Effect of ZYP on in vitro fertilization-embryo transfer outcomes in DOR

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Research Article

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Abstract

Background: Zishen Yutai Pill (ZYP), a traditional Chinese patent medicine, is often used for the prevention of recurrent miscarriage and threatened abortion. However, ZYP's beneficial role in embryo quality and pregnancy outcomes are unclear. At the same time, Bone morphogenetic protein 15 (BMP15) and Growth differentiation factor 9 (GDF9) play key roles in folliculogenesis. The expression of BMP15 and GDF9 is declined accompany with poorer oocyte quality in poor ovarian responders.The present prospective study attempted to explore ZYP's role in oocyte quantity,embryos quality, pregnancy outcomes and the expression of the BMP15 and GDF9 genes in follicle fluid (FF)in patients in patients with diminished ovarian reserve (DOR) receiving in vitro fertilisation-embryo transfer (IVF-ET).

Methods: The present prospective randomised controlled study was conducted in 120 patients with DOR who received the treatment using different protocols. The treatment group (group A) comprising 60 women received ZYP from the mid-luteal phase in the former cycle for nearly 20 days by using the GnRH antagonist protocol, whereas the control group (group B) comprising 60 women received the same protocol without ZYP. The number of oocyte received and high-quality blastocysts,and pregnancy results were contrasted between groups. Pregnancy outcomes included the clinical pregnancy rate, embryo implantation rate, multiple pregnancy rate, ectopic pregnancy rate, early abortion rate,ongoing pregnancy rate,live birth rate and other pregnancy complications.FF was collected after oocyte retrieval. Expression of the BMP15 and GDF9 in the FF was determined with enzymelinked immunosorbent assay (ELISA).

Results: The number of gathered oocytes and high-quality blastocysts was obviously greater in group A compared with that of group B ($P < 0.05$). These two groups shared a similar clinical pregnancy rate (both $P > 0.05$). The expression of BMP15 and GDF9 in the FF differed significantly among the two groups ($P < 0.05$).

Conclusion: ZYP significantly influences the acquisition of prominent follicles, resulting in high-quality embryos. Meanwhile,ZYP can enhance the expression of BMP15 and GDF9 in the FF. Therefore, ZYP application may significantly promote ovarian response and improve embryos' quality among patients with DOR by upregulating the expression of BMP15 and GDP9 .

Trial registration number: Chinese Clinical Trial Registry (ChiCTR2100048441)

Introduction

Diminished ovarian reserve (DOR) results in decreased fertility, despite the application of assisted reproductive techniques (ARTs). DOR poses a great challenge to in vitro fertilization (IVF) cycles. DOR refers to a gradual decrease in antral follicle counts (AFCs), accompanied by an increased follicle-stimulating hormone (FSH) level and a decreased anti-Mullerian hormone (AMH) level. In clinical practice, these patients can be divided into two groups, patients with premature ovarian insufficiency (POI) and patients poor ovarian response (POR) [1, 2]. Patients with DOR often exhibit POR, which significantly decreases the number of recovered oocytes and lowers the IVF success rate [3]. POR following IVF

treatments poses a great threat to patients and clinicians[4, 5]. Clinicians have been seeking efficient solutions for improving the results in poor responders, particularly for women with severe DOR [6, 7]. Women with DOR possess limited recoverable follicles regardless of aggressive gonadotropin stimulation. Numerous medicines are administered to patients with DOR for improving IVF outcomes.

