Comparison of Animal Models for Premature Ovarian Insufficiency Induced by Different Doses of Cyclophosphamide: a Network Meta-analysis

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Abstract

Introduction

Cyclophosphamide (CTX), is reported to be extensively used to establish POI animal model. But the most effective dose has not been systematically concluded yet. This systematic review and network meta-analysis is aimed to compare and rank the different doses of cyclophosphamide in the CTX-induced POI rat model.

Methods

Randomized controlled trials of CTX-induced rat POI model were searched in four databases from inception to December, 2021. A network meta-analysis was conducted to analyze the data of included publications. The quality assessment was assessed by SYRCLE's risk of bias tool. Data were analyzed with STATA 15.0 and Review Manager 5.3.

Result

205 records were searched and a total of 14 articles met inclusion criteria, Compared by Ovarian morphological changes, estrous cycle and hormone level (FSH, E_2, AMH), the loading dose of 200mg/kg CTX with the maintenance dose of 8mg/kg CTX for consecutive 14 days showed the best efficacy in inducing rat POI model.

Introduction

Premature ovarian failure (POF) is characterized by a state of female hypergonadotropic hypogonadism before the age of 40 years. It is more recently referred as Premature Ovarian Insufficiency (POI) due to the limitations of POF which cannot reflect the progression and diversity of the disease and only represents the terminal stage of ovarian failure[1]. POI is defined by at least four months of amenorrhea or oligomenorrhea with elevated follicle stimulating hormone (usually above 25 UI/L) detected on two occasions at least 4 weeks apart, in a woman before the age of 40[2]. The occurrence of POI is about 1% in a woman at childbearing age. Furthermore, women who suffer from POI may face up with different complications such as reduction of bone density, decreased cognitive function, sexual dysfunction, cardiovascular disease and other long-term complications[3], which seriously worsen women's quality of life. The pathogenesis of POI is multiple and complex while the mechanism is still unclear. About 65% of POI patients are idiopathic with no definite cause. Some factors lead to POI has been proved such as genetic factors including abnormalities (Turner syndrome, Fragile X syndrome), immune disorders (Cushing syndrome, autoimmune thyroiditis), and enzyme defects (galactosemia). [4]It has also proved that cigarette smoking, chemicals, pesticides and viruses may have adverse effects on ovarian function, resulting in POI. Meanwhile, radiotherapy, chemotherapy, and pelvic surgery, such iatrogenic factors can also induce POI[5].

Due to the diversity and uncertainty of the pathogenesis of POI, there were different ways to induce POI animal models such as oophorectomy, D galactose, chemotherapy, immunosuppressive drugs which can help us comprehensively explore the hypothesized pathogenesis. As the survival rate of patients after chemotherapy has increased with the increasing incidence of malignant tumors and immune diseases (such as systemic lupus erythematosus) in young women, the damage of female gonads caused by chemotherapy has gradually become a clinical concern. Although chemotherapy induced POI animal model can't provide a whole picture of POI, it is still an effective way to study and prevent ovarian function injury associated with chemotherapy. Cyclophosphamide (CTX), a representative chemical drug, was the first chemotherapy drug to be associated with POI[6] and now has been extensively used to establish POI animal model. It is necessary to evaluate the efficacy of CTX at different doses, so as to provide reference for the construction of animal model of chemotherapy-induced POI animal model. In this systematic review and network meta-analysis, we summarize these CTX-induced POF rat models and make a comparison to screen out the most suitable method.

Materials And Methods

Strategy for article retrieval

We systematically searched relevant studies including Medline (PubMed), Web of Science, and Chinese databases including China National Knowledge Infrastructure (CNKI) and China Journal Full-text Database (CJFD) up until the December 2021 by using the following keywords: "premature ovarian failure OR premature ovarian insufficiency OR primary ovarian insufficiency OR premature menopause OR gonadotropin resistant ovary syndrome OR hyper gonadotropic ovarian failure AND Cyclophosphamide OR CTX AND animal model OR animal models" in the title/abstract field. For the electronic search, reference management software (NoteExpress) was used. Publications from Medline (PubMed), Wed of Science Chinese databases were imported to this software, and duplicates were excluded.

