

Research Article

DE-T1 on the Blastocyst obtained Rate and Live Births Rates in Women Receiving IVF-ET Treatment

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Abstract

Background: Our objective was to retrospectively analyze the influence of DE-T1, a type of amino-polysaccharide extracted from dandelions, on the rates of blastocysts obtained and live births in women undergoing IVF-ET treatment.

Methods: This was a retrospective cohort study, con-

ducting a total of 1014 patients over the age of 30, who received IVF treatment at Hanabusa Women's clinic from Aug. 1, 2012 to Feb. 29, 2020. The patients were divided into two groups, based on their own choice regarding DE-T1 supplementation, which is available as over the counter medicine at the Clinic. The two groups' overall rates of blastocysts obtained were compared and the rates of blastocysts

obtained in patients with different ages and Anti-Mullerian hormone (AMH) levels were also compared using an Intention-to-treat (ITT) analysis. Among the patients who completed embryo transfers (ET), the live birth rate was compared between the supplementation group and the non-supplementation group using a Chi-squared test.

Results: The blastocyst obtained rate in the supplementation group was 75.98%, which was significantly higher than that of 57.28% in the non-supplementation group ($P=2.4\times 10^{-9}$). The blastocyst obtained rate across the range of ages in the supplementation group were significantly higher than those of the non-supplementation group (≥ 30 and < 35 years of age: 90.97% vs 74.32%, $P=0.001$; ≥ 35 and < 40 years of age: 82.40% vs 69.79%, $P=0.010$; ≥ 40 and < 43 years of age: 72.90% vs 52.11%, $P=0.002$; ≥ 43 years of age: 53.29% vs 22.95%, $P=5.7\times 10^{-5}$). The blastocyst obtained rate for both AMH levels in the supplementation group were significantly higher than those of the non-supplementation group (AMH ≤ 1.1 : 56.47% vs 40.44%, $P=0.002$; AMH > 1.1 : 88.48% vs 71.08%, $P=2.52\times 10^{-7}$). The live birth rate of the supplementation group was significantly higher than that of the non-supplementation group (57.53% vs. 40.0%, $P=0.045$).

Conclusion: DE-T1 supplementation might be an influence on improving the blastocyst obtained rate and live birth rates in women receiving IVF-ET treatment. DE-T1 supplementation might be of benefit to women of different ages and AMH levels.

Keywords: DE-T1; Fertility; Blastocyst obtained Rate; Live Birth Rate; AMH

1. Introduction

Due to social development, there has been an increase in delayed marriage and childbearing among women. It is well known that women's fertility declines with age [1]. With an increase in infertility among women, assisted reproductive technologies (ARTs) are being used more widely than ever. The assisted reproductive health care industry requires the collection of a large number of mature oocytes, and gonadotropins (Gns) are in common use. Many women of advanced reproductive age and women with poor ovarian functionality suffer from poor ovarian responses, and therefore do not respond adequately to Gns. They cannot produce high quality ovum by means of superovulation treatment, while the cultivation of high quality blastocysts, impregnation and childbirth are also out of reach. Consequently, obtaining mature oocytes and the successful cultivation of embryos, especially blastocysts, is vitally important to in vitro fertilization and embryo transfer (IVF-ET).

Dandelion (*Taraxacum mongolicum* Hand.-Mazz.) has been used as a traditional herb by the Chinese and other ancient cultures for centuries. The chemical composition of dandelion has become a hot topic for research in recent years. It has been reported that dandelion polysaccharide has efficacious properties, as an antioxidant, anti-free-radical, in the protection of the liver, for hypoglycemic activity, for promoting growth factor secretion, antitumorigenesis and more [2-6]. DE-T1 is a type of amino-polysaccharide extracted from dandelions. Our previous in vitro studies confirmed that it could promote the growth of granular cells, inhibit the apoptosis of granular cells, and regulate the balance of female hormones [7-8]. Shawkea T-1 (Tokujun Co., Ltd), with the essential

component DE-T1, is an assisted reproductive supplement which has been used in Japan for 23 years, and for over 9 years in China.

In this study, we aimed to retrospectively analyze the influences of DE-T1, combined with ovulation stimulants, on the blastocyst obtained rate and the live birth rates, to investigate if DE-T1 supplementation would have had an impact on the blastocyst obtained rates and the live birth rates of women receiving IVF-ET treatment. This study was approved by the Institutional Review Board (IRB) of Hanabusa Women's Clinic (approval number: 2021-09).