Zishen Yutai Pill (ZYP), which was included in the National Essential Medicine List of China after 2018, was one of the representative TCM preparations used in IVF It contains 15 Chinese herbs, namely *Semen Cuscutae, Polygonum Multiflorum,Radix Rehmanniae Preparata, Fructus Amomi, ginseng, Artemisiae Argyi Folium, HerbaTaxilli, CollaCoriiasini, cornu cervi degelatinatum, Atractylodes macrocephala, Morinda officinalis, Codonopsis Radix, Dipsaci Radix, Eucommia ulmoide*s *and Fructus Lycii*, respectively[8, 9]. Studies have suggested the utility of ZYP in improving endometrial receptivity; preventing recurrent miscarriage; and treating threatened abortion, luteal phase defect menstrual disorder, and ovarian dysfunction [10–12].ZYP may promote the embryonic implantation rate among patients receiving embryo transfer [13].ZYP exhibits no reproductive toxicity to embryonic and foetal developments and can be safely used at the clinical dose [14]. However,the mechanism of ZYP effects on the endometrial receptivity and ovarian function is still nuclear. A previous study showed that a high-dose ZYP may upregulate levels of transforming growth factor-beta (TGF)-β and improve oocyte function[15]. BMP15 and GDF9 are essential members of the TGF-β superfamily,which have been identified to play vital roles in the regulation of folliculogenesis by paracrine/autocrine mechanisms[16–21].At the transition of the primary follicle to the secondary follicle stage,GDF9 has been affected follicular development. Subsequently,GDF9 was closely related to granulosa cells(GC) differentiation, especially in the transition from preantral GC to cumulus cell (CC)[22]. BMP15 promotes follicle maturation, improve oocyte developmental competence, prevents GC apoptosis and implicated the determination of ovulation quota[18, 20, 23, 24]. Abnormal expression of BMP15 and GDF9 and genetic variants may be predisposed to follicle atresia and early exhaustion of ovarian reserve [25–27]. Further more, BMP15 and GDF9 in follicular fluid (FF) have been closely related to poorer oocyte quality and outcome of treatment for patients undergoing IVF-ET treatment[28].

This research attempted to explore ZYP’s effect the quality of oocytes on patients with DOR.We also performed the analysis of BMP15 and GDF9 in the follicular fluid to establish the mechanisms of ZYP in improving IVF outcomes in infertile women with DOR.

Materials And Methods

Study population

The current single-blinded clinical experiment was conducted among 120 patients with DOR who visited the Affiliated Reproductive Hospital of Shandong University for their infertility treatment from April 2020 to March 2021 after institutional ethics clearance. These patients were first evaluated and then randomised into two groups. The experimental procedures conformed to the Helsinki Declaration. The current research is documented in Chinese Clinical Trial Registry (ChiCTR2100048441) .

DOR in the present study was defined as follows: (i) a former POR ≤ 3 oocytes under the traditional stimulation protocol; (ii) AFC < 7; (iii) serum AMH level < 1.1 ng/mL. Patients aged more than 40 years(After the age of 40, the number of follicles decreased significantly, the quality of follicles decreased, and the problem of abnormal menstruation increased.); diagnosed as having endometriosis or polycystic ovary syndrome (PCOS); having a history of ZYP supplementation therapy; without definite endocrine disorders such as thyroid and adrenal; and having a history of ovarian therapy were excluded from this research. All the participants were ranked sequentially after gaining their written informed consent.

Sample size calculation

According to previous literature [29], the average number of oocytes retrieved in DOR patients was 3.2 ± 1.5. Due to the lack of data on the number of oocytes retrieved after taking Zishen Yutai Pills in patients with DOR, it is hypothesized that the number of oocytes retrieved after taking Zishen Yutai Pills can increase by 1. Using the sample size calculation software PASS 11.0, a power of 0.9 and a significance level of 0.05 were set to include 51 patients in each group. Assuming a 15% dropout rate, a total of 120 patients were required in both groups, with 60 patients in each group.

Of the 120 patients, three patients from group A (failed fertilization) and one patient from group B (high-quality embryo failure) were not included (Fig. 1). Finally, 57 patients from group A and 59 patients from group B were included in this study.

