

Impact of Elevated Oestrogen Levels on hCG Trigger Day on Clinical Pregnancy Outcome and OHSS Incidence in Long-Acting GnRHa Down-Regulated IVF/ICSI-ET Cycles

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Background: The relevance of oestrogen (E₂) levels on human chorionic gonadotropin (hCG) trigger day and clinical pregnancy outcomes was unclear. This study aimed to investigate whether E₂ levels on the hCG trigger day affect pregnancy outcome in long-acting gonadotropin releasing hormone agonist (GnRHa) down-regulated in-vitro fertilization/intracytoplasmic sperm injection-embryo transfer (IVF/ICSI-ET) cycles.

Methods: This is a retrospective study including 6967 patients undergoing their first long-acting GnRHa down-regulated IVF/ICSI-ET cycles at the Reproductive Medicine Centre of Nanjing Drum Tower Hospital from January 2016 to December 2021. Smooth curve fit analyses were conducted to identify the relationship between serum E₂ levels and the clinical pregnancy rate (CPR), along with the live birth rate (LBR). Threshold effect analyses were conducted to figure out potential cut-off values. Further investigation was also carried out for different types of embryos.

Results: Both CPR and LBR declined as the serum E₂ levels on the hCG day elevated. CPR reduced significantly when the serum E₂ levels exceeded 5000 pg/mL. While considering the influence of different types of transferred embryos, the blastocyst transfer was more vulnerable to the elevation of serum E₂ levels than cleavage-stage embryos, and CPR of blastocyst transfer decreased obviously when serum E₂ levels were equal to or greater than 5000 pg/mL. Meanwhile, a higher E₂ level leads to a higher incidence of moderate or severe ovarian hyperstimulation syndrome (OHSS).

Conclusion: The CPR decreased significantly as E₂ levels increased with serum E level higher than 5000 pg/mL on the hCG day, which was more obvious in blastocyst transfer than cleavage-stage embryo transfer. In addition, the incidence of moderate-to-severe OHSS also significantly increased. Therefore, the whole embryo freezing might be a better choice in patients undergoing long-acting GnRHa down-regulated IVF/ICSI cycles with higher serum E₂ level on the hCG trigger day instead of embryo transfer.

Keywords: oestrogen level, hCG trigger day, clinical pregnancy rate, long-acting GnRHa, blastocyst transfer, OHSS

Background

Blastocyst implantation requires synchronization of blastocyst and receptive endometrium, which is precisely regulated by maternal hormones. Under the physiological condition, progesterone (P) and oestrogen (E₂) together mediate successful blastocyst implantation. Preimplantation E₂ secretion optimizes the progesterone-primed receptive uterus and direct structural and functional changes in the uterus that lead to uterine receptivity,^{1,2} while altered hormonal environments may change the



uterine receptivity. Previous studies have shown that levels of E_2 determine the duration of the implantation window within a narrow range.³ Oestrogen extends the window of implantation at low levels, whereas higher E_2 levels can rapidly close the window and alter expression of genes related to endometrial receptivity, and thereby affect implantation.⁴

Previous studies suggested that the endometrium is influenced by the direct effect of controlled ovarian hyperstimulation (COH) in in-vitro fertilization/intracytoplasmic sperm injection and embryo transfer (IVF/ICSI-ET) cycles. Comparing to natural cycles, supra-physiological state is inevitable under COH.^{5,6} Previous studies have suggested that a higher serum P level around the time of frozen embryo transfer, when it is above the threshold level, might be associated with increased rates of pregnancy.⁷ When serum P levels exceed 1.5 ng/mL, an increase in these levels may result in a decreased pregnancy rate.⁸ Other data also suggest that for patients undergoing the gonadotropin releasing hormone (GnRH) antagonist protocol with cleavage-stage embryo transfer, the serum P level on the day of human chorionic gonadotropin (hCG) administration does not significantly affect clinical outcomes.⁹ However, our previous study indicated that elevated serum P levels on the hCG day during GnRH agonist (GnRHa) down-regulated IVF-ET cycles could decrease the clinical pregnancy rate of blastocysts.¹⁰ However, there is limited evidence of the impact of COH E_2 levels on pregnancy outcomes. Exposure to high levels of E_2 could be one of adverse impacts of COH on pregnancy outcomes.^{11,12} The exposure may lead to pregnancy complications and adverse neonatal outcomes, such as dysfunction of coagulation and fibrinolysis,¹³ higher miscarriage rate and lower live birth rate.^{14–16} While some early studies showed that elevated E_2 level was not detrimental to IVF outcomes.^{17,18}