2. Materials and Methods

2.1 Study design

This was a retrospective cohort study aiming to investigate the clinical outcome to DE-T1 supplementation. Patients were free to choose whether to take DE-T1 oral solution. Patients who chose to purchase and take Shawkea T-1, containing the essential ingredient DE-T1, were assigned to the supplementation group. Patients who chose not to take DE-T1 were assigned to the non-supplementation group. The rates of blastocysts obtained and the live birth rates of both groups were analyzed. The effect modifiers included age and Anti-Mullerian hormone (AMH) value.

2.2 Setting and case recruitment

All infertility patients from China who received IVF treatment at Hanabusa Women's Clinic (Kobe, Japan) for IVF-ET treatment, between Aug. 1, 2012 and Feb. 29, 2020 were subjected for the analyses. They were aged 30 or above. There was no restriction regarding the cause of their infertility, including complaints such as ovarian dysfunction, polycystic ovary syndrome, uterine leiomyoma, adenomyosis,

fallopian tube blockage, and basic diseases. Thorough informed consent, in which all patients were informed that their clinical data might be used for future medical research, was obtained from all of the patients. All of the patients agreed to the collection and analysis of their data [9]. Only patients with incomplete general information and patients who conceived naturally during the treatment period were excluded from this study. Patients whose embryos were transported to other institutions or provinces were excluded from the analysis of live birth rates.

2.3 Interventions

The supplementation group took Shawkea T-1 (a brown liquid with a smoky taste, batch number: 6120801011-6200929064, manufactured by Tokujun Co., Ltd, address: 4-2-15 Gokodori, Chuo-ku Kobe city, Hyogo, Japan) containing the essential component DE-T1 (an amino-saccharide extracted from dandelions). Patients aged between 30 and 34 years took 300ml (100ml per bag) daily. Patients aged between 35 and 40 years took 400ml daily. Patients aged over 40 years took 500ml daily. Data regarding DE-T1 administration, including the administration period and the administration dose of each patient in the administration group were obtained by telephone follow-up with medical assistants and the purchase of records provided by Tokujun Co., Ltd. The median (Q25, Q75) duration of administration among the 712 patients in the administration group was 5 (3, 7) months. All treatments and embryo culturing were performed by Hanabusa Women's Clinic's team of embryologists and IVF physicians. No fixed course of supplementation or treatment was set for the participants of this study. All patients were able to choose whether to have multiple oocyte retrieval and ET cycles

during the observation period, in accordance with their individual outcomes in terms of oocyte retrieval, embryo culturing and transplantation. Frozen embryo transplants were conducted for all patients in this study. This reduced both potential research biases as well any disturbance to ovarian stimulation, serum hormone levels, or the endometrial factors involved in fresh embryo cycles by means of artificial endometrial cycle preparation.

2.3.1 Main outcome measures:

- 1) The blastocyst obtained rate for both groups
The proportion of patients who obtained at least one blastocyst by the end of the observation period was calculated for each group. The blastocyst obtained rate = the number of patients who obtained at least one blastocyst at the end of the observation period/the total number of patients in the group $\times 100\%$.
- 2) The live birth rates of both groups
The proportion of patients who delivered a liveborn infant at ≥ 28 weeks' gestation was calculated for each group following the conclusion of their treatment. The live birth rate = the number of patients who delivered a liveborn infant at the end of the observation period/the total number of patients who had embryos transferred in the group $\times 100\%$.

2.3.2 Secondary outcome measures:

- 1) The blastocyst obtained rate of different age groups
All patients were divided into the following age groups: ≥ 30 to < 35 years, ≥ 35 to < 40 years, ≥ 40 to < 43 years, and ≥ 43 years. The blastocyst obtained rate for each age range

in the administration group and the control group were compared.

- 2) The blastocyst obtained rate in relation to different AMH values

All patients were grouped according to their AMH levels ($AMH \leq 1.1$ and $AMH > 1.1$). The blastocyst obtained rates of patients with these two different AMH values were compared, between the administration group and the control group.

2.4 Statistical methods

Patients who stopped treatment or were lost to follow-up during the observation period were also considered as effective cases in the calculation of outcome statistics and data analysis. Intention-to-treat (ITT) analysis was used in the analysis of the results. Data was analyzed using IBM SPSS Statistics for Windows, Version 23.0 (IBM Co., Armonk, NY, USA). Continuous variables were expressed as mean \pm SD, if data was normally distributed. Data not normally distributed was presented using the median (Q25, Q75). Categorical variables were expressed as counts and percentages. Data regarding patients' age was represented by "mean \pm SD", and an independent sample t test was used to determine statistical significance. Data on patients' AMH levels was represented by [median (Q25, Q75)], and non-parametric tests were used to determine statistical significance. Frequencies and percentages were used to calculate the blastocyst obtained rate and the live birth rate, and the Chi-square test was used to determine statistical significance. Two-tailed p-values less than 0.05 were considered statistically significant.