Study interventions

Each participant was informed about the study procedure before initiation of the study. According to a computer-generated randomisation list, 60 patients were randomised into the ZYP group to receive ZYP 5 g three times daily from the mid-luteal phase prior to the IVF cycles for nearly 20 days. On the other hand, 60 patients were randomised into the control group to directly enter the IVF cycles. All patients received the antagonist protocol. The protocol involves first injecting patients with 150–300 IU of gonafine to stimulate ovulation, the dose of GnRH-A was 0.25 mg/day when the diameter of the dominant follicle ≥ 11–12 mm or LH > 10 IU/L (or > 2 times the baseline level) or E2 > 200–300 pg/ml. If the maximal follicular diameter amounted to 14 mm, 0.25 mg of the GnRH antagonist, cetrorelix, was administered subcutaneously per day till the late ovarian stimulation period. If more than one mature follicle of a diameter ≥ 18 mm could be detected through ultrasound, ovulation was triggered through a 8,000-IU intramuscular injection of human chorionic gonadotropin (HCG). Oocyte puncture received 36 h HCG application through guided transvaginal ultrasonography.Clean follicular fluid was obtained from leading follicle of who undergoing IVF-ET treatment by trans-vaginal ultrasound-guided puncture for BMP-15 and GDF-9 determined.Fluid samples from the first aspirated follicle and without any visible blood contamination were carefully collected. Samples were centrifuged at 2000 r/min for 15 minutes and stored at -80°C for further examination.

At most two embryos were transplanted until the third day following the puncture. Moreover, remaining embryos were incubated. If blastocysts developed, the embryos were frozen. Luteal phase support was

started from the oocyte puncture date, and all patients were administered dydrogesterone (Abbott, the Netherlands) 20 mg twice daily and 200 mg urtogestan vaginal suppositories (Laboratoires Besins International, France). Serum beta HCG levels were evaluated 12–14 days following embryo transfer for the confirmation of biochemical pregnancy. Progestin support and ZYP lasted until the late 12-week gestation period after a successful pregnancy. Clinical pregnancy was defined as visualiz. Ongoing pregnancy was defined as the presence of a fetus with heart motion at 12 weeks of gestation. Live birth was defined as delivery of any viable infant at 28 weeks or more of gestation after embryo transfer. Transfer of embryos were evaluated by the ongoing pregnancy rate, number of gathered oocytes, fertilized oocytes, and frozen embryos, clinical pregnancy rate, implantation rate, multiple pregnancy rate, early miscarriage rate, and ectopic pregnancy rate.

Study outcomes

The primary outcome measure was the number of oocytes retrieved and high-quality embryos and follicular fluid BMP15 and GDF9 in patients with DOR. Secondary outcomes fertilization rate, clinical pregnancy rate, embryo implantation rate, ongoing pregnancy rate, miscarriage rate, ectopic pregnancy rate, pregnancy complications, live birth rate. Criteria for secondary outcome are presented in Table 1 of the Supplementary Appendix.

Table 1
Baseline Characteristics of the Study Population*

Index	Group A N = 60	Group B N = 60	P value
Mean Age(yr, x̄ ± s)	33.55 ± 3.99	33.97 ± 3.50	0.241
BMI(kg/m^2,x̄ ± s)	23.75 ± 3.35	23.62 ± 3.54	0.500
Primary infertility (No.,%)	15(25%)	21(35%)	0.319
Basal FSH(IU/L, x̄ ± s)	8.12 ± 2.62	8.01 ± 2.15	0.970
Basal LH(IU/L, x̄ ± s)	4.97 ± 2.89	4.18 ± 1.55	0.231
Basal E2(pg/mL, x̄ ± s)	40.38 ± 15.68	43.22 ± 20.54	0.924
Basal T(ng/dL, x̄ ± s)	19.17 ± 12.00	20.25 ± 13.72	0.890
basal AFC(No.,x̄ ± s)	5.70 ± 1.52	5.63 ± 1.56	0.746
AMH(ng/mL, x̄ ± s)	0.79 ± 0.31	0.71 ± 0.27	0.108
Proportion of IVF(No.,%)	43(71.7%)	49(81.7%)	0.280
Proportion of ICSI(No.,%)	17(28.3%)	11(18.3%)	0.280

**Plus-minus values can be indicated by means ± SD. No significant intergroup differences existed in baseline characteristics(p>0.05). FSH, follicle-stimulating hormone; LH, luteinising hormone; T, total testosterone; E2, oestradiol; AMH, anti-Müllerian hormone;IVF, in vitro fertilization; ICSI, Intracytoplasmic sperm injection*

†BMI, body mass index can be calculated as the weight (kilograms) divided by the square of height (metres).

