Pretreatment With a Long-acting GnRH Agonist in Early Follicular Phase Increases Clinical Pregnancy Rate in Frozen-thawed Embryo Transfer Cycles

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Research

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Abstract

Background: It is controversial whether gonadotropin-releasing hormone agonist (GnRHa) pretreatment can benefit the pregnancy outcomes in frozen-thawed embryo transfer (FET) cycles. In most of studies, GnRHa was administered during the mid-luteal phase for pretreatment. Few studies focus on FET cycles with GnRHa administered in early follicle phase.

Methods: The retrospective cohort study was conducted in a university-affiliated IVF center. 630 patients in the GnRHa FET group and 1141 patients in the hormone replacement treatment (HRT) FET without GnRHa group from October 2017 to March 2019 were included. The menstruation cycle of these patients was irregular.

Results: There were no differences observed between the two groups in patient's characteristics. However, the GnRHa FET group showed a higher percentage of endometrium with triple line pattern (94.8% vs 89.6%, $p < 0.001$) on the day of progesterone administration, and an increased implantation rate (34.7% vs 30%, $p < 0.01$), biochemical pregnancy rate (60.6% vs 54.3%, $p = 0.009$), and clinical pregnancy rate (49.8% vs 43.3%, $p = 0.008$), as compared to that in the HRT FET cycles with similar endometrial thickness, ectopic pregnancy rate, and early miscarriage rate. Binary logistic regression analysis showed the GnRHa FET group to be associated with an increased chance of clinical pregnancy rate compared with HRT FET without GnRHa group ($P = 0.014$, odds ratio [OR] 1.30, 95% confidence interval [CI] 1.06-1.61).

Conclusions: Pretreatment with a long-acting GnRHa in early follicular phase can improve the clinical outcome of the FET cycles. However, further randomized control trials (RCTs) will be needed to verify these results.

Background

Frozen-thawed embryo transfer (FET) allows for the embryos to be generated by *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI), which can then be frozen to be transferred after several months or years [1]. FET has been found to increase the cumulative pregnancy rate after one cycle of ovarian stimulation and oocyte retrieval [2]. FET also reduces the risk of ovarian hyperstimulation syndrome (OHSS). Embryos from the freeze-all strategy cycle, such as mild controlled ovarian stimulation (COS) cycle, progestin primed ovarian stimulation (PPOS) cycle, and cancelled fresh cycles resulting from other conditions, including extremely high levels of serum progesterone, can be transferred during FET cycles. In recent years, the rates of FET cycles have increased in Europe, from 28% in 2010 to 32.3% in 2011 [3]. However, there is currently little consensus on the most efficient method for endometrial preparation in FET cycles. Nature cycles are mainly used in ovulatory women while hormone replacement treatment (HRT) cycles have been utilized in anovulatory patients or in time controlled situations. In HRT FET, estrogen and progesterone are administered in a sequential regimen, and it aims to suppress the development of the dominant follicle and mimic the hormone exposure of the endometrium. Initially, estrogen was administered for more than 12 days to induce the proliferation of the endometrium. Then,

progesterone was administered to initiate the secretory changes associated with the endometrium reaching its optimal thickness, as observed by ultrasound. Based on the days of progesterone supplementation, the synchronously developed embryo was thawed and transferred [1].

At the beginning, to mimic the procedure of the fresh cycles, a gonadotropin-releasing hormone agonist (GnRHa) was applied and started from mid-luteal phase downregulate the pituitary GnRH receptors and prevent the follicular growth. For the subsequent menstrual cycle, estrogen and progesterone were administered sequentially, and the cycle is denoted as the HRT cycle. Then, in the HRT cycle without GnRHa, the development of the dominant follicles was also found to be suppressed, providing a more economic, convenient, and comfortable procedure for IVF patients, in addition to being less time consuming. Therefore, HRT cycle FET without GnRHa has become a common method for endometrial preparation in anovulatory patients. El-Toukhy et al reported that HRT FET with short-acting GnRHa daily starting in the mid-luteal phase of the menstrual cycle achieved significantly higher clinical pregnancy and live birth rates than without GnRHa suppression [4]. However, the other studies [5–12] found different results with similar clinical pregnancy rates. A Cochrane review [2] reported that HRT alone was associated with a similar clinical pregnancy rate, as compared to HRT with GnRHa suppression. Recently, an RCT[13] reported pretreatment with long-acting GnRHa after 5–7 days of oral dydrogesterone for patients with polycystic ovary syndrome (PCOS) did not improve clinical pregnancy rate in HRT FET cycle. In most of these studies, GnRHa was administered during the mid-luteal phase for pretreatment. Few studies focus on FET cycles with long-acting GnRHa administered in early follicle phase.

