

Investigation for Anxiety and Depression Situation in Couples Receiving in vitro Fertilization and Embryo Transfer (IVF-ET) with Donor Sperm and Associated Influencing Factors

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Background: This study aimed to explore the current situation of anxiety and depression on day of transfer in couples receiving in vitro fertilization and embryo transfer (IVF-ET) with donor sperm and analyze its influencing factors.

Methods: This study selected 187 IVF-ET administered couples who received donor sperm from our Hospital from August 2021 to July 2022. On the day of IVF-ET with donor sperm transplantation, patients were investigated with general data questionnaire, self-rating anxiety scale (SAS) and self-rating depression scale (SDS) to analyze their anxiety and depression scores and their influencing factors.

Results: Scores of anxiety and depression on the day of transplantation in patients receiving IVF-ET with donor sperm were 43.98 ± 6.80 and 46.03 ± 10.61 respectively, which were higher compared to levels of Chinese health norm ($P < 0.05$). The anxiety score of patients' spouses was 41.23 ± 6.69 and depression score was 44.23 ± 11.65 , which were higher compared to levels of Chinese health norm ($P < 0.05$). Score of anxiety and depression of women was significantly higher compared to those of their spouse ($P < 0.05$). Anxiety and depression scores of women in non-pregnant group were significantly higher compared to those in pregnant group ($P < 0.05$). Regression analysis showed that education level and annual family income were influencing factors of anxiety and depression scores of IVF-ET with donor sperm couples on the day of transfer, and number of eggs taken and transfer times were influencing factors of depression scores of IVF-ET with donor sperm patients on the day of transfer.

Conclusion: Psychological state of couples receiving IVF-ET with donor sperm was significantly affected, especially in female side. Medical staff should focus on patients with low education level, low family income, and more times of transfer and egg retrieval, and take targeted intervention measures to keep them in good psychological state, which is conducive to improving pregnancy outcome.

Keywords: in vitro fertilization and embryo transfer with donor sperm, day of transfer, anxiety, depression, psychological state

Introduction

According to statistics, the proportion of infertile couples in the world is about 10%–15%.^{1,2} Among infertile couples, male factors account for about 30%–40%, while azoospermia accounts for about 7%–14% of male infertility.³ For irreversible azoospermia patients with female infertility, conventional in vitro fertilization and embryo transfer (IVF-ET) after artificial insemination with donor sperm (AID) treatment is ineffective.⁴ IVF-ET with donor sperm refers to the process of placing the donor sperm and the extracted egg in vitro to fertilize them, and then transferring the developed embryo back to the mother's womb to develop it into a fetus.⁵

For most couples, infertility is a kind of psychological trauma, and the treatment of infertility is also a kind of psychological stress. A large number of studies at home and abroad show that couples receiving IVF-ET generally have anxiety and depression, and women's anxiety and depression are more serious than their spouses during the treatment cycle.^{6–8} In addition to the particularity of sperm source of couples receiving sperm donation and assisted pregnancy, the psychological burden of their women is more obvious and complex than other infertility, and many economic, social, marital and family problems cause their anxiety and depression.⁹ Some domestic scholars have reported that the anxiety level at different treatment stages of IVF is related to the treatment outcome, especially the standard score of anxiety during embryo transfer is significantly negatively related to pregnancy, indicating that anxiety affects the treatment effect to a certain extent and reduces the clinical pregnancy rate.¹⁰ Good psychological state is one of the important factors to improve clinical pregnancy rate and pregnancy outcome.

This study is to investigate the anxiety and depression of the couples who received IVF-ET with donor sperm on the day of transfer. At the same time, the influence of demographic sociological factors on their anxiety and depression was discussed. This paper will provide scientific basis for improving the psychological status of the couples who have received IVF-ET with donor sperm.