To further assess the relevance of E_2 levels on the hCG trigger day and clinical pregnancy outcomes, we conducted a retrospective study at Reproductive Medicine Centre of Nanjing Drum Tower Hospital. This study mainly focused on infertile women undergoing the long-acting GnRHa down-regulation protocol in IVF/ICSI-ET cycles.

Methods

Study Population

We performed this retrospective cohort study of patients undergoing their first long-acting GnRHa IVF/ICSI-ET cycles from 2016 to 2021 at Nanjing Drum Tower Hospital. A total of 6967 cycles were included. The study is approved by the Nanjing Drum Tower Hospital ethics committee. Patients with these following characteristics were excluded: (1) over 45 years old; (2) ICSI cycles with sperm from testicular sperm aspiration (TESA) or percutaneous epididymal sperm aspiration (PESA); (3) cancelled oocyte retrieval cycles or oocytes freezing cycles; (4) canceled embryo transfer cycles with sudden factors.

Long-Acting GnRHa Protocol for COH

A long-acting GnRHa (triptorelin acetate, decapeptyl, Ferring GmbH, 1.875 mg or 3.75 mg) was administered in the early follicular phase. The levels of sex hormone, such as E_2 , P, follicle-stimulating hormone (FSH), luteinizing hormone (LH) and the diameter and number of follicles, were measured after pituitary down-regulation. The following stimulation protocol was selected according to female age, body mass index (BMI), antral follicle count (AFC) and serum hormone (FSH, LH, E_2 , P) levels. Recombinant FSH (rFSH, Gonal-F, Merck Sereno, 75–300 IU) was administered, and recombinant LH (rLH, Luveris, Merck Sereno, 75–150 IU) or human menopausal gonadotropin (HMG, Menotropins for Injection, Livzon Pharm, 75–300 IU) was added as appropriate. When 1–2 dominant follicles reached 18 mm, recombinant hCG (rhCG, Merck Sereno, 250 μ g, i.h.) or hCG (Chorionic Gonadotropin for Injection, Livzon Pharm, 5000–10000 IU, i.m.) was injected to trigger ovulation. The oocytes were collected 36–38 hours later. Mature oocytes (metaphase II, MII) were fertilization and cultured to different embryo stages (cleavage-stage embryos or blastocysts).

Embryo Transfer and Pregnancy Detection

The abdominal ultrasound-guided fresh embryos transfer was conducted on the 3rd (cleavage-stage embryo) or 5th day (blastocyst) after oocyte retrieval. If there were a large number of high-quality embryos on the 3rd day (>3 for patients less than 38 years old; >4 for patients over 38 years old), we usually cultured the embryos to blastocysts and conducted the blastocyst transfer on the 5th day. If the number of high-quality embryos was not enough on the 3rd day, we usually directly transferred the cleavage-stage embryos. Serum β -human chorionic gonadotropin (β -hCG) levels were detected 12–14 days after embryo transfer to determine biochemical pregnancy. Patients with positive β -hCG results may undergo

ultrasound examination 28–30 days after embryo transfer. Clinical pregnancy was confirmed by one or more gestational sacs. The patients were continuously followed up until achieving live birth. The clinical pregnancy rate (CPR) is defined as the ratio of clinical pregnancy cycles to the total embryo transfer cycles. Live birth rate (LBR) is defined as the ratio of deliveries after 28 weeks to total embryo transfer cycles. The early miscarriage rate was defined as the ratio of early miscarriage cycles to total clinical pregnancy cycles. The CPR was the major outcomes of this study.