Inclusion and exclusion criteria of the articles

The inclusion criteria were defined as follows: (1) randomized controlled trials (2) animal models were CTX-induced rat models through intraperitoneal injection meanwhile the control group was injected with the same dose of normal saline;(3) articles which can provide the first author, year of publication and country.

The exclusion criteria were defined as follows: (1) reviews, case reports, letters; (2) repeated publications; (3) non-randomized controlled trials; (4) animal except rats;(5) No specific FSH and E_2 values.

Data extraction

To avoid bias, two independent researchers(Qi and Zhu) conducted data extraction and resolved conflicts through the consensus of research group .For each eligible study, the following information was extracted: (1)general study characteristics (the first author and the year of publication);(2) animal characteristics (the animal species, the design of animal experiments, the animal model used, and the follow-up);(3)number and appearance of ovarian follicles (primordial, primary, preantral, antral, atretic follicles);(4)hormonal findings, including ovarian follicles stimulating hormones (FSH), estrogen (E_2), luteal hormones(LH), and anti-Mullerian hormone (AMH); (5)body weight; (6) changes in estrus cycles.

Quality assessment

The quality assessment worked separately by two independent researchers (Qi and Zhu). All studies included for this meta-analysis were appraised through the SYRCLE's risk of bias tool which was based on the Cochrane risk of bias (RoB) tool and has been adjusted for aspects of bias that specialize in animal intervention studies which includes the assessment of selection bias(Sequence generation, Baseline characteristics, Allocation concealment), performance bias(Random housing, Blinding), detection bias(Random outcome assessment, Blinding), attrition bias(Incomplete outcome data), reporting bias(Selective outcome reporting), and other bias[7].

Data synthesis and statistical analysis

Stata15 software was used to perform the meta-analysis of the various outcome indicators. standardized mean difference (SMD) and 95% confidence interval (confidence Interval, CI) of continuous variables (FSH, E_2, LH, AMH) were retrieved. To obtain comparability and prevent from the differences in each modeling duration and time point of intervention between different trials, hormone data which was measured at the 28th day (4 weeks) after the first intervention day was applied in statistics. Heterogeneity between studies was performed using forest plots, Q tests, and I^2 tests. The critical value of the Q-test was set at 0.1. I^2 was used as the index to evaluate study heterogeneity, as low (0 to 49%), moderate (50 to 74%) or high (75 to 100%). The fixed effects model (FEM) was used in the case of low heterogeneity; the causes and sources of heterogeneity need to be analyzed when the heterogeneity was evaluated as moderate, otherwise, a random effects model was used for meta-analysis. A P value <0.05 indicated that the difference was statistically significant. Publication bias was assessed by funnel plots. A network plot was made through Stata15.0—where the larger dot indicated the larger sample size of the intervention; the thicker lines indicated the higher number of RCTs of the intervention. The relative ranking of the different doses of CTX-induced POI rat model for each outcome were estimated by using the distribution of the ranking probabilities and the surface under the cumulative ranking curve (SUCRA) probabilities to offer a summary statistic for the cumulative ranking[8]. SUCRA values of 100% and 0% were ranked as the best and worst intervention. Review Manager (RevMan) [Computer program], Version 5.3 was used for quality assessment and the literature quality score figure. Engauge Digitizer 10.8 software was used to collect data from the statistical graphs when the related data was merely found in figures.

Results

Results of articles retrieval

A total of 205 articles were acquired from the search on search of Medline (PubMed), Web of Science, and Chinese databases including China National Knowledge Infrastructure (CNKI) and China Journal Full-text Database (CJFD). After removing the duplicates ,43 articles were selected for further evaluation. A total of 16 articles were excluded by screening titles and abstracts. After carefully reviewing the full-text review ,13 studies were excluded due to the discrepancy of inclusion and exclusion criteria mentioned above. 14 studies[9–22] were finally selected for meta-analysis. A PRISMA flow diagram describing the process of literature search and selection is presented in Fig. 1. All the included articles are 14 RCTs. Among these 14 studies, the intervention method can be classified as one-time injection group (83.52mg/kg,100mg/kg) and multiple injections group (The loading dose were 50mg/kg or 200mg/kg with the maintenance dose was 8mg/kg for the consecutive 14 days). Thus, we divided these 14 studies into 4 groups:50mg/kg+8mg/kg*14d;200mg/kg+8mg/kg*14d;83.52mg/kg ;100mg/kg.SD rat accounted for 59.9% of the total number of rats used; Wistar rat made up 37.4% of the total. The basic characteristics of these articles are described in Table 1. The hormonal profiles of these articles are showed in Table 2.