Embryo grade

Gardner grading criteria were used and divided into six periods according to blastocoel formation. Stage 1: Early cavitary blastocyst with blastocyst cavity less than 1/2 of the embryo volume. Stage 2: blastocyst cavity coelom greater than or equal to 1/2 of the volume; Stage 3: fully expanded blastocyst, blastocyst cavity occupies the embryo; Stage 4: expanded blastocyst, blastocyst cavity volume greater than early embryo, zona thinning; Stage 5: blastocyst being hatched, trophoblast begins to break through the zona pellucida; Stage 6: hatched blastocyst, blastocyst is completely hatched from the zona pellucida. Stage 3 to 6 blastocysts need to score the inner cell mass and trophectoderm cells, and the inner cell mass is scored, A: the inner cell mass is tight and the number of cells is large; B: the inner cell mass is loose and the number is small; C: the number of the inner cell mass is very small; the score of the trophectoderm, A: the number of cells is large and a tightly arranged cell layer is formed; B: the number of cells is small and the arrangement is loose; C: the trophectoderm is composed of sparse cells. In our

center, blastocysts with recovered blastocyst standard ≥ stage 4 (4BC) and blastocysts with score ≥ stage 4 (4BC) are defined as high-quality blastocysts.

Determination of BMP15 and GDF9 in follicular fluid by ELISA

The concentrations of BMP15 and GDF9 in FF were measured with the ELISA Kit (Jiang's biological,Shanghai, China) by enzyme-linked immunoassay technique,according to the manufacturer's instructions.The standard use of both reagents was to make serial dilutions, i.e., 2000, 1000,500, 250, 125, 62.5pg/ml, and the working solution of 2000 pg/ml before the measurement. The FF samples were defrosted and then mixed together, and 50μl FF was added to each well in duplicate. The absorbance value was measured at 450 nm with a tecan infinite F50 microplate spectrophotometer (Longyue Biological Technology Development Co., Ltd., Beijing, China). The detection range of BMP15 and GDF9 was set as 0 ~ 2000 pg/ml, 0 ~ 48pg/ml respectively. Such samples were diluted 2 times, and 50 μl diluted FF was then added to each well.

Statistical analysis

Continuous variables can be indicated by mean ± standard deviation (SD), whereas the intergroup difference between these variable was compared using Student's t-test. Qualitative variables are presented as the proportion and were interpreted using the χ^2 test. SPSS version 22.0 was applied in statistical analysis, and a P value of < 0.05 denoted statistical significance.

Results

Differences between treatment and control groups in terms of the baseline features such as mean age, body mass index (BMI), primary infertility, number of IVF procedures, AMH levels, FSH levels, leutenising hormone (LH) levels, and T hormone levels were statistically nonsignificant. Intergroup differences in the primary infertility ratio and fertilisation type were also statistically nonsignificant (Table 1).

Although fertility outcomes including endometrial thickness, fertilisation rate, number of transferred embryos, clinical pregnancy rate, implantation rate, multiple pregnancy rate, early miscarriage rate, ectopic pregnancy rate ,ongoing pregnancy rate, live birth rate and pregnancy complications were found to vary between groups, such differences were statistically nonsignificant (Table 2). Patients undergoing ZYP treatment exhibited greater number of collected oocytes and high-quality embryos compared with the control group, and corresponding differences were statistically significant($P < 0.05$). After standardised ovarian stimulation, the transplant was cancelled for four patients, of which one patient exhibited fertilisation failure and two patients in the treatment group and one patient in the control group exhibited high-quality embryo failure. No adverse event was observed in the present study.