Materials and Methods

Subjects

This study adopted the convenient sampling method. In this study, 187 couples who received IVF-ET with donor sperm from the Reproductive Center of Guangdong Provincial Reproductive Hospital from August 2021 to July 2022 were selected. Inclusion criteria: ① Having full cognitive and behavioral abilities, being informed and willing to participate in this study. ② No history of serious physical disease, mental disease and nervous system disease. ③ No history of major psychological trauma. ④ Couples of patients on the day of transplantation in tube cycle management.

General Surgery Parameters

The general surgery parameters, including education level, age, number of husband visits, occupational status, annual family income, number of eggs taken, number of transplants and number of births, were collected and analyzed in this study.

Self-Rating Anxiety Scale (SAS)

SAS scale was compiled by Zung in 1971.¹¹ SAS scale is used to measure degree of individual anxiety, which can better reflect the subjective anxiety of subjects in the past week. The SAS scale includes 20 items and adopts a four-level scoring method. All items are assigned 1–4 points from “no or little time” to “most or all time”, of which 15 items are scored forward and 5 items are scored backward. The anxiety standard score (rounded) was adopted and assigned as rough score (sum of all item scores) \times 1.25,¹² the higher the score, the higher the anxiety level. The results of domestic norm showed that no anxiety was 25–49, mild anxiety was 50–59 points, moderate anxiety was 60–69 points, and severe anxiety was 70–100 points.¹³ In this study, Cronbach α coefficient was defined as 0.825.

Self-Rating Depression Scale (SDS)

SDS scale was compiled by Zung¹¹ in 1965, to evaluate the level of individual depression. SDS has been widely used in the screening of outpatients, evaluation of emotional status, investigation and scientific research. SDS scale consists of 20 items, of which 10 items are positive and 10 items are negative. All entries are assigned 1–4 points from “none or occasionally” to “always”. The depression standard score (rounded) was adopted and assigned as rough score (sum of all items) \times 1.25.¹⁴ The results of the Chinese norm showed that the cut-off value of SDS was 53 points, mild depression was 53–62 points, moderate depression was 63–72 points, and severe depression was 73–100 points. The higher the score was, the more serious the degree of depression was. In this study, Cronbach α coefficient was defined as 0.877.

Data Collection Method

After obtaining the consent of the patient couples, the researcher who has received professional training will distribute the questionnaire and carry out the measurement. The purpose and significance of the study were explained to the couples and the confidentiality of the data was explained using the unified guidance. After obtaining informed consent, the questionnaires were distributed, and the couples were filled out independently and recycled on the spot. A total of 202 questionnaires were issued and 187 valid questionnaires were recovered, with a feedback rate of the questionnaires of 92.6%.

Statistical Analysis

The SPSS 20.0 software was used to analyze the data. The scores of anxiety and depression of subjects with different characteristics were compared using two independent samples Student's *t* test or one-way ANOVA. The IVF with donor sperm patients were grouped according to the pregnancy outcome, and the standard scores of anxiety and depression on the day of transplantation were compared with the Chi-square test. The *p* level less than 0.05 is defined as significant difference.

Results

Anxiety and Depression Scores of Couples Receiving IVF-ET with Donor Sperm

The standard score of anxiety and depression on the day of transplantation in patients administered IVF-ET with donor sperm was 43.98 ± 6.80 and 46.03 ± 10.61 respectively, which were significantly higher than the literature norm (29.78 ± 10.46 for anxiety and 41.88 ± 10.57 for depression)¹⁵ (Figure 1, $P < 0.05$). The standard score of anxiety and depression of the spouse of the IVF-ET with donor patients was 41.23 ± 6.69 and 44.23 ± 11.65 respectively, which were significantly higher than the literature norm level¹⁵ (Figure 1, $P < 0.05$). Compared with the anxiety and depression of the couple on the day of transfer, the anxiety and depression score of the female was significantly higher than that of her spouse, the difference was statistically significant (Figure 1, $P < 0.05$).