Statistical Analysis

To investigate the relationship between serum E_2 levels on the hCG administration day and CPR in long-acting GnRHa down-regulated IVF/ICSI-ET cycles, a smooth curve fit analysis utilizing a generalized additive model (GAM) was performed. The analysis of threshold effects was further conducted to figure out the cut-off value of serum E_2 levels. We used univariate analysis to identify potential factors influencing clinical pregnancy outcomes and included them (female age, basal FSH level, AFC, initial Gn dose, Gn duration, P level on the hCG day, number of oocytes retrieved, number of transferred embryos, types of embryos transferred and endometrial thickness) as covariates in subsequent statistical analyses to minimize the impact of confounding factors. Patients were therefore divided into two groups based on their serum E_2 levels on the hCG day (Group 1 < 5000 pg/mL; Group 2 \geq 5000 pg/mL). In addition to clinical pregnancy outcomes, we specifically compared the incidence of ovarian hyperstimulation syndrome (OHSS) between groups, and categorized the severity of OHSS based on patients' clinical conditions.¹⁹ We also performed smooth curve fit analyses of serum E_2 levels and CPR respectively in patients with different types of embryos transferred: cleavage-stage embryos and blastocysts. Variables are presented as the mean \pm standard deviation (SD). $P < 0.05$ was considered statistically significant. Our analyses were performed with R software version 3.6.0 (<http://www.R-project.org>) and EmpowerStats software (www.empowerstats.com, X&Y solutions, Inc. Boston MA).

Results

Relationship Between Serum E_2 Levels of hCG Day and CPR/LBR

The smooth curve fitting results demonstrated that the CPR maintained relatively stable in the first segment of the curve (Figure 1 and Table 1, aOR: 1.005, 95% CI: 0.999–1.009, $p = 0.053$) but followed by a significant decline as the E_2 levels

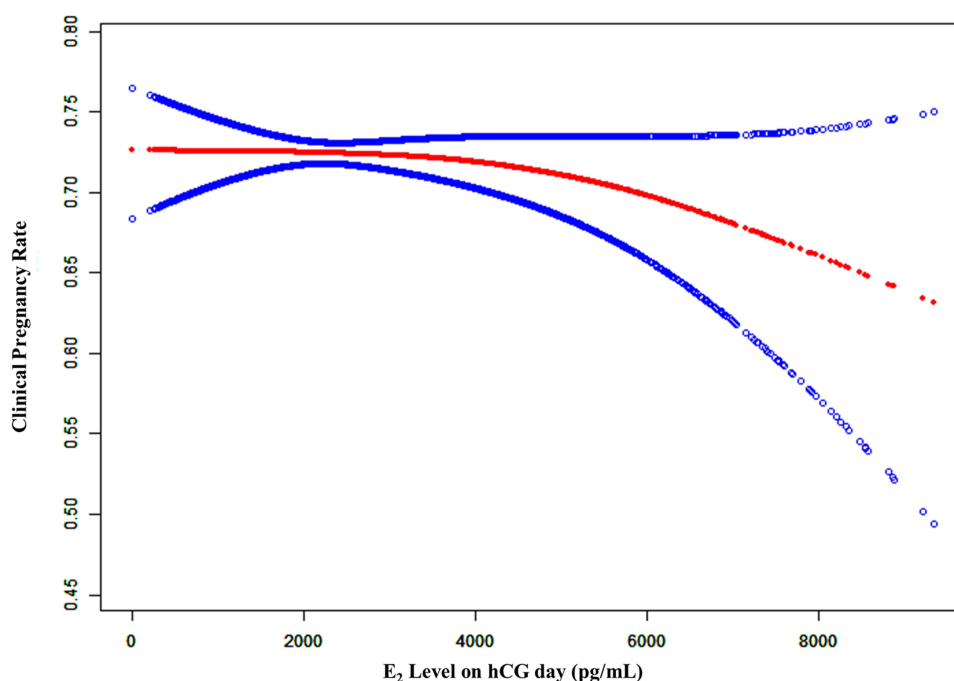


Figure 1 The illustrated curved line shows the relation between the E_2 level on the hCG day and CPR. The area between two dotted lines is expressed as the 95% CI.