Table 2
Therapeutic Outcome in the Study Population*

Outcome	Group A N = 60	Group B N = 60	*P* value
Endometrial thickness on hCG† trigger day(cm, x̄ ± s)	1.02 ± 0.19	0.95 ± 0.17	0.257
oocytes retrieved(No.,x̄ ± s)	5.28 ± 2.16	4.05 ± 1.88	0.020
high quality embryos (No., x̄ ± s)§	2.68 ± 1.45	2.15 ± 0.93	0.010
Fertilization rate(No., %)	263/317(83.0%)	211/243(86.8%)	0.237
embryos transferred(No., %)	1.65 ± 0.58	1.58 ± 0.53	0.335
Single embryo transfer(No., %)	15/57(26.3%)	23/59(39.0%)	0.169
Double embryo transfer(No., %)	42/57(73.7%)	36/59(61.0%)	0.169
frozen embryos(No., x̄ ± s)	0.90 ± 1.36	0.53 ± 0.75	0.046
Clinical pregnancy‖(No., %)	26(43.3%)	26(43.3%)	1.000
Implantation‡(No., %)	32/99(32.3%)	30/95(31.6%)	1.000
Multiple pregnancy ¶(No., %)	7/26(26.9%)	4/26(15.4%)	0.499
Pregnancy loss(No., %)	6/26(23.1%)	6/26(23.1%)	1.000
Ectopic pregnancy(No., %)	1/26 (3.8%)	0	1.000
Ongoing pregnancy**(No., %)	19/26 (73.1%)	20 /26(76.9%)	1.000
Live birth rate(No., %)	19(31.7%)	19(31.7%)	1.000
Pregnancy complications***(No., %)	3/26(11.5%)	1/26(3.8%)	0.610

** Plus–minus values are indicated by means ± SD.*

†hCG, human chorionic gonadotropin.

‖Clinical pregnancy referred to observations about the gestational sac using ultrasonography.

‡Embryo implantation rate was measured as number of observed intrauterine gestational sacs divided by the number of transferred embryos.

¶ Multiple pregnancy rate was calculated as number of multiple pregnancy divided by the number of clinical pregnancy. All the multiple pregnancies are twins.

*** Ongoing pregnancy suggested the foetal heartbeat detected using ultrasonography until the 12th week of gestation.*

Outcome	Group A N = 60	Group B N = 60	*P* value
§ *The number of high-quality embryos was calculated as the total number of excellent embryos per group and the number of excellent embryos per capita per group.*			
*** *There were 2 women with gestational diabetes mellitus(GDM)and 1 woman with gestational hypertension in Group A,and 1 woman with preeclampsia in Group B.*			
Data are number/total number or number (%) unless stated otherwise.			

As shown in Fig. 2, the concentration of BMP15(401.99 ± 266.63pg/ml vs. 199.14 ± 181.26 pg/ml) and GDF9 (7.25 ± 3.97pg/ml vs. 4.33 ± 2.33 pg/ml) in FF have differed significantly between the two groups ($P < 0.01$), with an obvious trend of ZYP group in DOR patients.

Discussion

Ovarian reserve reflects the reproductive potential and ART outcomes in women [30, 31]. DOR is frequently encountered during infertility treatment [32]. However, no unified definition of DOR is available [33]. AMH and age are considered to be the most valuable factors that affect ovarian response during IVF cycles[34]. Therefore, all the patients engaged with the present study were aged less than 40, with a serum AMH level of < 0.5–1.1 ng/mL. Numerous solutions are available for promoting the ovarian response and increasing the pregnancy outcome among the patients receiving IVF [35]. GH significantly increases the ovarian response among patients with POR who are about to receive IVF [36]. Dehydroepiandrosterone (DHEA) supplementation improves the serum AMH level and increases the embryo score for patients with DOR [37]. However, studies regarding ZYP efficacy in patients with DOR are rare. ZYP is considered gentle and safe.

In traditional Chinese medicine theory, the kidney stores the essence and is vital for reproduction; thus, the fundamental physiological processes of women have some connections with the kidney, and sufficient kidney essence is vital for a successful pregnancy. Under conventional Chinese medicine theory, POR belongs to the category of ‘infertility’, ‘hypomenorrhea’, ‘amenorrhea’, and ‘menopausal syndrome’. Additionally, POR is considered to have a close relation with the spleen and deficiency of kidney energy [38]. Therefore, improved physical conditions in the kidney and spleen can lead to improved POR. ZYP (‘zishen’ means ‘tonifying the kidney’) comprises 15 herbs in combination with other natural substances, and such compound has been considered conducive for tonifying the kidney and invigorating the spleen. Thus, ZYP might have a positive effect on patients with DOR.