Comparison of Anxiety and Depression Scores of Patients Receiving IVF-ET with Donor Sperm with Different Characteristics on Transplant Day

In this paper, anxiety and depression scores were used as dependent variables, and general data of patients were used as independent variables for univariate analysis. The results showed that there was a statistically significant difference in

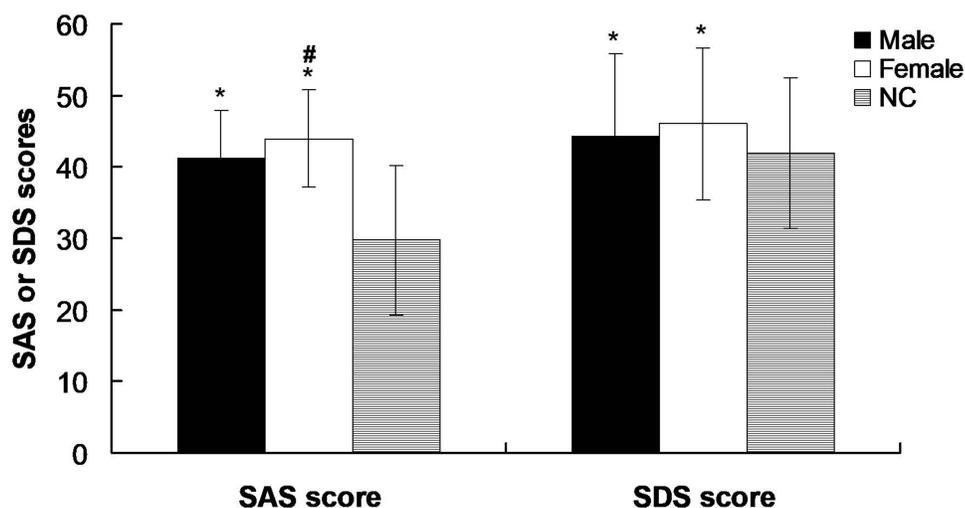


Figure 1 Comparison between anxiety and depression scores of couples receiving IVF-ET with donor sperm and Chinese health norm. IVF-ET: in vitro fertilization and embryo transfer. * $p < 0.05$ versus NC group, # $p < 0.05$ versus Male group.

Abbreviations: SAS, self-rating anxiety scale; SDS, self-rating depression scale; NC, normal control.

SAS anxiety scores among patients receiving IVF-ET with donor sperm with different education levels ($X^2=4.068$, $P=0.019$) and different family income ($X^2=3.576$, $P=0.015$) (Table 1). There were statistically significant differences in SDS depression scores among patients receiving IVF-ET with donor sperm with different education levels ($X^2=5.269$, $P=0.006$), different family income ($X^2=6.998$, $P=0.000$), and different times of egg retrieval ($X^2=4.365$, $P=0.038$) and transplantation times ($X^2=3.970$, $P=0.021$) (Table 1). Therefore, the education levels and family income are factors affecting anxiety and education levels, family income, egg retrieval times, and transplantation times are factors affecting depression, for the patients receiving IVF-ET with donor sperm.

Comparison of Anxiety and Depression Scores of Spouses of Patients Receiving IVF-ET with Donor Sperm with Different Characteristics on Transplant Day

The scores of anxiety and depression were used as dependent variables, and the general information of the patient's spouse was used as independent variables for univariate analysis (Table 2). The results showed that there were significant differences in SAS anxiety scores between spouses of patients receiving IVF-ET with donor sperm with different

Table 1 Comparison of Anxiety and Depression Standard Scores of IVF-ET with Donor Sperm Patients with Different Characteristics of Donor ($n=187$, mean \pm SD)

