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UROLOGY - LETTER TO THE EDITOR

Hormonal stimulation of spermatogenesis: a new way to treat the infertile male with non-obstructive azoospermia?

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Dear Editor,

We herein report a full-term delivery of a healthy child obtained with aid of intracytoplasmic sperm injection (ICSI) in an infertile couple with non-obstructive azoospermia (NOA) and severe endometriosis, following hormonal stimulation of spermatogenesis, cryopreservation of ejaculated spermatozoa using the cell sleeper method, and subsequently ICSI.

Case presentation

A 28-year-old male and his 32-year-old spouse were admitted to The Fertility Clinic Skive due to primary infertility. The couple had tried to conceive for 2 years. The medical history of the male revealed bilateral cryptorchidism and testicles which only fully descended to the scrotum at the age of 12. The reason why the condition was left untreated is unknown. No previous medical treatment or surgery was recorded. The clinical examination of the patient revealed a normal virilized male with both testicles in the scrotum, both having a volume of approximately 8 ml. We noted no abnormalities of the epididymides, and both vas deferens were present and normal. No varicocele was palpable on examination of the spermatic cords. His body mass index was 26.6.

The patient had had his semen examined on multiple occasions, all of which revealed a normal volume ejaculate with azoospermia even after centrifugation and examination of the pelleted sediment. His endocrine profile was within the normal range as follows: FSH level 11.6 IU/l, LH level 8.0 IU/l, E2 level 111 pmol/l, total testosterone (TT) 18.2 nmol/l, prolactin 253 mIU/l, and TSH level 4.08 mIU/l. The genetic analysis showed a normal karyotype 46,XY and no Y chromosome microdeletions. To further explore the azoospermia, a diagnostic testicular sperm aspiration using a fine needle was performed, revealing a few immotile and one motile sperm. As we were able to retrieve spermatozoa by testicular biopsy, tissue was not examined further, so no histopathological diagnosis was set.

After discussing the therapeutic options with the couple, we started medical treatment as detailed in Table 1. Blood parameters were monitored every 3–4 weeks, and the hCG dose was adjusted as needed to keep total testosterone levels between 23 and 29 nmol/l. For this, blood was taken in the morning (around 10 am) and after 2 days of hCG administration. When the basal FSH levels dropped below 1.5 IU/l, we added recombinant FSH. Both hCG and FSH were administered on the same day.

Semen specimens were obtained for analysis on a monthly basis, and after a total of three months of combined therapy with rec-hCG and rec-FSH, a few motile spermatozoa were seen in the ejaculate. Initially, five specimens were frozen using the conventional slow freezing procedure, all of which contained a few motile spermatozoa. However, we were not able to retrieve motile spermatozoa from thawed samples. Subsequently, we changed the cryopreservation method to rapid freezing using a new carrier for small numbers of spermatozoa (Cell Sleeper, Nipro, Japan). A total of five cell sleepers with 26, 17, 13, 36 and 20 motile spermatozoa, respectively, was

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Table 1 Hormone levels during the course of treatment

Medication	Date (treatment duration)	FSH (IU/l)	LH (IU/l)	Estradiol (pmol/l)	TT (nmol/l)	Semen analysis	Action taken
1560 IU rec-hCG[1] × 2we	April 2017 (4 weeks)	1.6	1.6	236	33	NA	hCG dose decreased due to high TT
780 IU rec-hCG × 2we	May 2017 (8 weeks)	3.5	1.9	168	40	NA	No dose adjustment
780 IU rec-hCG × 2we	June 2017 (12 weeks)	1.2	0.7	107	25	Azoospermia	rec-FSH added due to low FSH levels
780 IU rec-hCG × 2we 150 IU rec-FSH × 2we	July 2017 (16 weeks)	2.2	0.5	80.5	10.2	Azoospermia	No dose adjustment
780 IU rec-hCG × 2we 150 IU rec-hFSH[2] × 2we	August 2017 (20 weeks)	1.5	0.5	49.7	9.7	Azoospermia	hCG and FSH doses increased due to low TT and FSH, respectively
1560 IU rec-hCG × 2we 225 IU rec-FSH × 2we	September 2017 (24 weeks)	1.8	< 0.3	230	35	Few ejaculated sperm; sperm freezing started*	hCG dose decreased due to high TT
1040 IU rec-hCG × 2we 225 IU rec-FSH × 2we	October 2017 (28 weeks)	2.4	< 0.3	104	16.3	Few ejaculated sperm; sperm freezing continued	No dose adjustment
1040 IU rec-hCG × 2we 225 IU rec-FSH × 2we	November 2017 (32 weeks)	1.7	< 0.3	111	19.6	Few ejaculated sperm; sperm freezing method changed**	No dose adjustment
1040 IU rec-hCG × 2we 225 IU rec-FSH × 2we	January 2018 (36 weeks)	2.5	< 0.3	111	23	Few ejaculated sperm; sperm freezing continued**	No dose adjustment
1040 IU rec-hCG × 2we 225 IU rec-FSH × 2we	February 2018 (40 weeks)	NA	NA	NA	NA	Few ejaculated sperm; five cell-sleepers stored with motile sperm	Pregnancy achieved by ICSI using frozen-thawed ejaculated sperm; treatment discontinued
Treatment discontinued	March 2018	8.8	7.9	69.2	13	NA	Ongoing pregnancy

× 2we: twice a week

ICSI intracytoplasmic sperm injection, *NA* not available, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone, *TT* total testosterone

*Conventional liquid nitrogen vapor freezing method; **cell sleeper freezing method;

[1]Rec-hCG (Ovitrelle®, Merck) was self-administered subcutaneously (SC) using a pen injector device. The dose was set by turning the dose setting knob clockwise; each 'click' corresponding to ~ 260 IU

[2]Rec-FSH (Gonal-F®, Merck) was self-administered subcutaneously (SC) using a pen injector device. The dose was set by turning the dose setting knob clockwise until the desired dose appeared on the dose display

cryopreserved as previously described (1). The cell sleeper is a novel non-biological device in which individual spermatozoa can be frozen in micro-droplets (Supplementary Fig. 1). In this case, we had a post-thaw sperm recovery rate of 54%. The spouse had four metaphase II oocytes retrieved after controlled ovarian stimulation, which were injected with thawed motile spermatozoa. The two pronuclei (2PN) fertilization rate was 50%, and two blastocysts were vitrified on day 5 of embryo culture. A 4AB blastocyst was transferred in a subsequent frozen/thawed cycle, resulting in the birth of a healthy child.