In fresh IVF cycles, Hu'et al reported long-acting GnRHa long protocol with administration in follicular phase can increase endometrial thickness, implantation and clinical pregnancy rate than short-acting GnRHa long protocol with administration in luteal phase. There were similar number of retrieved oocytes and embryos transferred between two protocols and lower rate of high quality embryos in the former. It was probably administration of GnRHa in follicular phase can improve endometrial receptivity. In FET cycles, it was reported in most of study that GnRHa was administrated during the mid-luteal phase. It is unknown that whether pretreatment in follicular phase could benefit the outcome of pregnancy. Moreover, it is hard for patients to recognize the mid-luteal phase and complicated to induce menstruation for patients with PCOS. Thus, this study aimed to investigate the clinical outcome of the FET cycles after administration of long-acting GnRHa in early follicular phase.

Materials And Methods

Study population and inclusion criteria

Patients who underwent FET were retrospectively investigated from October 2017 to March 2019 at a university-affiliated IVF center. This study was approved by the Institutional Review Board (No.20190815) and was conducted according to the principles laid out in the Declaration of Helsinki. The inclusion criteria was as follows: (1) patients received FET cycles with GnRH agonist pretreatment (GnRHa FET) and HRT FET cycles without GnRH agonist (HRT) for endometrial preparation; (2) patients were 45-years

old or less; (3) at least one grade I, II, or blastocyst embryo was transferred. Embryos were graded according to the Garden score system; (4) endometrial thickness on the day of progesterone supplementation was ≥ 6.5 mm. Patients with IUA and untreated hydrosalpinx were excluded. Patients who received two and more GnRHa treatments were also excluded.

Endometrial preparation protocols

HRT FET without GnRH agonist

The HRT cycle was mainly performed in patients with abnormal ovulation and irregular menstrual cycles. On the third day of menstruation, ultrasound was performed to visualize any ovarian cysts to exclude the possibility of ovarian cysts in the experimental groups. Estradiol valerate ((Progynova, Bayer) or white estradiol tablet (Femoston, Abbott) was administered at an initial dose of 2 mg bid, which was increased to 3–4 mg bid as necessary, while transdermal estradiol gel (Oestrogel, Besins, France) was administered at an initial dose of 1.5-2 slide calipers bid. Estrogen was administered for at least 12 days. The thickness of the endometrium was monitored. If it reached at least 6–7 mm and no dominant follicle was found, vaginal micronized progesterone supplementations were administered at a dose of 400 mg bid for endometrial conversion. Cleavage embryos were transferred on the fourth day of progesterone administration and the blastocyst embryos were transferred on the sixth day.

FET with GnRH agonist

On the second day of menstruation, ultrasound was performed to visualize any ovarian cysts to rule out the presence of ovarian cysts in the experimental groups. Then, 3.75 mg of leuporelin was administered. If no dominant follicles were observed in the ultrasound 14 days later, estrogen and progesterone were administered, and the embryo was transferred, as described above. If dominant follicles were found, HMG was used to promote the development of follicles as necessary, after which ovulation was triggered by HCG with the thickness of the endometrium reaching at least 6 mm. After ovulation, progesterone was added for endometrial conversion. The cleavage and blastocyst embryos were transferred on the third and fifth day postovulation, respectively.

Outcomes

Twelve days after embryo transfer the concentration of HCG in the serum was tested. If the pregnancy test was positive 28–35 days after ET, transvaginal ultrasound was performed to confirm clinical pregnancy. Luteal support was continued up to 2 months after FET. The clinical pregnancy rate was the primary outcome considered by this study. The secondary outcomes were the thickness of the endometrium on the day of progesterone supplementation and the implantation rate. We defined miscarriages as spontaneous clinical pregnancy losses before 12 weeks of gestation. Implantation rates were defined as the ratios of the number of gestational sacs over the number of transferred embryos. Clinical pregnancy was defined as a visible yolk sac observed using ultrasound.