Table 1

The basic characteristics of included studies

Author	Year	rat(sex, strain,age, weight)	Number of each group(control/treatment	Way of CTX administrated	loading dose of CTX on the first day	maintenance dose of CTX	Intervention duration(day)
Asmaa et al.	2010	Female,Wistar,8-10 weeks old,200-250g	15/15	Intraperitoneal injection	50mg/kg	8mg/kg	15
Wang et al.	2013	Female,Wistar,12 weeks old,180-200 g	16/16	Intraperitoneal injection	50mg/kg	8mg/kg	15
Hala et al.	2016	Female,Wistar,6-10 weeks old, 200-250g	12/12	Intraperitoneal injection	50mg/kg	8mg/kg	15
Fu et al.	2017	Female,Wistar,N/A,180-200 g	20/20	Intraperitoneal injection	50mg/kg	8mg/kg	15
Li et al.	2017	Female,SD,10-12 weeks old,235-245g	40/40	Intraperitoneal injection	50mg/kg	8mg/kg	15
Fu et al.	2018	Female,SD,12 weeks old,202-254g	20/20	Intraperitoneal injection	50mg/kg	8mg/kg	15
Li et al.	2019	Female,SD,10-12 weeks old,N/A	24/24	Intraperitoneal injection	50mg/kg	8mg/kg	15
Tang et al.	2021	Female,SD,8-10 weeks old,240-260g	16/16	Intraperitoneal injection	50mg/kg	8mg/kg	15
Feng et al.	2020	Female,SD,8-10 weeks old,240-260g	12/12	Intraperitoneal injection	83.52mg/kg	/	1
Xia et al.	2020	Female,SD,N/A,200-240g	6/6	Intraperitoneal injection	100mg/kg	/	1
Zheng et al.	2021	Female,SD,12 weeks old,240-280g	15/15	Intraperitoneal injection	100 mg/kg	/	1
Song et al.	2016	Female,Wistar,8 weeks old,180-200g	10/10	Intraperitoneal injection	200mg/kg	8mg/kg	15
Rauf et al.	2018	Female,Wistar,12-16 weeks old,NA	10/10	Intraperitoneal injection	200mg/kg	8mg/kg	15
Noha et al.	2021	Female,Swiss albino,8 weeks old,130-170g	6/6	Intraperitoneal injection	200mg/kg	8mg/kg	15

Table 2

The hormonal profiles of included studies

Author	Year	Number of each group(control/treatment	Intervention method	FSH (IU/L) (control/treatment	E_2 pg/ml) (control/treatment	AMH ng/ml) (control/treatment
Asmaa et al.	2010	15/15	50mg/kg+8mg/kg*14d	4.02±0.07/15.28±0.35	60.51±1.48/24.63±0.45	/
Wang et al.	2013	16/16	50mg/kg+8mg/kg*14d	3.27±0.02/14.67±0.76	60.03±0.09/23.66±0.32	/
Hala et al.	2016	12/12	50mg/kg+8mg/kg*14d	7.46±1.71/19.15± 2. 43	68.66±6.65/35.21±3.93	/
Fu et al.	2017	20/20	50mg/kg+8mg/kg*14d	8.47±0.65/26.39±0.57	57.00±1.57/25.48±1.51	/
Li et al.	2017	40/40	50mg/kg+8mg/kg*14d	28.38±0.30/77.10±5.94	30.22±2.19/18.18±1.42	0.37±0.02/0.21±0.02
Fu et al.	2018	20/20	50mg/kg+8mg/kg*14d	9.56±0.02/13.23±0.12	146.07±0.12/87.57±0.13	/
Li et al.	2019	24/24	50mg/kg+8mg/kg*14d	17.88±3.00/73.50±9.66	31.28±0.32/18.03±0.21	0.38±0.04/0.21±0.06
Tang et al.	2021	16/16	50mg/kg+8mg/kg*14d	4.58±0.09/7.10±0.02	140.29±2.08/123.02±1.01	1.00±0.09/0.98±0.02
Feng et al.	2020	12/12	83.52mg/kg	26.4±5.16/33.42±2.76	107.98±9.82/90.56±8.09	0.59±0.09/0.44±0.07
Xia et al.	2020	6/6	100mg/kg	24.00±6.00/28.20±2.40	167.0±2.6/134.0±3.20	/
Zheng et al.	2021	15/15	100mg/kg	17.42±0.53/30.90±2.05	280.97±20.45/138.42±9.34	/
Song et al.	2016	10/10	200mg/kg+8mg/kg*14d	13.29±0.33/18.16±1.66	52.34±10.96/35.35±4.56	1.79±0.11/1.29±0.09
Rauf et al.	2018	10/10	200mg/kg+8mg/kg*14d	30.42±1.62/53.22±0.78	81.5±4.91/41.5±2.86	7.03 ± 0.82/3.29±0.30
Noha et al.	2021	6/6	200mg/kg+8mg/kg*14d	15.0±7.0/52.0±31.0	183.5±17.7/85.3±10.2	/