Table 1 Threshold Effect Analysis of E₂ Level (100 pg/mL) of hCG Day on the Clinical Pregnancy Rate

Outcome	Clinical Pregnancy		
	OR	95% CI	p Value
Model I (linear)			
Linear effect	1.002	(0.998, 1.006)	0.389
Model II (polyline)	OR	95% CI	p Value
Predicted threshold (K, E ₂ level, 100 pg/mL)	50		
Effect 1 (<K)	1.005	(0.999, 1.009)	0.053
Effect 2 (>K)	0.981	(0.963, 0.999)	0.036
Variability of effectiveness	1.144	(1.016, 1.272)	
Logarithmic likelihood ratio test	0.022		

Abbreviations: E₂, oestrogen; hCG, human chorionic gonadotropin; OR, odds ratio; CI, confidence interval; K, predicted threshold.

climbing (Figure 1 and Table 1, aOR: 0.981, 95% CI: 0.963–0.999, p = 0.036). In addition, the LBR followed similar trend (Figure S1).

The analysis of threshold effects further indicated that the inflection point occurred when the serum E₂ concentration on the hCG day was equal to 5000 pg/mL (Table 1). Therefore, patients were divided into two groups according to different E₂ levels: Group 1 with E₂ levels less than 5000 pg/mL, Group 2 with E₂ levels greater than or equal to 5000 pg/mL (Table 2). The female age, basal FSH levels, basal E₂ levels and gonadotropin (Gn) duration was comparable between two groups.

Table 2 Characteristics of Patients with Different Serum E₂ Levels (5000 pg/mL) on hCG Day

Variable E ₂ Level on Trigger Day (ng/mL)	Group 1 < 5000	Group 2 ≥ 5000	p Value
Cases (n)	6582	385	
Age (y)	29.7 ± 3.7	29.3 ± 3.7	0.06
BMI (kg/m ²)	23.46 ± 4.42	21.95 ± 3.21	<0.01
Basal FSH (mIU/mL)	6.93 ± 1.73	6.92 ± 1.56	0.95
Basal E ₂ (pg/mL)	43.52 ± 51.60	47.00 ± 37.52	0.19
AFC (n)	20.7 ± 5.9	22.5 ± 6.1	<0.01
Initial Gn dose (IU)	130.23 ± 37.52	122.40 ± 29.31	<0.01
Total Gn dose (IU)	2024.1 ± 707.6	1795.9 ± 583.1	<0.01
Gn duration (d)	12.3 ± 2.6	12.5 ± 2.4	0.24
E ₂ level on trigger day (pg/mL)	2446.98 ± 1099.90	5907.60 ± 893.40	<0.01
P level on trigger day (ng/mL)	0.65 ± 0.42	0.85 ± 0.67	<0.01
Number of oocytes retrieved (n)	11.3 ± 3.9	15.3 ± 3.7	<0.01
Number of available embryos (n)	4.9 ± 2.5	6.5 ± 3.2	<0.01
Available embryo rate (%)	60.7	56.7	<0.01
Number of transferred embryos (n)	1.52 ± 0.50	1.41 ± 0.49	<0.01
Types of embryos transferred			<0.01
Cleavage-stage embryo (n)	4956 (75.30%)	242 (62.86%)	
Blastocyst (n)	1626 (24.70%)	143 (37.14%)	
OHSS rate (%)	4.53 (298/6582)	11.95 (46/385)	<0.01
Mild OHSS	1.93 (127/6582)	1.56 (6/385)	0.61
Moderate OHSS	2.57 (169/6582)	9.88 (38/385)	<0.01
Severe OHSS	0.03 (2/6582)	0.51 (2/385)	<0.01

(Continued)

Table 2 (Continued).

Variable E ₂ Level on Trigger Day (ng/mL)	Group 1 < 5000	Group 2 ≥ 5000	p Value
Embryo implantation rate (%)	60.8 (6099/10,025)	65.1 (355/545)	0.05
Clinical pregnancy rate (%)	72.0 (4740/6582)	74.0 (285/385)	0.39
Early miscarriage rate (%)	7.0 (331/4740)	6.7 (19/285)	0.84
Live birth rate (%)	63.8 (4202/6582)	66.0 (254/385)	0.40

Abbreviations: E₂, oestrogen; BMI, Body Mass Index; FSH, Follicle-stimulating hormone; AFC, Antral follicle count; Gn, Gonadotropin; P, progesterone; OHSS, ovarian hyperstimulation syndrome.