Research has verified ZYP’s effect on infertility and miscarriage treatment [37, 39]. However, only few studies have used ZYP to treat patients with POR. A randomised controlled trial investigated the mechanism of ZYP supplementation during POR treatment. Liang et al. reported that the use of ZYP and ear-point pressing with coxherb seeds for the treatment of patients with reduced ovarian reserve greatly reduced FSH, FSH/LH, and estradiol levels during menstruation compared with the artificial cycle group.

Additionally, both life quality (traditional Chinese medicine syndrome scores) and clinical pregnancy rate rose considerably ($P < 0.05$) [40]. Another randomised controlled trial by Liu et al. reported that co-treatment using ZYP during controlled ovarian stimulation cycles might promote estradiol levels, clinical pregnancy, and implantation rates, whereas they observed no significant difference between the experimental and control groups concerning freeze-thaw embryo transfer of clinical pregnancy and implantation rates [41].

All transplanted embryos in present study were high quality embryos (cleavage embryos or a blastocyst), so pregnancy outcomes including clinical pregnancy rate, implantation rate and live birth rate between two groups were similar. However, the number of gathered oocytes and transferred embryos was greater among patients receiving IVF cycles along with ZYP treatment than those directly undergoing IVF cycles. And the expression of BMP-15 and GDF-9 in the ZYP treatment group were significantly higher than those in the control group.Thus, a combination of ZYP and Gn treatment is capable of improving the ovarian response to superovulation drugs in ART and also in DOR populations. Most importantly, Zishen Yutai Pills significantly increased the number of oocytes and high-quality embryos produced by patients. Therefore, ZYP is beneficial to follicle development. This finding is concurrent with those of several animal studies, which suggest that ZYP can increase the blood supply to the gonads and sexual organs of rabbits [42]. Kidney jing plays a crucial role in female reproduction. Several preliminary reports have suggested the efficacy of kidney-tonifying medicines in improvement of reproductive function. Li et al. verified the role of a kidney-tonifying medicine in increasing the number of ovarian follicles in the mouse model, which is in agreement with our experimental results [43]. Some studies have suggested that spleen-strengthening and kidney-reinforcing traditional Chinese medicines can slow down DOR progression and improve the ovarian reserve [44]. Hu et al. reported that kidney-tonifying medicines can recover oestrogen receptor expression in menopause [45]. Therefore, this medicine could be used as a therapy as a substitute of hormonal treatment.

Studies have reported the antioxidant potential of ZYP in removing free radicals [46]. However, the detailed molecular mechanisms through which ZYP effects the oocytes, cumulus cells, and granulosa cells are unknown. This is due to the oocyte-secreting factor that controls ovarian function during female reproduction. It not only modulates fate for somatic granulosa cells but also improves the quality and developmental competence for eggs. BMP15 and GDF9 are oocyte-secreted factors that play a leading role in controlling ovarian function in female reproduction, regulating the apoptosis of somatic granulosa cells and the quality and developmental competence of the oocyte. BMP15 and GDF9 can also enhance the effect of FSH aon GCs and provide more E2 for oocyte[47, 48]. Super-GDP9 is able to improve the rate of blastocysts in vitro maturation (IVM)[49]. The expression of the BMP15 and GDF9 genes was declined among patients with DOR[50, 51].The decline expression BMP15 and GDF9 may lead to abnormal folliculogenesis and poor oocyte quality.Gong et al also proved that such decline was associated with increased age,especially for those over 40[44]. BMP15 and GDF9 may provide more earlier biomarkers for fertility in DOR. In this study, ZYP could improve the expression of BMP15 and GDP9, and also increased the number of blastocysts. Therefore, ZYP improves the quality both oocyte and embryo by increasing

the expression of BMP15 and GDF9.However, the underlying molecular mechanism of ZYP actions in oocyte demands further research.

The present study has certain limitations. The sample was from single center, and most patients belonged to the same geographical area, which may have affected the results. More interventional trials are warranted to verify ZYP-related clinical relevance in promoting reproductive outcomes of the subpopulations. Additionally, the study on the security of the long-terms outcomes of ZYP on both mother and child was overlooked.

In conclusion, we determined the major effects of ZYP on recruiting dominant follicles from associated cohorts, leading to high-quality blastocysts by upregulating the expression of BMP15 and GDF9. Therefore, the administration of ZYP in an IVF cycle could considerably intensify the ovarian response in patients with DOR.