Statistical analysis

Statistical analysis was performed using SPSS 23.0 (IBM, Armonk, NY, USA). Measurement data are presented as the mean $\pm$ standard deviation (SD) and were analyzed by Student's t-test or Fisher's exact tests where appropriate. Enumeration data are presented as percentages (%) and analyzed using the Chi-square test. $p < 0.05$ was considered statistically significant. Binary logistic regression analysis was used to assess the association between the GnRHa and clinical pregnancy. We calculated odds ratios (OR) with 95% confidence interval (CI). Forest map was generated by GraphPad Prism 8.0.2 (GraphPad Software, Inc, USA).

Results

Patients characteristics

In total, 630 patients were included in the GnRHa FET group and 1141 patients were included in the HRT FET without GnRHa group. There were no difference between the two groups in terms of age, duration of infertility, type of infertility, percentage of PCOS, diminished ovarian reserves, endometriosis, scarred uterus, uterus malformation, or the grade and number of transferred embryos (Table 1).

Table 1
Characteristics of patients.

Characteristics	GnRHa FET (n = 630)	HRT without GnRHa (n = 1141)	<i>P</i>
Age	32.1 ± 5.2	32.4 ± 5.3	0.306
Duration of infertility	4.8 ± 3.6	5.1 ± 3.9	0.127
Type of infertility			0.108
Primary	310/630 (49.2)	516/1141 (45.2)	
Secondary	320/630 (50.8)	516/1141 (54.8)	
Number of pregnancies	1.2 ± 1.4	1.2 ± 1.5	0.245
Percentage of PCOS	106 (16.8)	208 (18.2)	0.459
Percentage of DOR	86 (13.7)	183 (16.0)	0.180
Percentage of scarred uterus	102 (16.2)	158 (13.8)	0.182
Percentage of endometriosis	23 (3.7)	36 (3.2)	0.578
Number of transferred embryos	1.7 ± 0.4	1.7 ± 0.4	0.890
Grade of transferred embryos			0.472
Cleavage I	160 (22.7)	277 (23.9)	
Cleavage II	331 (46.9)	548 (47.4)	
Blastocyst	215 (30.5)	332 (28.7)	
Note: GnRHa = gonadotropin-releasing hormone agonist; HRT = hormone replacement treatment; PCOS = polycystic ovary syndrome; DOR = decreased ovarian reserve			

Table 2
Outcome of FET with or without GnRHa.

Outcome	GnRHa FET (n = 630)	HRT without GnRHa (n = 1141)	P
Endometrial thickness	9.4 ± 1.5	9.3 ± 1.5	0.215
Endometrial pattern			0.000
Triple line	597 (94.8)	1022 (89.6)	
No triple line	33 (5.2)	119 (10.4)	
Biochemical pregnancy rate	382 (60.6)	619 (54.3)	0.009
Implantation rate	425/1225 (34.7)	602/2011 (30.0)	0.00
Clinical pregnancy rate	314 (49.8)	494 (43.3)	0.008
Ectopic pregnancy rate	8 (1.3)	17 (1.5) ^a	0.707
Early miscarriage	34/630 (5.4)	60/1141 (5.3)	0.901
Note: FET = frozen-thawed embryo transfer; GnRHa = gonadotropin-releasing hormone agonist; HRT = hormone replacement treatment; ^a Included two heterotopic pregnancies.			

Outcomes

The GnRHa FET group produced a higher percentage of endometrium with triple line pattern (94.8% vs 89.6%, $p < 0.001$) on the day of progesterone administration, as well as an increased implantation rate (34.7% vs 30%, $p < 0.01$), biochemical pregnancy rate (60.6% vs 54.3%, $p = 0.009$), and clinical pregnancy rate (49.8% vs 43.3%, $p = 0.008$), as compared to the HRT FET group; however, the endometrial thickness, ectopic pregnancy rate, and early miscarriage rate were similar.