Total dose of Gn was greater in Group 1. The serum P level on the trigger day was higher in Group 2. Considering that the increase in P levels on hCG day may be related to high E₂ levels, we further conducted an interaction analysis. The results indicated that P levels on hCG day had no effect on the relationship between E₂ levels on hCG day and CPR (Table S1, P interaction = 0.88). It is worth noting that AFC, number of retrieved oocytes and number of available embryos were significantly higher in Group 2, while available embryo rates were significantly lower. The OHSS rate was also significantly elevated when serum E₂ levels exceeded 5000 pg/mL. When serum E₂ levels exceed 5000 pg/mL, the incidence of OHSS also significantly increases, particularly the proportion of moderate-to-severe OHSS cases.

Analysis of the E₂ Levels on the hCG Day and CPR for Different Types of Transferred Embryos

Subgroup analysis was carried out according to different types of transferred embryos. As shown in Figure 2, when the serum E₂ levels exceeded 5000 pg/mL, patients with blastocysts transfer undergone a significant decline in CPR as serum E₂ level on the hCG day increased (Table 3, aOR: 0.974, 95% CI: 0.949–0.999, p < 0.044), whereas the increase of E₂ levels seemed to have little effect on CPR of blastocyst transfer when serum E₂ levels were less than 5000 pg/mL

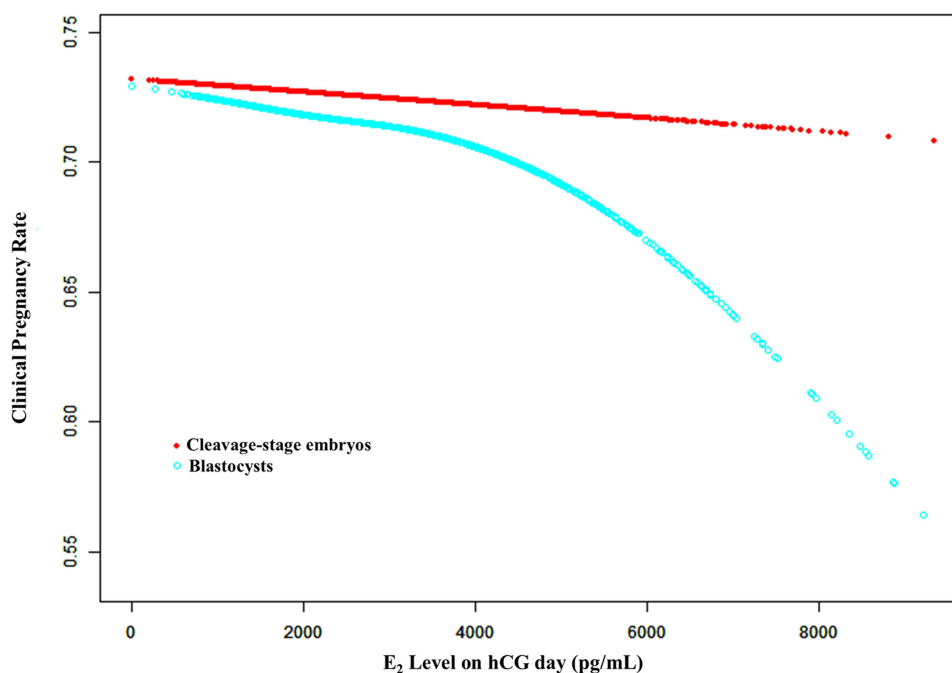


Figure 2 The illustrated curved line shows the relation between the E₂ levels on hCG day and CPR of different types of embryos transferred. The area between two dotted lines is expressed as the 95% CI.

Table 3 Threshold Effect Analysis of E₂ Level (100 pg/mL) of hCG Day on the Clinical Pregnancy Rate of Different Types of Transferred Embryos

Outcome	Clinical Pregnancy					
	Cleavage-Stage Embryos			Blastocysts		
Types of Transferred Embryos	OR	95% CI	p Value	OR	95% CI	p Value
Model I (linear)						
Linear effect	1.002	(0.997, 1.007)	0.364	1.000	(0.993, 1.007)	0.987
Model II (polyline)						
Predicted threshold (K, E ₂ level, 100 pg/mL)	50					
Effect 1 (<K)	1.004	(0.998, 1.009)	0.178	1.006	(0.997, 1.015)	0.200
Effect 2 (>K)	0.987	(0.962, 1.013)	0.330	0.974	(0.949, 0.999)	0.044
Variability of effectiveness	1.171	(1.020, 1.322)		1.175	(1.033, 1.317)	
Logarithmic likelihood ratio test	0.253			0.040		

Abbreviations: E₂, oestrogen; OR, odds ratio; CI, confidence interval; K, predicted threshold.

