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☐ TITLE

Is immunotherapy effective on the treatment of secretory azoospermia ?

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☐ DISCLOSURES RELEVANT TO THIS PAPER

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☐ STRUCTURED ABSTRACT

Aims of the study

To assess the effectiveness of medicine and immunotherapy on secretory azoospermia.

Methods used to conduct the study

The husband with azoospermia was 63 years old and his 35-year-old wife presented secondary infertility for 5 years. The male had operation and chemotherapy of stomach cancer five years ago. After operation and chemotherapy,the male made the treatment on immunotherapy until today. In this treatment, andriol and aescuven forte was given. The total treatment were fourteen months. Semen, blood hormone and pregnancy were detected.

Results of the study Six months after treatment, sperm appeared in the semen（2018-12-13：sperm concentration 3×106/ml，PR 0%），ICSI was made while ET was failure. 1 year later, the semen was changed greatly (2019-01-29：sperm concentration 43×106/ml，PR 15%，acrosin 43.1μIU/106), second ICSI was made and TET was failed. In May,2020, third cycle ICSI was made,frozen embryo was transplanted in December and in March the wife was conceived for morn than four months. ☐

Conclusions

We have discussed and published the medicine treatment of azoospermia in 2017 while it is the first time to find the relationship between the spermatogenesis and immunotherapy. Form this

case, it could been concluded that Aescuven Forte with Testosterone Undecanoate is good for the patient with testicle dysfunction while the time of medicine treatment is better for more than six months. Immunotherapy might be good for spermatogenesis and further study needs to be made.

☐ WHAT'S KNOWN? (what is already known about this subject?)

The medicine treatment of azoospermia has been published by us in 2017 on AJM. It is already known that Aescuven Forte was good for azoospermia.

☐ WHAT'S NEW? (what does this study contribute to the literature?)

Though we had known that Aescuven Forte was good for azoospermia, the treatment about the testicular failure is still hard to the andrologist. This study contributed new way to the literature which is Aescuven Forte with Testosterone Undecanoate to treat patient with azoospermia. On the other side, this study also contribute to the literature that immunotherapy might be helpful to spermatogenesis.

☐ MAIN BODY OF MANUSCRIPT, INCLUDING FIGURES AND TABLES

Though we have published an article to talk about some effective treatments of secretory azoospermia[(1)], the management is still a challenging especially for the cases with complicated medical background or the cases with loss of spermatogenic cells and extremely elevated follicle-stimulating hormone (FSH) level. In this report, we describe a successful treatment for a secondary SA patient who is undergoing cell immunotherapy after stomach cancer resection and chemotherapy. Our findings may contribute to the determination of treatment strategy for refractory SA patients.

Case Presentation

1.Basic information

A male patient aged 63 years and his 35-year-old wife presented with the complaints of secondary infertility for 5 years though they had a son together in 2014. The patient was treated with stomach cancer resection followed by six cycles of chemotherapy in 2016. After chemotherapy, he continued cell immunotherapy to date. The male partner also had a history of hypertension and diabetes for 20 years and 10-year androgen replacement therapy for climacteric symptoms.

2.Test Results

The first test of the patient's semen indicated semen volume with 0.6ml. There were no sperm detected in the semen after centrifugation (table 1)and in the follow-up examination of urine after

ejaculation. All seminal biochemical indicators revealed normal value. Other laboratory evaluations indicated a normal total testosterone level, elevated serum FSH concentration while PSA in serum with 1.397ng/ml concentration(table 2). Scrotum ultrasound revealed no abnormality.

Treatment

3.1Fertility treatment

In prevent of the testosterone negatively regulate FSH, the first treatment was to stop androgen replacement therapy and to take Aescuven Forte (300mg,bid.Cesra Arzneimittel Gmbh & Co. KG). Three months later, semen volume was increased while there were still no sperm in the semen. At the same time, serum FSH level was increased while the T level reduced.

Because the laboratory evaluation after the initial treatment indicated an abnormally increased serum FSH level and decreased T level, androgen was added. Te final fertility treatment was set as Aescuven Forte (0.3,bid)with Testosterone Undecanoate (Zhejiang Medicine Co. Ltd., 40mg , bid) .The total treatment time was 14 months.

3.2 Cord Blood Immunotherapy

The patient was treated with stomach cancer resection followed by six cycles of chemotherapy in 2016. Accompany with chemotherapy,he took immunotherapy at the same time. After the chemotherapy, he continued cord blood immunotherapy to date.