Binry logistic regression analysis was performed to evaluate each variable's effect on clinical pregnancy (Table 3) and forest map was generated (Fig. 1). Age, PCOS, number of transferred embryos, grade of transferred embryos, endometrial thickness and using GnRHa were significantly associated with clinical pregnancy. GnRHa FET group was associated with an increased chance of clinical pregnancy compared with HRT FET without GnRHa group ($P = 0.014$, odds ratio [OR] 1.30, 95% confidence interval [CI] 1.06–1.61).

Table 3
Multivariable logistic regression analysis for clinical pregnancy.

Covariate	OR (95% CI)	P
Age	0.92(0.90–0.95)	0.000
Duration of infertility	1.02(0.99–1.05)	0.178
Type of infertility		
Primary	Reference	
Secondary	0.84(0.64–1.10)	0.178
Number of pregnancies	0.91(0.66–1.27)	0.59
Number of abortions	1.17(0.85–1.63)	0.34
Number of deliveries	0.99(0.67–1.46)	0.964
PCOS		
NO	Reference	
YES	1.47(1.13–1.93)	0.005
Endometriosis		
NO	Reference	
YES	0.55(0.30–0.99)	0.047
Number of transferred embryos	2.14(1.65–2.77)	0.000
Grade of transferred embryos		0.000
Cleavage	Reference	
Blastocyst	1.99 (1.56–2.55)	0.000
Endometrial thickness	1.10(1.03–1.18)	0.007
Endometrial pattern		
Triple line	1.36 (0.94–1.96)	0.099
No triple line	Reference	
Protocol		
With GnRha	1.28(1.04–1.58)	0.020
Without GnRha	Reference	
Note: OR = odds ratio; CI = confidence interval; PCOS = polycystic ovary syndrome; DOR = decreased ovarian reserve; GnRha = gonadotropin-releasing hormone agonist; HRT = hormone replacement treatment;		

Interestingly, we found that 1–3 dominant follicles grew in 37 patients, 14 days post-GnRHa administration. The age of these patients was 34.1 ± 4.8 years. The endometrial thickness was 9.4 ± 1.5 mm on the day of progesterone administration with a rate of triple line pattern as 81.1%. The clinical pregnancy rate was 54.1% after the transfer of 1.9 ± 0.3 embryos, and the early abortion rate was 10.8% (Supplementary Tables 1 and 2).

Discussion

In spite of the widespread application of FET cycles, no significant improvement in terms of the pregnancy rate has been observed in comparison to the fresh cycles. The present study found that the GnRHa FET group produced a significantly higher percentage of endometrium with triple line pattern, and an increased implantation and clinical pregnancy rate, as compared to the HRT FET cycles without GnRHa group. Logistic regression analysis showed the GnRHa FET group to be significantly associated with an increased chance of clinical pregnancy compared with HRT FET without GnRHa group. These results demonstrated that the administration of a single dose of long-acting GnRHa in early follicular phase of the same FET cycle can improve the clinical outcome of HRT cycles, possibly by improving the receptivity of the endometrium. The relatively large amount of data provide evidence for the clinical application of this method in FET cycles. However, RCTs will be needed to prove the results presented in this study.

The method using FET with pituitary downregulation has been used before the HRT cycles without GnRHa suppression. There is no consensus on which method is consistently better for the outcome of pregnancy. As compared to a natural or modified natural cycle protocol, data from a retrospective 1391 cycles reported that the synthetic HRT protocol with GnRHa was associated with a higher live birth rate in the blastocyst-stage of the FET cycles [14]. However, there were no differences in the reproductive outcomes between the two methods in majority of studies, which included patients with regular ovulation [15–19]. Similar negative results were observed between the GnRHa HRT and HRT alone cycles in patients with regular menstrual cycles [5, 8] and those with functioning ovaries [7, 9, 11]

It has been reported that pituitary suppression when initiated in the middle luteal phase in HRT cycles results in higher clinical pregnancy and live birth rates than the HRT cycles without prior GnRHa therapy in patients with regular menstrual cycles [4]. Hebisha et al. reported that the administration of a GnRHa for HRT FET during endometrial preparation increased the implantation and pregnancy rates in patients with undefined ovary functions [10], with a similar pregnancy outcome being observed in other studies [6, 12]. Our study also found that the downregulation of FET produced a higher percentage of endometrium with triple line pattern, and a higher implantation and clinical pregnancy rates as compared to the HRT FET cycles without GnRHa. These patients had abnormal ovulation and irregular menstrual cycles.