Discussion and future perspectives

Urologists and reproductive specialists are often challenged when facing patients with severe male infertility scenarios [1, 2]. In particular, the treatment of men with

NOA demands a deeper insight [3]. In such cases, the hormonal stimulation of spermatogenesis is still being explored. Thus, there is still little knowledge regarding the type of patient who might benefit from medical treatment, the optimal medication, the regimen, and the duration of treatment. Moreover, few reports exist regarding the pregnancies and births obtained after treatment. As for the type of azoospermic patient most likely to respond to medical treatment, it seems that males with the combination of basal FSH within normal ranges and hypospermatogenesis or late maturation arrest might respond most optimally to hormonal stimulation with exogenous gonadotropins [4, 5].

In primates, both FSH and testosterone (T) can qualitatively initiate, maintain, and re-initiate spermatogenesis. Under physiological circumstances, only the combination of adequate levels of endogenous testosterone (T) and FSH yields quantitatively normal germ cell numbers [6]. T and FSH act via different mechanisms; FSH acts directly on the Sertoli cells within the seminiferous tubules, and in the immature testis FSH can also stimulate the Leydig cells. In contrast, T acts on spermatogenesis indirectly via somatic testicular cells in the final phase of spermatogenesis. T is further converted to dihydrotestosterone (DHT) and estradiol; however, the role of estradiol in spermatogenesis remains unclear. Finally, LH acts on Leydig cells, inducing the synthesis and release of T.

Currently, hormonal stimulation of spermatogenesis is based on basic physiology, whereas the potential beneficial role of medications remains unclear [4]. It is generally believed that medical treatment is ineffective in the presence of high circulating gonadotropin levels. However, the FSH and LH pulse amplitudes are relatively low in such cases, resulting in low endogenous circulating T as well as intratesticular testosterone (ITT) levels [7]. In particular, the low ITT level is incapable of stimulating the spermatogonial DNA synthesis and spermiogenesis properly in patients with residual spermatogenic activity [7].

Several drugs like clomiphene citrate, tamoxifen, urinary and recombinant gonadotropins have been proposed by different authors (reviewed by Esteves [3]); however, based on a recent study [5], it seems rational to initiate treatment with hCG, which is very similar to native LH in molecular composition. The aim is to increase serum T and ITT levels and through negative feedback of the pituitary to suppress endogenous FSH thus up-regulating FSH receptors.

In the present case, the level for initiation of rec-FSH treatment was set at < 1.5 nmol/l [4]. The serum T level was kept under the upper limit between 23 and 29 nmol/l, according to the recommendations of a previous study [8]. As for duration, it may take two or more months before basal FSH levels decrease below 1.5 IU. Subsequently, treatment with rec-FSH and hCG should continue for at least 3 months to optimally support spermatogenesis in consecutive spermatogenesis cycles.

Previous experience shows that serum FSH levels around 3 UI/l are suitable to sustain spermatogenesis [3]. In the present case, rec-FSH, 150 IU, twice weekly, was used as a starting dose for 7 weeks and the dose was subsequently increased to 225 IU, twice weekly; however, others proposed a higher dose of 300 IU every other day for 4–5 months [9]. As for the increased peripheral serum T conversion to estradiol, which may be eventually seen after hCG treatment, this might impact the ITT level due to the inhibitory effects of E2 on the pituitary. For this reason, an aromatase inhibitor could be beneficial to maintain high circulating T as well ITT levels [3]. However, in the present case, we did not apply an aromatase inhibitor because the T/E2 ratio remained adequate.

We initially used the conventional slow-freezing method, but it turned out to be unsuccessful due to the inability to retrieve motile sperm after thawing. In conventional freezing protocols, dilution of semen with relatively large cryoprotectant volumes can make it difficult to find viable sperm post-thawing in cases where few spermatozoa are present in the neat ejaculated sample. Thus, post-thaw sperm washing and centrifugation are required to concentrate the few spermatozoa, but these steps may harm sperm motility and viability, making ICSI more challenging. In contrast, the cell sleeper method in our case proved to be a reliable method with a high sperm recovery rate after thawing in line with previous reports [4, 10].

An important clinical question to ask is how many sperm samples should be frozen before the long and expensive treatment of the male is terminated? Until now, there are no clinical recommendations; however, in the present case we cryopreserved a total of five cell sleepers which should secure the chance of providing the couple with more than one child without having to treat the male again.

Conclusions

This report describes successful hormonal stimulation of spermatogenesis in a non-obstructive azoospermic male, in whom individual spermatozoa were frozen on cell sleepers. With this procedure, the couple subsequently obtained a live birth after their first ICSI embryo transfer. Although more cases are needed to draw conclusions, we suggest that the combination of male hormonal stimulation, the cell sleeper method, and ICSI treatment might give new hope for biological parenting in such couples, who would otherwise be referred to either sperm donation or adoption.

Compliance with ethical standards

Conflict of interest None.

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