Items	n	SAS Scores	t/ X^2	p	SDS Scores	t/ X^2	p
Education degree			4.068	0.019*		5.269	0.006**
Junior high school/below	71	45.08 \pm 6.89			49.07 \pm 10.77		
Technical secondary school/high school	50	44.50 \pm 6.68			44.27 \pm 10.23		
College degree or above	66	41.70 \pm 6.40			43.86 \pm 9.90		
Age (years)			0.409	0.665		0.414	0.662
29	66	44.47 \pm 6.22			46.56 \pm 9.65		
30–34	79	43.46 \pm 7.00			45.14 \pm 10.74		
≥ 35	41	44.13 \pm 7.44			46.59 \pm 11.86		
Accompanied by husband (times)			0.356	0.701		0.094	0.91
0–5	22	43.81 \pm 6.88			45.11 \pm 10.58		
6–10	36	44.84 \pm 7.10			46.25 \pm 10.81		
>10	129	43.77 \pm 6.73			46.12 \pm 10.64		
Occupational status			1.488	0.208		0.531	0.713
Company or enterprise	46	42.41 \pm 6.69			44.39 \pm 9.41		
Government/public institution	23	45.08 \pm 6.78			44.74 \pm 11.80		
Temporarily unemployed	47	43.33 \pm 7.00			46.66 \pm 10.70		
Liberal professions	39	44.46 \pm 6.58			46.55 \pm 10.64		
Self-operated/self-employed	32	45.77 \pm 6.73			47.27 \pm 11.19		
Annual household income (ten thousand)			3.576	0.015*		6.998	0.000**
<5	50	46.46 \pm 7.27			50.22 \pm 10.69		
5–10	89	43.55 \pm 6.70			46.42 \pm 10.23		
11–30	37	42.24 \pm 6.12			41.33 \pm 10.13		
>30	11	42.05 \pm 4.72			39.66 \pm 5.31		
Number of eggs taken (times)			3.035	0.083		4.365	0.038*
1	146	43.52 \pm 6.83			45.18 \pm 10.40		
≥ 2	41	45.60 \pm 6.51			49.06 \pm 10.91		
Transplantation (times)			0.444	0.642		3.97	0.021*
1	91	43.88 \pm 7.05			45.59 \pm 10.45		
2–3	83	43.82 \pm 6.49			45.29 \pm 10.05		
≥ 4	13	45.69 \pm 7.16			53.88 \pm 12.79		
Number of births			–0.25	0.803		0.155	0.877
0	128	43.89 \pm 6.73			46.03 \pm 10.09		
>1	59	44.16 \pm 7.01			45.77 \pm 11.59		

Notes: * $p < 0.05$ and ** $p < 0.01$ represented significant significances for comparison between groups.

Abbreviations: IVF-ET, in vitro fertilization and embryo transfer; SD, standard deviation; SAS, self-rating anxiety scale; SDS: self-rating depression scale.

Table 2 Comparison of Anxiety and Depression Standard Scores of IVF-ET with Donor Sperm Patients' Spouses with Different Characteristics of Donor (n=187, mean±SD)

Items	n	SAS Scores	t/ X^2	p	SDS Scores	t/ X^2	p
Education degree			4.109	0.018*		6.933	0.001**
Junior high school/below	52	41.78±7.39			47.10±11.17		
Technical secondary school/high school	76	42.51±5.68			46.08±10.54		
College degree or above	59	39.30±7.00			39.94±12.28		
Accompanied by husband (times)			0.686	0.505		0.342	0.71
0–5	22	41.76±6.78			43.47±11.54		
6–10	36	42.25±6.31			45.65±11.55		
>10	129	40.86±6.80			43.97±11.76		
Occupational status			1.897	0.113		1.597	0.177
Company or enterprise	71	41.15±5.97			43.58±11.46		
Government/public institution	14	37.50±7.37			37.68±12.53		
Temporarily unemployed	7	41.61±9.81			43.39±10.50		
Liberal professions	50	42.81±6.94			45.60±11.42		
Self-operated/self-employed	45	40.70±6.51			45.91±11.79		
Annual household income (ten thousand)			7.927	0.000**		8.094	0.000**
<5	50	44.15±7.37			48.65±11.31		
5–10	89	41.29±5.68			45.01±10.74		
11–30	37	38.65±6.48			39.24±12.37		
>30	11	36.14±6.03			34.66±6.07		
Number of eggs taken (times)			1.698	0.194		0.637	0.426
1	146	40.89±6.69			43.87±11.68		
≥2	41	42.43±6.67			45.52±11.60		
Migration times (times)			1.168	0.313		2.23	0.11
1	91	40.82±6.83			45.24±11.82		
2–3	83	41.27±6.66			42.45±11.17		
≥4	13	43.85±5.76			48.56±12.47		
Number of births			0.32	0.749		0.422	0.674
0	128	41.40±6.42			44.69±10.96		
>1	59	41.06±7.39			43.86±13.08		