Abbreviations

DOR, diminished ovarian reserve; ARTs, assisted reproductive techniques; IVF, in vitro fertilization; AFCs, antral follicle counts; FSH, follicle- stimulating hormone; AMH, anti-Mullerian hormone; POI, premature ovarian insufficiency; POR, poor ovarian response; ZYP, Zishen Yutai Pill;BMP15: Bone morphogenetic protein 15; GDF9: Growth differentiation factor 9;ELISA: Enzyme-linked immunosorbent assay; POR: Poor ovarian response; FF: Follicle fluid;GC:granulosa cells;CC:cumulus cell;TGFβ: Transforming growth factor β; PCOS, polycystic ovary syndrome; BMI, body mass index; E2: Estradiol;LH, leutenising hormone; DHEA, Dehydroepiandrosterone.

Declarations

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Data availability statement

Overall datasets involved in the research have been incorporated by this paper/ supplementary material.

Author contributions

FLia was responsible for research concept and design in the paper. XL,ZW,HX,YZ and JX were responsible for acquiring, analyzing and interpreting data, as well as writing the draft for this paper. HL and YS were responsible for manuscript review and amendment. The authors approve of the issue of the manuscript.

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Availability of data and materials

Overall datasets involved in the research have been incorporated by this paper/ supplementary material.

Ethics and dissemination: The experiment had solicited approval from the Institutional ethics committee of the Affiliated Reproductive Hospital of Shandong University. All the participants provided written informed consent. This survey was conducted as per the Declaration of Helsinki and relevant amendments.

Consent for publication

Not applicable.

Conflict of Interest Statement

It is announced that this study had been performed without commercial or fiscal relations which might be considered to be potential conflicts of interest.