(Table 3, aOR: 1.006, 95% CI: 0.997–1.015, $p = 0.200$) and cleavage-stage embryo transfer under any levels of E₂. When it comes to live birth rate, both blastocyst and cleavage-stage embryo transfer were negatively influenced by high serum E₂ levels on hCG day and presented linear effect (Figure S2), which was still more significant in blastocyst transfer.

Discussion

Comparison with Other Studies

Our study suggested that excessive levels of E₂ on the hCG day impaired CPR and LBR in patients undergoing long-acting GnRHa down-regulated IVF/ICSI cycles. Despite that our conclusion has been backed up by several research,^{20,21} there do exist several studies revealed that high serum E₂ levels on the trigger day is not associated with clinical outcomes. Discrepancies may be caused by variations in ovulation stimulation protocols. DNA micro-arrays showed that GnRH antagonist protocol was more similar to natural cycles comparing to GnRHa protocol, which may result in the non-significance of elevating of E₂ levels.¹⁸

One of the studies recruiting 3393 infertile women undergoing the long-acting GnRHa protocol revealed that elevated serum E₂ levels on hCG day had no detrimental effect on clinical pregnancy and birth outcomes, and the implantation rate, clinical pregnancy rate, and live birth rate were even significantly higher in the high E₂ group than in the low E₂ group ($p < 0.05$).²² The divergence of our conclusion may attribute to the difference standard of subgrouping. Percentile analysis was often used to define elevation of the E₂ level on hCG day,^{23,24} but there is still no agreement on standard definition of elevated serum E₂ levels. Since levels of E₂ vary from different populations depending on their baseline conditions and ovarian stimulation protocols, the definition of elevated E₂ levels should be chosen carefully, which may otherwise influence the conclusion. Former mentioned studies categorized patients into high and low E₂ groups by using the 50th or 95th percentile. However, our study, instead of defining the elevation of E₂ levels by percentile analysis, performed threshold effect analysis to define the cut-off value. 5000 pg/mL is extremely high levels of E₂ comparing to cut-off values in other studies, which presented apparent and significant effect on CPR and LBR, this value may be more reasonable in clinical assessment of E₂ levels on the hCG day under the long-acting GnRHa down-regulated protocol.

Although some studies might not consider serum E₂ levels as a good predictor of live birth rate or other clinical outcomes, their results still demonstrated negative effects of high E₂ levels on hCG day.^{25,26} For example, the study²⁵ showed that patients with >75th percentile oestrogen levels had lowest rates of clinical pregnancy, embryo implantation and live birth (though without statistical significance), and the 51st-75th percentile group showed significantly highest rates of low birth weight infants and premature delivery.

Possible Mechanisms of the Effect of Excessive E₂ Levels on IVF/ICSI Outcomes

The super-physiological E₂ level is inevitable during the COH cycles for IVF/ICSI. It disturbs successful pregnancy by exerting its influence on micro-environment depending on hormones. Research mainly focused its effect on oocytes/embryo quality and endometrial receptivity. The development of oocytes is closely related to the level of E₂, and the level of E₂ in follicular fluid is relevant to that in serum.²⁷ In that case, elevated oestrogen levels might alter oocyte production and maturity, and therefore further impair the embryo quality. Yet its effect on available embryos is still undetermined. Several studies^{22,28} along with ours revealed that high levels of E₂ might bring higher mean numbers of oocytes and mature oocytes. In addition, our study suggested that the available embryo rate was higher in the lower E₂ group.