Notes: * $p < 0.05$ and ** $p < 0.01$ represented significant significances for comparison between groups.

Abbreviations: IVF-ET, in vitro fertilization and embryo transfer; SD, standard deviation; SAS, self-rating anxiety scale; SDS, self-rating depression scale.

education levels ($X^2=4.109$, $P=0.018$) and different family income ($X^2=7.927$, $P=0.000$). Moreover, there were significant differences in SDS depression scores between spouses of patients receiving IVF-ET with donor sperm with different education levels ($X^2=6.933$, $P=0.001$) and different family income ($X^2=8.094$, $P=0.000$). These results suggest that education levels and family income are the influencing factors of depression in the spouses of patients receiving IVF-ET with donor sperm.

Comparison of Anxiety and Depression Scores of Patients Receiving IVF-ET with Donor Sperm with Different Pregnancy Outcome

In this study, patients receiving IVF-ET with donor sperm were divided into non-pregnancy group and pregnancy group according to pregnancy outcome. The anxiety and depression scores of the two groups were compared on the transplant day. The results showed that the SAS anxiety scores of the patients in the non-pregnant group were significantly higher than those in the pregnant group on the day of transfer (Table 3, $t=3.729$, $P=0.000$). Furthermore, the SDS depression scores of the patients in the non-pregnant group were significantly higher than those in the pregnant group on the day of transfer ($t=2.593$, $P=0.010$). Therefore, anxiety and depression may be negative factors that affect the pregnancy outcome of IVF-ET with donor sperm patients.

Table 3 Comparison of Anxiety and Depression Standard Scores of IVF-ET with Donor Sperm Patients on the Transplantation Day According to Pregnancy Outcome (mean±SD)

Groups	Case (n)	SAS Scores	SDS Scores
Non-pregnant group	66	46.41±6.83	48.71±10.49
Pregnant group	121	42.66±6.43	44.57±10.43
t		3.729	2.593
p		0.000**	0.010*

Notes: * $p < 0.05$ and ** $p < 0.01$ represented significant significances for comparison between groups.

Abbreviations: IVF-ET, in vitro fertilization and embryo transfer; SD, standard deviation; SAS, self-rating anxiety scale; SDS, self-rating depression scale.

Discussion

IVF-ET technology can be divided into husband sperm source and donor sperm source in terms of sperm source. This study involved 187 IVF-ET administered couples who received donor sperm (IVF-ET with donor sperm). Generally, the population administering IVF-ET treatment with donor sperm generally enters in vitro fertilization cycle after undergoing artificial insemination with donor sperm (AID) in early stage or due to male factors, such as azoospermia. In this study, the patients have already tried other treatment methods or assisted by other reproductive technologies stipulated by our country, and only entered the in vitro fertilization and embryo transfer cycle with donor sperm (IVF-ET with donor sperm) with strict indications in this study. Furthermore, literature reports suggest that supplementing with inositol can improve ovarian reserve in female patients.^{16,17} At present, there are indeed applications of inositol and coenzyme Q10 in clinical practice to improve ovarian reserve, but they are all auxiliary treatment methods.^{18,19} Although the importance of evaluation for psychological variables in infertility, even no studies evaluated and compared the effectiveness of different treatments or interventions.²⁰ However, if we want to fundamentally improve pregnancy outcomes, assisted reproductive technology, such as IVF-ET with donor sperm, is still an essential treatment measure.

