embryos [1].

However, a putative local hyperthermia might be not the only reason for a poor fertilization outcome after gastrointestinal infection. There are strong evidences for the mammalian system, that local infections, even far outside the reproductive tract—such as mastitis in cows—have a strong negative impact on reproduction [6]. Pro-inflammatory cytokines have the capability to influence early oocyte maturation. Exposure of porcine oocytes to an elevated TNF- α concentration was reported to cause a reduction in their maturation from GV stage to MII stage and increase the proportion of oocytes with abnormal chromosome alignment and cytoskeleton structure [2,7]. In concordance to these observations, TNF- α knockout mice reveal increased fertility [3]. TNF- α is assumed to influence oocyte maturation by decreasing the percent of oocytes that cleave and develop following fertilization [9]. Additionally, among the

molecules that could potentially disrupt oocyte and embryonic function are endotoxins, which are source of diarrhea and emesis in food poisoning and gastrointestinal infections.

Oocyte recruitment and selection spans approximately 2–3 months. In first case the time in between the second and third attempt of IVF/IMSI could thus have sufficed for the patient to sort out impaired follicles. Although these two cases cannot substitute studies with high number cases, we think that it would be important to consider patient's state of health during the time period of follicle stimulation regarding possible infections. Detailed effects of putative infections on follicle development should be monitored. In this context oocyte quality and fertilization outcome need to be elucidated.

References

1. Aroyo A, Yavin S, Arav A, Roth Z. Maternal hyperthermia disrupts developmental competence of follicle-enclosed oocytes: in vivo and ex vivo studies in mice. *Theriogenology*. 2007;67:1013–21.
2. Baka S, Malamitsi-Puchner A. Novel follicular factors influencing oocyte developmental potential in IVF: a review. *Reprod Biomed Online*. 2006;12:500–6. review.
3. Cui LL, Yang G, Pan J, Zhang C. Tumor necrosis factor α knock-out increases fertility of mice. *Theriogenology*. 2011;75:867–76.
4. Hansen PJ. Effects on heat stress on mammalian reproduction. *Philos Trans R Soc Lond B Biol Sci*. 2009;364:3341–50. Review.
5. Hansen PJ, Drost M, Rivera RM, Paula-Lopes FF, Al-Katanani YM, Krininger 3rd CE, Chase Jr CC. Adverse impact of heat stress on embryo production: causes and strategies for mitigation. *Theriogenology*. 2001;55:91–103. Review.
6. Hansen PJ, Soto P, Natzke RP. Mastitis and fertility in cattle—possible involvement of inflammation or immune activation in embryonic mortality. *Am J Reprod Immunol*. 2004;51:294–301. Review.
7. Ma CH, Yan LY, Qiao J, Sha W, Li L, Chen Y, Sun QY. Effects of tumor necrosis factor- α on porcine oocyte meiosis progression, spindle organization, and chromosome alignment. *Fertil Steril*. 2010;93:920–6.
8. Soto P, Natzke RP, Hansen PJ. Identification of possible mediators of embryonic mortality caused by mastitis: actions of lipopolysaccharide, prostaglandin F $_{2\alpha}$, and the nitric oxide generator, sodium nitroprusside dihydrate, on oocyte maturation and embryonic development in cattle. *Am J Reprod Immunol*. 2003;50:263–72.
9. Soto P, Natzke RP, Hansen PJ. Actions of tumor necrosis factor- α on oocyte maturation and embryonic development in cattle. *Am J Reprod Immunol*. 2003;50:380–8.
10. Vanderzwalmen P, Hiemer A, Rubner P, Bach M, Neyer A, Stecher A, Uher P, Zintz M, Lejeune B, Vanderzwalmen S, Cassuto G, Zech NH. Blastocyst development after sperm selection at high magnification is associated with size and number of nuclear vacuoles. *Reprod Biomed Online*. 2008;17:617–27.