3. Results

Data from 1014 Chinese female patients who received IVF-ET treatment at Hanabusa Women’s Clinic was analyzed in this study. 712 patients were assigned to the supplementation group, and 302 patients were assigned to the non-supplementation group. 231 patients completed embryo transfers. Of these, 186 were from the supplementation group, while 45 were from the non-supplementation group. With the exception of those patients who had their embryos transported to other institutions or provinces, there were several main reasons why some patients did not have embryo transfers, as follows: 1) Some patients preferred to gather more embryos at a younger age prior to starting embryo transfers, to thus improve the success rate of ensuing embryo transfers, rather than proceeding to embryo transfer immediately upon the conclusion of one stimulation cycle. 2) Some patients opted to take several months to reach optimum physical condition, prior to commencing embryo transfer. 3) Some patients failed to

produce high quality embryos for transfer. During the observation period from Aug. 1, 2012 to Feb. 29, 2020, a medical assistant followed up on the treatment process by means of monthly telephone calls. There was no significant difference in the baseline characteristics of the participants between the administration and control groups (Table 1).

Figure 1 shows the main flow and results of this study. The blastocyst obtained rate of the supplementation group was significantly higher than that of the control group (P<0.05). Furthermore, the blastocyst obtained rates of the supplementation group of different ages and AMH levels were all significantly higher than those of the non-supplementation group (P<0.05) (Table 2). The live birth rate of the supplementation group was significantly higher than that of the non-supplementation group (P<0.05) (Table 3). Raw data are available as Supplementary Tables 1, 2 and 3.

Parameters	Administration group		Control group		P value
	N		N		
Age (years) (mean ± SD)	712	38.5 ± 4.6	302	38.2 ± 4.8	0.41
≥30 and <35	155	32.2 ± 1.4	74	32.0 ± 1.5	0.10
≥35 and <40	250	37.0 ± 1.4	96	36.5 ± 1.4	0.16
≥40 and <43	155	41.0 ± 0.8	71	41.0 ± 0.6	0.82
≥43	152	44.5 ± 1.7	61	45.0 ± 1.8	0.16
Patients received ET	186	36.1 ± 4.0	45	36.2 ± 3.9	0.84
AMH (ng/ml) [Median (Q 25, Q75)]	712	1.60 (0.68, 2.92)	302	1.26 (0.50, 2.89)	0.11
≤1.1	278	0.47 (0.20, 0.80)	136	0.41 (0.12, 0.78)	0.20
>1.1	434	2.56 (1.74,3.90)	166	2.64 (1.70,4.42)	0.58
Patients received ET	186	2.46 (1.46,4.18)	45	2.50 (1.19,4.87)	0.91
Partner’s age (years) (mean ±SD) *	711	41.0 ± 6.9	295	40.8 ± 7.3	0.72
Stimulation cycle	1523		690		
Conventional ovarian stimulation		39.86%		38.99%	0.70

Mild ovarian stimulation		60.14%		61.01%	
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Note: *,One case in the administration group and 7 cases in the control group lacked their partners' information

Table 1: Baseline characteristics in administration group and control group.