The results of this study showed that the standard score of anxiety and depression of patients receiving IVF-ET with donor sperm was 43.98 ± 6.80 and 46.03 ± 10.61 , respectively. Zhang et al²¹ analyzed 145 female assisted pregnancy patients and found that the standard score of anxiety and depression was slightly higher than those in this study. The standard score of anxiety and depression of their spouses on the day of transplantation was 41.23 ± 6.69 and 44.23 ± 11.65 , which were significantly higher than the Chinese health norm. In this study, the standard anxiety and depression scores of patients receiving IVF-ET are higher than their spouses, which is consistent with the results of Liu et al.²² Foreign research results have also reported that anxiety and depression are prevalent in patients with infertility.^{23,24} In this study, the anxiety and depression of the couples receiving IVF-ET with donor sperm were at a high level on the day of transplantation, which may be due to the following reasons: ① After administering IVF-ET with donor sperm, the patients are subjected to multiple psychological pressures brought about by traditional Chinese culture, family economy and the particularity of sperm source. ② Couples undergoing IVF-ET with donor sperm, as a special group in the treatment of assisted reproductive technology, need to receive sperm from the national human sperm bank. They often go through the process of in vitro fertilization with the authorization of the donor, which increases their economic expenses and the anxiety about the biological and genetic characteristics of the offspring, therefore causing the anxiety and depression. ③ Under the influence of traditional Chinese culture, infertility caused by male factors is often ignored, resulting in greater pressure on female patients during sperm donor assisted pregnancy.

The results of this study also showed that the annual income of the family is the main influencing factor of anxiety and depression of the couples receiving IVF-ET with donor sperm on the day of transfer. That is, the higher the annual income of the family, the lower the score of anxiety and depression of the couples receiving IVF-ET with donor sperm on the day of transfer. These results suggest that economic pressure and family income are the main influencing factors of anxiety and depression, which is similar to the research results of domestic scholars.^{25,26} The research results of Crawford et al²⁷ showed that due to the economic burden caused by high treatment costs, some infertile patients gave up treatment before getting a good pregnancy outcome. In this study, the highest proportion among the different families'

annual income groups is between 50,000 yuan and 100,000 yuan, up to 47.6%. This shows that the annual income of most couples receiving IVF-ET with donor sperm is at a relatively low level. However, the couples of IVF-ET with donor sperm have been busy with the diagnosis and treatment of infertility for a long time. Because of the consumption of time and energy, it is not conducive to the development of career and affects the annual income of the family, which will cause psychological stress, anxiety and depression for IVF-ET with donor sperm couples.

The results of this study showed that education level is the main influencing factor of anxiety and depression of couples receiving IVF-ET with donor sperm. The higher the education level, the lower the anxiety and depression scores of couples receiving IVF-ET with donor sperm on the day of transfer, which is consistent with the research reports of domestic scholars and others.^{21,28} The possible reason for this result is that couples with low educational level are more affected by traditional Chinese concepts and have fewer channels and ways to receive information, so they are more likely to have anxiety and depression. On the contrary, the higher the education level, the better the psychological response to the fact of infertility and the learning of more relaxation skills, the lower the score of anxiety and depression.

The results of this study showed that the number of eggs taken and the number of transplants were the main factors affecting the depression scores of patients receiving IVF-ET with donor sperm. The more the number of eggs taken and transferred, the higher the depression score of the patients receiving IVF-ET with donor sperm on the day of transfer, which is consistent with the research results of Wang et al.²⁸ In addition, Qi et al²⁹ confirmed that patients with a history of embryo transfer had higher depression scores. At this time, because of the increase in the number of egg retrieval and transfer operations, the patients have too much concern about the quality of their embryos, sperm quality and pregnancy outcome, and are prone to lose confidence in assisted reproductive therapy, resulting in higher depression scores. In addition, with the increase in the number of assisted reproductive surgery, the repeated expenditure of treatment costs has made the family economy worse, resulting in depression. Zhang et al³⁰ also confirmed that with the increase of treatment costs, the anxiety and depression of IVF with husband's sperm patients become more serious.