3.2.1 The treatment during chemotherapy

In order to cooperate with the chemotherapy, cell immunotherapy was given every 21 days. For each course of treatment, 30 billion autologous immno cells were transfused, which were completed in three times (every two days per time).

3.2.2 The treatment after chemotherapy

Cord blood immunotherapy after chemotherapy was given every month. For each course of treatment, 30 billion autologous cells were transfused, which were completed in three times (every two days per time).

All the treatment has been approved by the patient.

4Results

4.1

Six months after fertility treatment, sperms began to appear in the semen of the patient (table 1). Intracytoplasmic sperm injection (ICSI) had been employed in March of 2019. In vitro fertilization process began with a long-term ovarian stimulation, from which ten ova were harvested. Then eight were fertilized via ICSI, and two formed available embryos. However, the embryos arrested development in August the same year after tubal embryo transfer (TET). During the process of ICSI, the fertility treatment for the male patient was adjusted to oral Testosterone Undecanoate only. Because of the failure of TET, the patient took oral Aescuven Forte again for preparing a second cycle of ICSI. According to the results of semen analysis in November, the quality of the sperms was improved obviously(table 1).

4.2

Second ICSI was performed in 2020. The laboratory evaluation indicated that the quality of the sperms was improved obviously (table 1,3). From the process of long-term ovarian stimulation, 15 ova were harvested. Seven formed available embryos, and then five of which undergone blastocyst culture. Unfortunately, the process of TET was failed. The wife of the patient was in preparation for the next cycle of TET, and the patient stopped using oral Aescuven Forte.
In May,2020, third cycle ICSI was made,frozen embryo was transplanted in December and in March the wife was conceived for morn than four months.

Tabel 1 Follow-up of semen parameters in this patient

Time	Volume (ml)	Concentration ($\times 10^6$/ml)	PR+NP (%)	PR (%)	PH	Acrosin ($\mu IU/10^6$)
2018-05-29	0.6	0	0	0	7.3	
2018-06-08	1	0	0	0	7.4	
2018-07-10	1.4	0	0	0	7.3	
2018-09-22	1.4	0	0	0	7.7	
2018-12-13	1.4	3	0	0	7.3	
2019-02-11	0.9	2	0	0	7.4	
2019-03-04	0.8	4	11	2	7.2	
2019-11-12	1.5	43	30	15	7.4	43.1

Table 2 Follow-up of serum hormone concentrations

Time	FSH	LH	T	E2
2018-06-14	18.80	3.82	1.92	12.98
2018-07-17	22.4	5.43	1.20	<20
2019-11-29	21.3	4.57	2.22	<20

Table 3 Comparison of semen parameters in ICSI

Time	Semen masturbated			Semen treatment in vitro		
	Vomume (ml)	Concentration ($\times 10^6$/ml)	PR+NP(%)	volume (ml)	concentration ($\times 10^6$/ml)	PR+NP(%)
2019-03-15	0.7	3	0	0.1	1	0
2020-01-12	0.5	30	20	0.1	20	60

2020-04-14	1.0	1	1	o.4	7/HP	1/HP

Discussion

Through the analysis of the case, we obtained some clues of the treatment strategy for refractory SA patients.

The treatment for SA had been described in detail in our previous article(1). In this report, we mainly focus on three points, including the treatment course of SA, the role of FSH in evaluating the process of spermatogenesis, and the influence of cellular immune therapy on spermatogenesis.

The course of treatment is one of the principal factors which influence the therapeutic effects of fertility treatment. For humans, the duration of spermatogenesis cycle is variously estimated as taking 64 days which can be subdivided into four phases which last differing lengths of time. The last phase is called spermiogenesis. During this phase spermatids develop into mature, motile spermatozoa, also known as sperm cells. And this process takes 16 days. Considering the transport on ductal system, the minimum treatment course should take more than three months. In fact, for the patients suffering from oligospermia, because there are spermatogenic cells at different stages staying in seminiferous tubules, one-month treatment may bring an obvious improvement of semen quality; however, for the patients suffering from SA, because of the absence of spermatogenic cells at most spermatogenesis stages, the treatment should be a longer-term course. In this case, the sperm cells could be detected after around seven months of treatment. Then the count of sperm cells remained at a relatively stable level for five months. A further improvement of semen quality happened after twelve months of treatment.