Quality assessment of included studies

According to the SYRCLE's RoB tool, bias assessment revealed a low risk of 43.6%, unclear risk of 36.4%, and high risk of 20% among them. 14 Studies neither mentioned how allocation of animal was concealed and none of them housed rats randomly within the animal room. Only two studies [9]; [18]described that animal were selected at random for outcome assessment, and only one study[18] mentioned the outcome assessor was blinded. No article was found with incomplete outcome data or considered to show selective reporting so that attrition and reporting bias were low. The summary and details are shown in Fig. 2 and Fig. 3.

Ovarian morphological changes

The data of follicle counts of was extracted from 4 studies (Table 3). Results of the studies show that the intervention of CTX reduces the number of primordial and primary follicles and increases the number of atretic follicles.

Table 3
Follicle counts of 4 studies

Author	Year	Number	Intervention method	Primordial	Primary	Secondary	Antral	Atretic
Li et al.	2019	24	normal saline	18.04±0.11	17.28±0.21	20.28±0.02	9.68±0.08	5.17±0.65
		24	50mg/kg+8mg/kg*14d	12.46±4.38	15.89±0.02	8.89±0.01	5.17±0.21	4.72±0.01
Tang et al.	2021	16	normal saline	12.56±1.91	6.63±0.61	11.78±1.11	2.73±0.47	0.68±0.14
		16	50mg/kg+8mg/kg*14d	2.6±0.37	2.23±0.35	3.43±0.41	0.32±0.14	3.21±0.55
Song et al.	2016	10	normal saline	40.7±9.2	14.3±2.5	8.3±1.5	/	20.3±4.5
		10	200mg/kg+8mg/kg*14d	23.0±1.7	15.0±6.2	4.7±1.5	/	28.3±3.8
Noha et al.	2021	6	normal saline	2.6±1.34	3.8±1.47	2.5±0.52	1.5±0.52	0.6±0.51
		6	200mg/kg+8mg/kg*14d	0.5±0.52	0.5±0.52	0.4±0.41	0.2±0.22	2.8±0.63

Estrous cycle

Only seven studies examined the estrus cycles of rats under CTX exposure (Table 4). All of these studies reported changes in estrus cycles, Fu et al.[14] described that the estrous cycle of rats of the CTX group was prolonged significantly, especially the time of diestrus. The abnormal rate of estrous cycle of 50mg/kg+8mg/kg*14d group on average were 99.07% comparing to 63% of 83.52mg/kg group.

Table 4
Estrous cycle of 7 studies

author	year	Intervention method	abnormal rate of estrous cycle
Hala et al.	2016	50mg/kg+8mg/kg*14d	94.40%
Fu et al.	2017	50mg/kg+8mg/kg*14d	100%
Li et al.	2017	50mg/kg+8mg/kg*14d	100%
Fu et al.	2018	50mg/kg+8mg/kg*14d	100%
Li et al.	2019	50mg/kg+8mg/kg*14d	100%
Tang et al.	2021	50mg/kg+8mg/kg*14d	100%
Feng et al.	2020	83.52mg/kg	63%

Sex Hormone Levels

14 studies all reported the concentration of FSH(n=444) and E_2(n=444) of the rat serum 4 weeks after the first intervention. Six of the 14 studies reported the concentration of AMH(n=224) of the rat serum 4 weeks after the first intervention. Only two studies presented LH level which cannot be compared in groups. We applied the random-effects model to evaluate the differences in FSH, E_2 and AMH between the treatment group and control group.