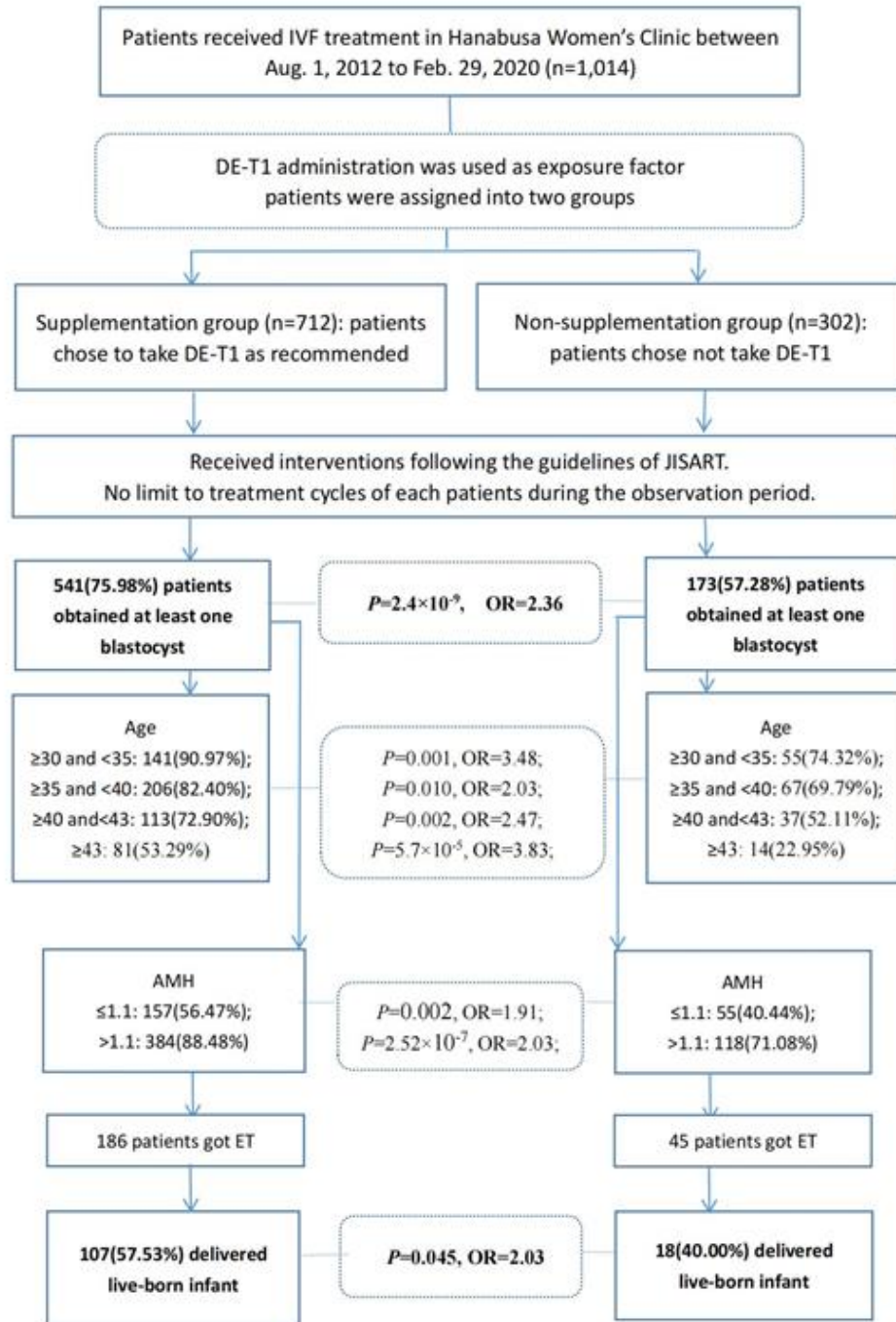


Figure 1: Flow chart and main results of the study.

	Administration group		Control group		P -value	OR
	N	Cases obtained Blastocyst (%)	N	Cases obtained Blastocyst (%)		
Toal	712	541 (75.98)	302	173 (57.28)	2.4×10 ⁻⁹	2.36
Age (years)						
≥30 to <35	155	141 (90.97)	74	55 (74.32)	0.001	3.48
≥35 to <40	250	206 (82.40)	96	67 (69.79)	0.010	2.03
≥40 to <43	155	113 (72.90)	71	37 (52.11)	0.002	2.47
≥43	152	81 (53.29)	61	14 (22.95)	5.7×10 ⁻⁵	3.83
AMHa (ng/ml)						
≤1.1	278	157 (56.47)	136	55 (40.44)	0.002	1.91
>1.1	434	384 (88.48)	166	118 (71.08)	2.52×10 ⁻⁷	3.12

Table 2: Comparison of blastocyst obtained rates between the two groups.

Group	N	Cases delivered live-born infant (%)	P -value	OR
Administration group	186	107 (57.53)	0.045	2.03
Control group	45	18 (40.00)		

Table 3: Comparison of the live birth rate between the two groups.