The results of this study also showed that the anxiety and depression scores of the patients in the non-pregnant group were higher than those in the pregnant group on the day of transplantation after IVF-ET with donor sperm. The research results of Ouyang et al¹⁰ showed that the anxiety standard score of the non-pregnant group during embryo transfer is significantly higher than that of the pregnant group, so anxiety affects the effect of assisted reproductive therapy to a certain extent. Zhang et al³¹ showed that the degree of anxiety and depression in the failed pregnancy group was higher than that in the successful pregnancy group, consistent with the conclusion of this study. Klonoff-Cohen³² had studied patients undergoing IVF-ET and showed that psychological factors can affect all aspects including transplantation and clinical pregnancy. The anxiety of the non-pregnant group during the embryo transfer period in this study may be due to the psychological burden caused by family expectations and external concerns, as well as concerns about the quality of embryos and sperm. The above factors are very likely to lead to anxiety and depression, which has a negative impact on pregnancy outcomes psychologically and physically.

To sum up, there are different levels of anxiety and depression on the day of transfer of IVF-ET with donor sperm couples, especially female patients. It is recommended that assisted reproductive practitioners focus on patients with low family income, low education level, and more times of egg retrieval and transplantation. The following measures can be taken in clinics: ① The reproductive center will incorporate psychological assessment into the routine assessment system, timely identify the anxiety and depression of the patients on the day of embryo transfer, give intervention, adjust the patients' psychology, and improve the pregnancy outcome. ② Assisted reproduction practitioners should pay special attention to female patients with repeated cycles who have a history of multiple egg retrieval and transfer operations and give psychological guidance. ③ In line with the general environment of China's vigorous encouragement of fertility, and with reference to the models of some regions and foreign countries, we actively call for the inclusion of some assisted reproductive technology treatment programs in medical insurance and obtain a certain proportion of reimbursement. At the same time, we will actively promote the launch of various public welfare programs to help pregnant women, so as to better alleviate the problems caused by economic factors to couples receiving IVF-ET with donor sperm.

This study also showed a few limitations. First, only one reproductive center in Guangdong province was investigated, which may have cultural differences and selective bias. Second, this study has not compared the anxiety and depression of IVF-ET with donor sperm with IVF-ET with husband's sperm couples on the day of transfer. In the future, we will carry out

investigation and research in cooperation with different regions and centers, expand the research sample, and further elaborate the anxiety and depression of the couples receiving IVF-ET with donor sperm on the day of transfer and the influencing factors, so as to provide scientific reference for improving the clinical pregnancy outcome of this group.

Conclusion

In total, scores of anxiety and depression on the day of patients receiving IVF-ET with donor sperm or their spouses were higher than the levels of Chinese health norm. Score of anxiety and depression of women was higher compared than their spouse. Anxiety and depression scores of non-pregnant women were higher than pregnant women. Education level and annual family income were influencing factors of anxiety and depression scores of IVF-ET with donor sperm couples, and number of eggs taken and transfer times were influencing factors of depression scores of IVF-ET with donor sperm patients. Therefore, the psychological status of the couples who received IVF-ET with donor sperm was significantly affected, especially in the female side. Medical staff should focus on patients with low education, low family income, and more times of transplantation and egg retrieval, and take targeted interventions to keep them in a good psychological state, which is conducive to improving pregnancy outcomes.

Data Sharing Statement

All data generated or analysed during this study are included in this published article.

Ethics Approval and Consent to Participate

This study has been approved by the Ethics Committee of the Guangdong Institute of Family Planning Science and Technology (Approval No. 2018-14). This study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants.

Consent for Publication

All participants signed a document of informed consent.

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Disclosure

The authors declare that they have no competing interests.

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