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Figures

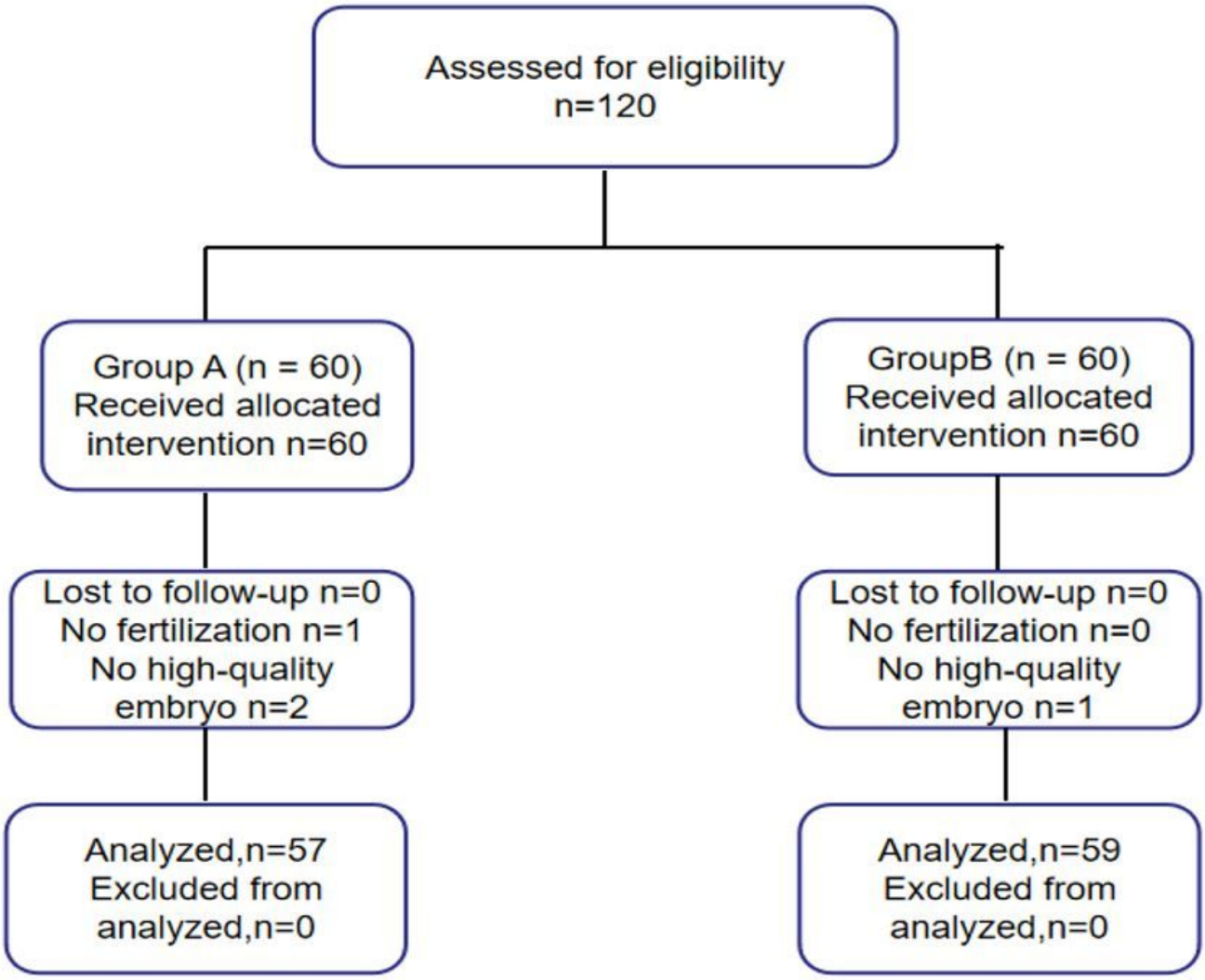

Figure 1

CONSORT Flow Diagram for Progress of the Participants of the Randomised Trial

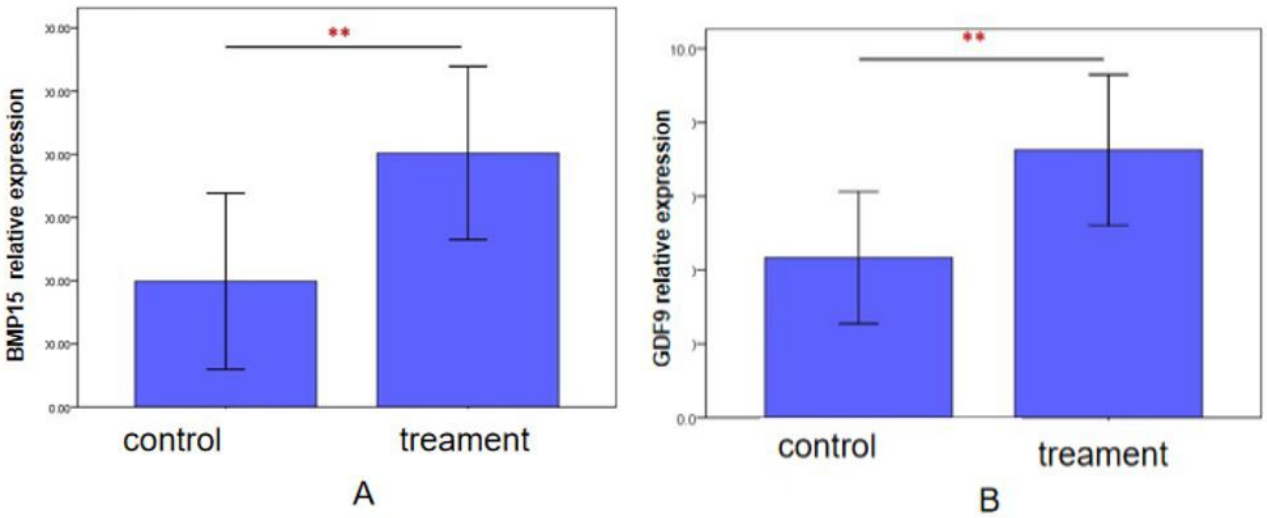

Figure 2

Expression lever of BMP-15 and GDF-9 in DOR follicular fluid in the control and treatment groups as A and B shown. The expression of BMP-15 and GDF-9 in the ZYP treatment group were higher than those in the control group. Each treatment was repeated 2 times. The data was analysed with an independent-samples T test. The difference between the two groups was significant ($P<0.01$).