FSH plays an important role in the process of diagnosis and treatment of SA. It has been suggested that normal dynamics of FSH is one of the necessary prerequisites of mormal spermatogenesis. FSH acts on the Sertoli cells of the seminiferous tubules to maintain the spermatogenesis. Then the activated Sertoli cells not only nourish the developing sperm cells, but also produce the inhibin B which suppresses FSH secretion from the pituitary gland. In the condition of spermatogenic failure, the production of inhibin B will be inhibited and the level of plasma FSH will increase. So the level of plasma FSH is also used as a gold standard of the diagnosis of SA. In the case, FSH had been tested three times. For the first time, the plasma FSH was at a high-normal level. Considering the medication history of the patient, the long-term

androgen replacement therapy might cause feedback inhibition on the hypothalamic-pituitary function, which influenced the elevation of FSH. This assumption was proved by an abnormally increased FSH in the second test one month after stopping androgen replacement therapy. Because the level of plasma androgen was also decreased into lower than normal, the supplement of androgen was given again. After a half-year treatment, sperm cells could be detected in the semen, which had been frozen for further use. After another two-month treatment, ICSI was employed, and available embryos were formed after in vitro fertilization. But because of the failure of TET, the treatment was postponed until the second treatment cycle in September of the next year. In November, after two-month re-treatment, the quality of sperms was obviously improved in the semen analysis. But there were very few improvements in the results of sex hormone analysis. Spermatogenesis is a complicated process. Our knowledge about it is quite limited. Though the changes of FSH level can't be used as an independent criterion for evaluating and analyzing the process of spermatogenesis, the FSH analysis is still an important criterion for the diagnosis and treatment of SA.

Whether the cell immunotherapy influenced the spermatogenesis or not is the question we are concerned about the most in this case. From the perspective of the treatment course, the first 6-month treatment successfully brought an improvement of the semen quality: the sperm cells could be detected in the semen samples, which means the fertility treatment given to the patient was effective. Unexpectedly, there was a significant improvement of semen quality after re-starting the fertility treatment, though there was a 3-month long pause before re-starting the treatment. Based on our previous experience, in some other cases, the effect of fertility treatment might last for a relative long time even after stopping the treatment. But for these patients, the quantity and quality of the sperm cells were always stable in that period. At a minimum, the two-step improvement of semen after treatment in this case is the first case to be reported. It is still unclear whether the improvement of semen has an close relationship with cord blood immunocyte import and whether the these immune cells import influenced the process of spermatogenesis or not. And there are still a lot of cases need to be collected and researches need to be conducted to explain this question.

Recently, researches about the influence of stem cell therapy on the process of spermatogenesis are limited only in animal researches[2,3]. Though some researchers have observed the

promotional effect of stem cells on spermatogenesis from studies on mouse[4], there is no published article about the influence of stem cells on spermatogenesis in clinical trials. In fact, for researches about the influence of stem cell therapy on spermatogenesis, there is still a big gap between animal research and clinical trials: on one hand, there are a lot of ethical problems need to be solved before clinical trials; on the other hand, there are vital biological questions need to be answered, such as how to give stem cell therapy to patients, where these stem cells will be transported, and what is the final destination of these stem cells. In fact, stem cells can not entry testicurlar tissue easily because of blood testis barrier only when the stem cells being injected into the testis. On this way, immune cells might be beneficial to the spermatogenesis.

However, there is no paper reported the relation between spernatogenesis and immune cells. Fortunately, we got the chance. In the current case, cell immunotherapy was necessary for the male's follow-up treatment of cancer. After he started fertility treatment, there was an overlap between these two treatments. Though the first improvement of his sperm quality happened after fertility treatment, the further significant improvement can't be wholly explained by oral medication, which made us focus on the influence of the combination between fertility treatment and immunotherapy.

According to a recent publication, the mechanism of cell immunotherapy is eliminating the inflammatory cells and balancing the immune system. In this way, immune cells might be beneficial to spermatogeneis. It should be conformed that immune cells is not bad to spematogenesis. However,we do not know how much degree the immune cells give the benefit to spermatogenesis. And we do not know by which way the immune cells make the benefit to the spernatogenesis. So it needs the further step to study the phenomenon. This case just give a new research direction, that is the influence of immunotherapy on the process of spermatogenesis.

5. Conclusion

Spermatogenesis is a complicated process. At present, knowledge about the mechanism and regulation is still limited.

We have discussed and published the medicine treatment of azoospermia in 2017[1] while it is the first time to find the relationship between the spermatogenesis and immunotherapy.

For this case, it could been concluded that for the patients with SA,especially the patient with testicular dysfunction, medical treatment would be a long-term process, the effective treatment

course might be ranging from six to twelve months. Besides, during the process of treatment, the level of FSH plays an important role in evaluating the testicular function. The therapeutic influence of immonotherapy on the process of spermatogenesis still mystical to us at present. Further study is still necessary to investigate.

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