4. Discussion

4.1 The main outcomes of this study

With the continuous development of ARTs, blastocyst culture has become more widely used. Meta-analysis has shown that when an equal number of embryos are transferred in a fresh IVF cycle, the probability of both live birth and clinical pregnancy is significantly higher if the embryo transfer is performed at the blastocyst stage rather than at the cleavage stage. This has been found to apply particularly to live birth rates [10]. Another unselected patient cohort study also concluded that the cumulative live birth rate per oocyte collection cycle is higher for blastocyst-stage transfers, as compared to cleavage-stage transfers [11]. It was found that IVF outcomes might be improved by

performing blastocyst-stage transfers as compared to cleavage-stage transfers. The embryo selection and implantation processes improve with blastocyst stage transfers, while there is a demonstrable decline in multiple gestations [12-16]. Several explanations have been proposed for the superiority of blastocyst-stage transfers in improving implantation rates [13], and the transfer of blastocysts has become the first choice of an increasing number of patients and reproductive medical centers.

In this study, the blastocysts obtained rate for the supplementation group was 75.98%, which was significantly higher than 57.28%, for the non-supplementation group (OR=2.36, P< 0.001). Given that live birth is the ultimate aim of ARTs, and the

defining goal of each patient, the live birth rate was used as one of the main outcomes in this study. The live birth rate of the supplementation group was significantly higher than that of the non-supplementation group ($P < 0.05$). This indicates that DE-T1 could improve not only the outcome for blastocyst culturing, but also the outcome of the pregnancy itself.

4.2 The influences of DE-T1 on patients of differing ages and AMH levels

Studies have shown that the non-separation of meiosis chromosomes increases with age. This, in turn, leads to an increase in aneuploidy and spontaneous abortion rates in early embryos [17-18]. The most common resulting abnormality has been found to be autosomal trisomy, which is caused by an abnormal arrangement of the chromosomes and the composition of the spindle matrix during meiosis in elderly female oocytes [18]. A 2013 study concluded that both the aneuploid rate and the no-euploid embryo rate rose in a mathematically predictable way with age [1].

In this study, we enrolled patients from 30 to 46 years of age. Considering the influence of age and AMH on blastocyst formation in IVF treatment, we further observed the influence of DE-T1 on patients of different age groups (≥ 30 to < 35 years; ≥ 35 to < 40 years; ≥ 40 to < 43 years of age and ≥ 43 years of age), and different AMH levels ($AMH \leq 1.1ng/ml$ and $AMH > 1.1ng/ml$). Our results showed that the blastocyst obtained rates of the supplementation group with differing ages and AMH levels were all significantly higher than those of the non-supplementation group ($P < 0.05$). These results suggest that women of differing ages and AMH values could all benefit from DE-T1 supplementation

during IVF treatment.

4.3 The mechanisms of DE-T1 on reproduction health

Follicular dysplasia has long been considered as one of the main causes of infertility. The causes of follicular dysplasia infertility are various. Congenital ovarian dysplasia, premature ovarian failure or ovarian lesions, excessive obesity or wasting, irregular menstruation and heredity, are the common risk factors [19]. A variety of drugs have solved the problem of ovulation disorder in recent years, but this kind of treatment has anti-hormonal influences and can lead to the depletion of estrogen levels [20]. The aforesaid anti-hormonal effects could inhibit endometrial DNA synthesis and cause a decrease in endometrial receptivity, while the depletion of estrogen levels could lead to increased cervical viscosity, which is not conducive to sperm passage. Therefore, any long-term use of ovulation promoting drugs tends to lower pregnancy rates and increase early abortion rates [21-22]. The Follicle-Stimulating Hormone (FSH) receptor is the only receptor present in granular cells. Our previous studies have confirmed that DE-T1 could increase the activity of FSH receptors on the surface of ovarian granulosa cells [7].

In this study, DE-T1 supplementation significantly increased the blastocyst obtained rate in women undergoing IVF treatment. We speculate that the mechanism might be related to an increase in the number of follicular granulosa cells and a delay in the death of granulosa cells, as well as a reduction in sinusoidal cell atresia, thus increasing the quantity of mature ovum cells. Growth hormone (GH) is also involved in many processes of human reproduction [23]. In the process of oocyte development, GH could

promote the maturity of oocytes and improve the receptor activity of granulosa cells. Further research has [24] found that the effect of GH on reproduction is mainly caused by promoting the secretion of insulin-like growth factor (IGF), which effectively enhances the activity of granulosa cells. It has been reported that dandelion polysaccharide could increase the activity of IGF related receptors [5]. This may also be a contributing factor in the beneficial impact of DE-T1 on reproductive health.