1. FSH

The CTX-induced group showed a clear increase in FSH compared to the normal saline group (SMD 4.86,95% CI4.32 to 5.30, p=0.000; I2=96.9%, p=0.000) (Fig. 4A). Furthermore, subgroup analysis based on CTX doses was completed. Moreover, network meta-analysis could allow provide rank possibility and SUCRA probability calculations. A network plot describing the corresponding comparisons within the network is presented in Fig. 5a. The details of rank possibility are showed in Fig. 6a. SURCA plot are showed in Fig. 7a. The rank plot indicated that the 200mg/kg +8mg/kg *14d group had the highest possibility of being the best dose (Rank 1 = 41.6), followed by the 50mg/kg +8mg/kg*14d group (Rank 2 = 42.2), 100mg/kg group (Rank 3 = 35.0), 83.52mg/kg group (Rank 4 = 20.8) and normal saline group(Rank5 = 51.6). The treatments were ranked as follow according to the SUCRA (Fig. 7a): 50mg/kg+8mg/kg*14d (SUCRA=78.6)>200mg/kg+8mg/kg*14d(SUCRA=76.5) > 100mg/kg (SUCRA=42.4) > 83.52mg/kg (SUCRA=38.4) > normal saline (SUCRA=14.0).

2. E_2

The CTX-induced group cleared a significant decrease in E_2 compared to the normal saline group (SMD -4.28,95% CI-4.77 to-3.79, p=0.000; I^2=96.9%, p=0.000) (Fig. 4B). Subgroup analysis based on CTX doses was also carried out. The specific networks were presented in Fig. 5b. The specific details of rank possibility are showed in Fig. 6b. SURCA plot are showed in Fig. 7b. The rank plot showed that the 100mg/kg group had the highest possibility of being the best treatment(Rank 1 = 43.6), followed by the 200mg/kg +8mg/kg*14d group (Rank 2 = 33.4), 50mg/kg +8mg/kg*14d group (Rank 3 = 46.2), 83.52mg/kg group

(Rank 4 = 27.0) and normal saline group(Rank5 = 66.5). The treatments were ranked as follow according to the SUCRA (Fig. 7b): 100mg/kg (SUCRA=77.9) >200mg/kg+8mg/kg*14d (SUCRA=75.5) > 50mg/kg+8mg/kg*14d (SUCRA=51.4) > 83.52mg/kg (SUCRA=36.6) > normal saline (SUCRA=8.6).

3. AMH

AMH is obviously decreased in treatment group comparing to the normal saline group (SMD -2.23,95% CI-2.63 to -1.83, p=0.000; I^2=96.0%, p=0.000) (Fig. 4C).6 studies can be divided into 3 subgroups by CTX doses, which were 50mg/kg+8mg/kg*14d group ,83.52mg/kg group and 200mg/kg+8g/kg*14d group. Subgroup analysis was also completed. The specific networks are illustrated in Fig. 5c. The rank possibility is presented in Fig. 6c. The rank possibility showed that the 200mg/kg +8mg/kg*14d group may have been the best in stability (Rank 1 = 75.5), followed by the 50mg/kg +8mg/kg*14d group (Rank 2 = 31.9), 83.52mg/kg group (Rank 3 = 16.0) and normal saline group (Rank 4 = 40.4). The SUCRA is presented in Fig. 7c and the treatments are ranked as follow: 200mg/kg+8mg/kg*14d (SUCRA=89.1) > 50mg/kg+8mg/kg*14d (SUCRA=39.0) > 83.52mg/kg (SUCRA=39.7) > normal saline (SUCRA=32. 1).