Differently, we administered long-acting GnRHa during menstruation in the same FET cycles, while short-acting GnRHa was administered on a daily basis in the middle phase in the three above mentioned

studies with positive outcomes [4, 10, 14]. Le et al administered medroxy-progesterone acetate for 10 days in order to induce menstruation and a half dose of long-acting GnRHa on the third day of medroxy-progesterone acetate. The administration of exogenous estradiol was initiated on the third day of menstruation. They found comparable pregnancy outcomes to those observed for modified natural cycles [20]. Nekoo et al and Prato et al administered 3.75 mg of long-acting GnRHa at the mid-luteal phase (day 21) of the previous cycle, resulting in a similar pregnancy rate between the HRT and GnRHa HRT FET cycles [5, 7]. Xie et al. administered 3.75 mg of long-acting GnRHa on day 3 of menstruation. After 28 days, estrogen and progesterone were administered for endometrium preparation. The data showed that the resultant clinical pregnancy and live birth rates were higher in the GnRHa HRT FET cycle than that in the HRT FET cycle. In Qi's study, 3.75 mg of long-acting GnRHa was injected in day 2 or 3 of menstruation. HRT was initiated 28 days later. They found pregnancy outcomes were improved in patients with endometriosis and PCOS[21]. However, in patients aged 38 years or older, Dong et al failed to find a significant difference in pregnancy and live birth rate between two groups[22]. An RCT reported that in patients with repeated implantation failure, short term GnRHa from 21 day of menstruation did not increase the pregnancy and implantation rates of subsequent HRT cycles[22]. In our study, we did not indicate the patients and there were no difference between the two groups in terms of patients parameters including ages, percentage of PCOS and endometriosis.

A 28 days interval between the administration of GnRHa and estrogen was thought to be sufficient as the pituitary suppression exerted by GnRHa was relieved upon embryo implantation, wherein the GnRHa-HCG system could play an important role in embryo implantation and development [23]. Palmerola et al administered 1000 mg of leuprolide acetate on day 2 of the cycle. Estrogen administration was initiated 14 days later when the rate of suppression was adequate. As a result, the implantation and the clinical pregnancy rates were found to be satisfactory [24]. Our results suggested that an interval of 14 days between the GnRHa and estrogen administration did no harm to the embryo implantation but rather increased the percentage of triple line endometrium, the implantation rate and clinical pregnancy rate. An interval of 14 days reduced the patient waiting time to start endometrial preparation. We also speculated that GnRHa does direct effect in endometrial development to improve receptivity. Maybe shorter interval or administration of GnRHa and HRT together can also improve pregnancy outcome of FET. It deserves to be studied in further.

Additionally, in this study, we found that 14 days after administering a single dose of GnRHa (3.75 mg), dominant follicles developed and the endometrium grew to a normal thickness in 37 patients. This is most likely due to the flare-up effect of GnRHa, which stimulated the development of the follicles. After direct HCG triggering or after necessary HMG stimulation, ET was found to produce 54.1% of CPR, suggesting that the downregulation of GnRHa did not affect embryo implantation at this situation; thus, the "cyst" need not to be punctured and the cycles need not to be cancelled under these conditions. However, the limited data available in our study suggests a tendency for a high early miscarriage rate. We cannot exclude the effect that downregulation by GnRHa could have on luteal function. The effect of an additional estrogen supplementation will need to be investigated in future studies.

The mechanism by which GnRHa improves the outcome of pregnancy in HRT FET cycles remains to be elucidated. The GnRH/ GnRHR system occurs in the endometrium, ovaries, and human preimplantation embryos. The expression of this system supports its physiological regulatory role in the functioning of the corpus luteum, endometrium receptivity, the capability of trophoblast invasion, and the processes related to embryo implantation [25, 26]. Some studies implied that GnRHa facilitates embryo implantation by enhancing luteal secretion. However, the results obtained in our study cannot be explained on that basis. In this study, the exogenous estrogen and progesterone were administered when there was no corpus luteum, except for the 37 patients who were undergoing ovulation. Meanwhile, after 8 weeks of administering long-acting GnRHa, the pituitary was found to begin to recover its functions [27]. In our study, ET was performed at about 31–40 days after administering GnRHa, which is when the pituitary was in a state of suppression and the corpus luteum could not be stimulated.