Impaired endometrial receptivity might play a more dominant role in high oestrogen led pregnancy failure. As previously mentioned, reaching the specific concentration and duration threshold is required for E₂ to sufficiently priming the endometrium,²⁹ and high levels of E₂ may rapidly close the window of implantation.³ Oestrogen primes the endometrium for implantation by inducing oestrogen receptor (ER) and progesterone receptor (PR) both in endometrium epithelial and stromal cells. Perturbations of oestrogen would impair implantation by affecting receptivity related gene expression,³⁰ like integrin β 1 and β 3, and consequently decrease rates of embryo attachment.³¹ Though the network of signaling pathways needs to be fully characterized,³² endometrium gene expression profiles has verified the significant difference between patients undergoing natural cycles and stimulated cycles, and high serum E₂ may well engage in the modulation.^{33,34}

Moreover, excessive E₂ level can also increase the risk of OHSS,^{22,25} which was also substantiated by our study and is worth much attention. Considering that the predictive cut-off value of 5000 pg/mL was calculated based on the clinical pregnancy rate as the outcome variable, we conducted additional threshold effect analyses to further explore the correlation between E₂ levels on hCG day and OHSS rate. The results indicated that when serum E₂ levels on hCG day were below 3500 pg/mL, the incidence of OHSS increases significantly with rising E₂ levels. Once E₂ levels on hCG day reach or exceed 3500 pg/mL, the OHSS incidence reaches a certain plateau and remains unaffected by further increases in E₂ levels (Table S2). Other studies also suggested that supra-physiological E₂ levels on the day of hCG trigger may indicate disrupted fetal growth and contribute to small size for gestational age and low birth weight.^{15,35} It has been shown in animal models that high E₂ levels in the early stage of pregnancy might diminish uterine artery remodeling and regulate placental extra villous trophoblast invasion,³⁶ which may account for its negative effect on pregnancy and birth outcomes.

Limitations

Although our study enrolled a large data set (n = 6967) to explore the possible effect of serum E₂ levels on hCG day on pregnancy outcomes, potential bias cannot be ignored considering our retrospective design. Another issue should be noted is that the population of higher E₂ levels group was smaller comparing to the lower E₂ levels group, which is another possible source of bias. In addition, AMH levels were failed to be included in our baseline characteristics, because the test did not widely carry out until recent years in our reproductive medicine centre. Due to the limitations of the data system, we were unable to directly obtain specific data on the type of Gn, early-onset and late-onset OHSS, which warrants further refinement in future studies. Additionally, our research focused on predicting OHSS risk at the embryo transfer stage under a specific COH protocol, but proactive prevention (prior to protocol selection) should be more critical. In future studies, we will further incorporate more fundamental indicators, such as BMI, AMH, and AFC, to estimate OHSS risk in advance and select more appropriate COH protocols, thereby effectively reducing OHSS risk. In a real-life setting, these results are cautiously applicable; however, further prospective investigations are needed to elucidate the impact of E₂ levels on clinical outcomes.

Conclusions

In conclusion, our study indicated that for patients undergoing IVF/ICSI with the long-acting GnRHa down-regulated protocol, when the serum E₂ level on hCG day reached or exceeded 5000 pg/mL, the clinical pregnancy rate significantly decreased as E₂ levels raised, particularly in patients receiving blastocyst transfer. Concurrently, the risk of moderate-to-severe OHSS markedly increases in patients with higher E₂ levels on hCG day. At this point, for such patients, elective cryopreservation of all embryos may be the preferable option.

Abbreviations

E₂, oestrogen; hCG, human chorionic gonadotropin; GnRHa, gonadotropin-releasing hormone agonist; OHSS, ovarian hyperstimulation syndrome; IVF-ET, in vitro fertilization-embryo transfer; ICSI, intracytoplasmic sperm injection; CPR, clinical pregnancy rate; LBR, live birth rate; P, progesterone; COH, controlled ovarian hyperstimulation; TESA, testicular sperm aspiration; PESA, percutaneous epididymal sperm aspiration; FSH, follicle-stimulating hormone; LH, luteinizing hormone; BMI, body mass index; AFC, antral follicle count; HMG, human menopausal gonadotropin; GAM, generalized additive model; Gn, gonadotropin; SD, standard deviation.

Data Sharing Statement

The datasets generated and analysed during the current study are not publicly available due to the special requirements of our hospital and our reproductive medicine center for the disclosure of patients' clinical data but are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

This retrospective study received ethical approval from the ethics committee of Nanjing Drum Tower Hospital. All patient couples provided written informed consent. All methods were carried out in accordance with relevant guidelines and regulations. Our study complies with the Declaration of Helsinki.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

Yuan Yan, Jingwen Jiang and Jie Mei are co-first authors for this study. The authors report no conflicts of interest in this work.

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