Discussion

Cyclophosphamide, as a wide use of chemical drug for the treatment of cancers and auto-immune diseases, is continually to be regarded as one of the most gonadotoxic agents[23].Studies proved that CTX can increased granulosa cell apoptosis and follicular atresia on growing follicles in human ovarian tissue in vitro[24].The possible molecular pathways of CTX-induced ovarian damage were demonstrated in recent studies(1)DNA damage: CTX treatment can lead to an upregulation in granulosa cell apoptosis with the induction of DNA breaks[25]; [26]. 2) Inflammation response: The levels of pro-inflammatory cytokines IL-6 ,IL-8 and TNF-α were increased in CTX-induced mice serum[27]. (3) MTOR/PTEN/Akt pathway: The induction of Akt, mTOR and FOXO3a was found in granulosa cells with 24h CTX exposure[28]. The schematic model of the oocyte apoptotic pathway induced by CTX are showed in Fig. 8.CTX was widely used to simulate POI which induced by the ovarian-toxicity of chemotherapy.

This systematic and network meta-analysis compared different characteristics of rat POI model induced by different doses of CTX on the morphological, hormonal, and clinical manifestations. To the best of our knowledge, this network meta-analysis is the first to evaluate the effect of CTX-induced rat POI model. Network meta-analyses could compare the effects of interventions more directly and be able to provide the rank possibilities and SUCRA probabilities of compared interventions to improve the accuracy of the assessment[29].

14 studies were involved in this meta-analysis and be grouped into (1) 50mg/kg+8mg/kg*14d(8 studies);(2) 200mg/kg+8mg/kg*14d (3 studies);(3) 83.52mg/kg (1 study);(4) 100mg/kg(2 studies). While our static analysis still showed a high heterogeneity after performing subgroup analysis. Possible explanations are as follows: Firstly, none of the study described whether rats were housed randomly and only one study mentioned the outcome assessor was blinded which may contribute to heterogeneity and bias. Secondly, follicles can be counted differently since researchers may have diverse principles of identification. Thirdly, each study used different kits or methods for hormone measurement.

The changes of follicle imply the variation of ovary because the ratio of primary follicle to primordia follicle reflects the process of follicle transformation. The apoptosis of granulosa cells is the main mechanism of diminished ovarian reserve and transformation of the atretic follicles. All of the studies which showed the specific number of primordial, primary, secondary, Antral and atretic follicles elucidated that CTX intervention can damage the follicles resulting in the decrease of primordial follicles and an increase in the number of atretic follicles. Since there were only 4 studies gave the exact number, we just extract the data without comparing in subgroup.

In female rats the correct time of estrus cycle is dependent on the normal development of the hypothalamic-pituitary-ovarian axis[30]. Abnormal rates of estrous cycle reflect the failure of ovary function. 50mg/kg+8mg/kg*14d group showed 99.07% rate of abnormal estrous cycle which were obviously more than the rate of 83.52mg/kg group.

Hormone level is an important and sensitive indicator of ovary function. Clinical studies showed that patients in the POI group were found to have significantly higher FSH and LH serum concentrations and lower E_2 serum levels, when compared to healthy controls.[31] Since the early menopausal transition is associated with a sharp rise in serum FSH levels, even when serum estrogen levels remain within normal limits, the FSH serum level is the gold standard test in POI diagnosis[32]. Signs indicating a reduced follicle number may already be expressed by elevated early follicular FSH levels, so we choose FSH as an indicator of the efficacy of POI modeling[33]. And the Changes in the level of AMH as an indicator of the ovarian follicular reserve was also investigated since AMH can be a predictor for POI patients[34]. All of the studies included presented the concentration of FSH and E_2. We compared those quantitative values by forest plot, ranking possibilities and SUCRA possibilities. Results demonstrated that 200mg/kg +8mg/kg*14d group had the highest possibility of being the best dose to elevate FSH and reduce AMH. Although 100mg/kg group ranked first in the effectiveness of lowering E_2 level, 200mg/kg +8mg/kg*14d group ranked second with narrow margin. Therefore, the loading dose of 200mg/kg CTX with the maintenance dose of 8mg/kg CTX may have the highest possibility to induce rat POI model.

Since none of the study mentioned the mortality of rats and no incomplete outcome data was found. We detected the 200mg/kg as a safe dose for CTX-induced POI model.

In addition, 50mg/kg +8mg/kg*14d group ranked first in FSH SUCRA possibilities and ranked second in ranking possibilities of FSH and AMH. While 50mg/kg was the lowest dose of these subgroup, the number of its studies included was the most which indicated the availability and efficacy of the doze. In other words, the 50mg/kg concentration of CTX can successfully form POI rat model at the lowest cost.