A meta-analysis [28] including six RCTs showed that a single or continuous injection of short-acting GnRHa during the luteal phase for luteal support increased the pregnancy and live birth rates in both the agonist and antagonist protocols. Fujii et al continuously administered GnRHa during the luteal phase until 14 days after oocyte retrieval in the long protocol IVF [29]. The serum concentrations of estradiol and progesterone on the day of embryo transfer and 7 days after oocyte retrieval were similar to those obtained using the long protocol alone. However, the implantation and live birth rates of the GnRHa group were significantly higher. A possible explanation could be the direct action of the GnRHa in regulating embryo invasion and development, endometrial receptivity, and cross talk between the embryo and the endometrium. In a murine model, ovarian stimulation decreased the expression of the endometrial expression of integrin beta-3 subunit, leukaemia inhibitory factor, and the implantation rate during the implantation window. These were partially restored by the administration of the GnRHa. These results suggest that the GnRHa plays an important role in improving the endometrial receptivity [30]. An *in vitro* study [31] demonstrated that the GnRH-II agonist promoted the cell motility of human decidual endometrial stromal cells by binding to the GnRH-I receptor, leading to the phosphorylation of protein kinase 1/2 and the activation of MMP-2 and MMP-9. These findings suggest that the GnRH-II agonist has a strong effect on the endometrial decidualization and embryo implantation. In this study, an increased percentage of triple line endometrium after GnRHa suppression suggests an improved endometrial receptivity. Meanwhile, GnRHa may improve the invasive and secretory capabilities of the embryonic trophoblast cells, thus facilitating embryo implantation and cross talk in the uterus. After administration of GnRHa during pregnancy, the serum levels of human chorionic gonadotropin (hCG) were found to be significantly elevated. The results indicated that GnRH could stimulate the release of hCG by the trophoblast cells of the placenta [32]. Peng et al showed that GnRH-induced RUNX2 expression could strengthen the invasive capacity of the human extravillous trophoblast cells by modulating the expression of MMP9 and MMP2 [33].

This study has several limitations. Since it was a retrospective study and not a prospective randomized study, undetected biases may occur. Thus, we performed logistic regression analysis to reduce biases. A relatively large number of patients were included under strict criteria. We did not evaluate some of important data, such as we did not compare the live birth rates between the groups, and we did not

conduct routine tests to determine the serum hormone levels on the days of progesterone supplementation, embryo transfer, implantation window, or pregnancy testing. Owing to the small patient sample size, the clinical outcomes between GnRHa + ovulation and GnRHa + HRT were inconclusive. However, the measurements of endometrial thickness and clinical pregnancy rate were satisfactory in these groups of patients. The rate of miscarriage need be evaluated in further studies.

In conclusion, the administration of a single dose of long-acting GnRHa during the early follicular phase and initiating HRT 14 days later can improve the clinical outcome of the FET cycles. The mechanism of this process may rely on the direct effect of the GnRHa on the regulation of embryo invasion and development, endometrial receptivity, and the cross talk between the embryo and the endometrium. However, further RCTs will be needed in order to validate the results presented in this study.

Abbreviations

GnRHa: gonadotropin-releasing hormone agonist; FET: frozen-thawed embryo transfer; OR: odds ratio; CI: confidence interval; RCTs: randomized control trials; IVF: in vitro fertilization; ICSI: intracytoplasmic sperm injection; OHSS: ovarian hyperstimulation syndrome; COS: controlled ovarian stimulation; PPOS: progestin primed ovarian stimulation; PCOS: polycystic ovary syndrome; SD: standard deviation; hCG: human chorionic gonadotropin.

Declarations

Acknowledgements

Not applicable.

Authors' contributions

Y.P.L conceived and designed the study. All the authors analysed and interpreted the data and wrote the manuscript. B.X contributed to data collection and performed the statistical analysis. All the authors approved the final version of the manuscript.

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Availability of data and materials

The data generated and analyzed in this study will be availed upon request by the corresponding author

Ethics approval and consent to participate

This study was approved by the ethics committee of reproductive Medicine Center, Xiangya Hospital, Central South University (No.20190815).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests

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Figures

Figure 1

Metanalysis

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