To analyze the process by which DE-T1 improves live birth rates: firstly, DE-T1 significantly increased the blastocyst obtained rate of the patients in the supplementation group. Thus, the patients had more blastocysts available for ET, and therefore more chance of delivering an infant. Secondly, our previous studies have confirmed that DE-T1 can regulate the activity of multiple receptors in the ovaries and uterus, and balance the secretion of female reproductive hormones, which play a key role in ovum growth, embryo implantation, and fetal development [7-8]. In this way DE-T1 improves the quality of ova during IVF treatment and promotes high-quality fertilized embryos. We hypothesize that the improvement of live birth rates by DE-T1 supplementation was achieved through these two functions: increasing the number of blastocysts obtained and improving the quality of those blastocysts.

4.4 Research design and future prospects

With the increasing application of evidence-based methods in the field of medical research, randomized controlled trials (RCTs) and cohort studies have been widely used in clinical research. Intervention cohort studies are the main source of evidence for causal association, especially when randomized controlled

trials are difficult to carry out due to ethical limitations [25-26]. Considering patients' subjective desires regarding their treatment and patient compliance, in addition to the need for long-term follow-ups and complicated interventions, we employed the methods of a retrospective cohort study to observe the influence of DE-T1 on the blastocyst obtained rates and live birth rates of women receiving IVF treatment.

We did not take the quantity of oocytes as an observation indicator for this study. As mentioned above, blastocysts are more valuable than oocytes or early embryos. Blastocysts are also acknowledged to be superior in the prediction of the therapeutic effects of any regimen or protocol under study, and in terms of pregnancy outcomes. Furthermore, as this study was a retrospective cohort study, the related influencing factors, such as the oocyte retrieval cycles and the oocyte retrieval protocols of the two groups, were not completely controlled or consistent for either group. Consequently, using the quantity of oocytes as an indicator would have introduced bias and other challenges in the assessment and interpretation of the research outcomes. In this study, we retrieved 4,155 oocytes from 712 patients in the supplementation group, over the course of 1,523 ovarian stimulation cycles, with an average of 2.72 oocytes obtained per cycle. For the non-supplementation group, 1,746 oocytes were collected from 302 patients over 690 cycles, with an average of 2.53 oocytes obtained per cycle. This evidence was in line with our more long-term clinical observations, suggesting that DE-T1 could improve both the quality and quantity of oocytes. Patients who struggled to attain successful oocyte retrieval and blastocyst culturing generally resorted to several cycles of oocyte retrieval, rendering their courses of treatment relatively long. We

aim to explore optimal courses of supplementation for patients of differing ages and AMH values in the future, to enable us to prescribe individualized supplementation protocols for each patient.

In this study, we observed 1014 cases over an observation period of approximately 8 years. The supplementation group underwent a total of 1523 stimulation cycles. Using real-world research methodologies, we didn't set any strict inclusion criteria for the participants. In consequence we suggest that the results of this study may more truly reflect the influence of DE-T1 on women with different ages and AMH values who receive IVF-ET treatment. Reproduction is a complex physiological process. The mechanisms by which DE-T1 achieves its multitarget, synergistic influences on the hypothalamus, pituitary glands, ovaries, uterus and other organs requires further study.

5. Conclusions

A retrospective cohort study was conducted to explore the influences of DE-T1 on blastocyst obtained rates and live birth rates of women receiving IVF treatment. Our results showed that DE-T1 could significantly increase both the blastocyst obtained rate and the live birth rate, and furthermore, that women of differing ages and AMH values could all benefit from DE-T1 supplementation.

Declarations

Ethics approval and consent to participate

This study was approved by the Institutional Review Board (IRB) of Hanabusa Women's Clinic (approval number: 2021-09). Thorough informed consent, in which all patients were informed that their clinical data might be used for future medical research, was obtained from all of the patients. All of the patients

agreed to the collection and analysis of their data. All the methods were performed in accordance with relevant guidelines and regulations.

Statement

All the authors confirmed that all methods were performed in accordance with the relevant guidelines and regulations in this research.

Consent for publication

Not applicable

Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary information file.

Competing interests

The authors declare that they have no competing interest.

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No funding was obtained for this study.

Authors' contributions

H.S. is the corresponding author and primarily responsible for writing and editing the manuscript. H.S and S. J. wrote the main manuscript text and S. J performed the statistical analyses. Y.Y., T.N. and L.B contributed with data acquisition and reviewing the manuscript text. X.D., D.Y., J.O., S.K., and M.S. contributed with reviews and revisions of the manuscript text. All authors read and approved the final version of the manuscript.

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Supplementary

Here, is the list of [supplementary files](#). Click to download.

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