This meta-analysis also encountered several limitations. Firstly, only 14 studies could be included and the sample size might not be large enough due to the strict inclusion criteria and not enough studies available. Secondly, the conversion of data and different methods or kits of each study might normally cause

bias. Thirdly, the quality assessment of these studies needs further development.

Conclusion

In conclusion, our results provide a comparison of animal models for premature ovarian insufficiency induced by different doses of cyclophosphamide. According to the network analysis, the loading dose of 200mg/kg CTX with the maintenance dose of 8mg/kg CTX for consecutive 14 days might be the best way to induce POI rat model. In addition, the loading dose of 50mg/kg CTX with the maintenance dose of 8mg/kg CTX can establish a relatively stable POI rat model at the lowest cost. These findings could provide researchers with appropriate CTX dosage of inducing POI animal models. Due to the unclear pathogenesis of POI, animal model of POI is a powerful tool to uncover this issue. We expect more comprehensive studies to verify our findings.

Declarations

Data availability

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

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Author contributions

Y.-W.Q. had the idea for the article and performed the literature search, data analysis. Y.-W.Q. and Y.-M.Z. conducted systematic literature selections and meta-analysis. Y.-W.Q. drafted the manuscript. B.L. critically revised the manuscript. All authors approved the final version of the manuscript.

Competing interests

All other authors declaim no conflicts of interest.

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Figures

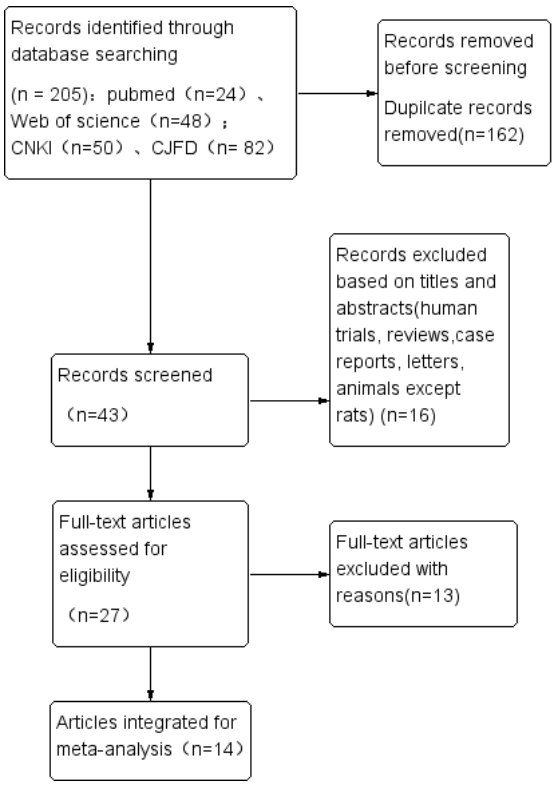

Figure 1

Flow diagram

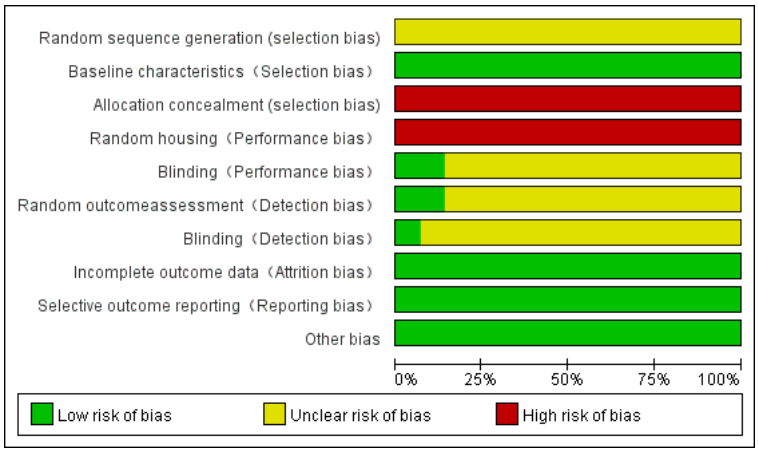

Figure 2

Risk of bias summary

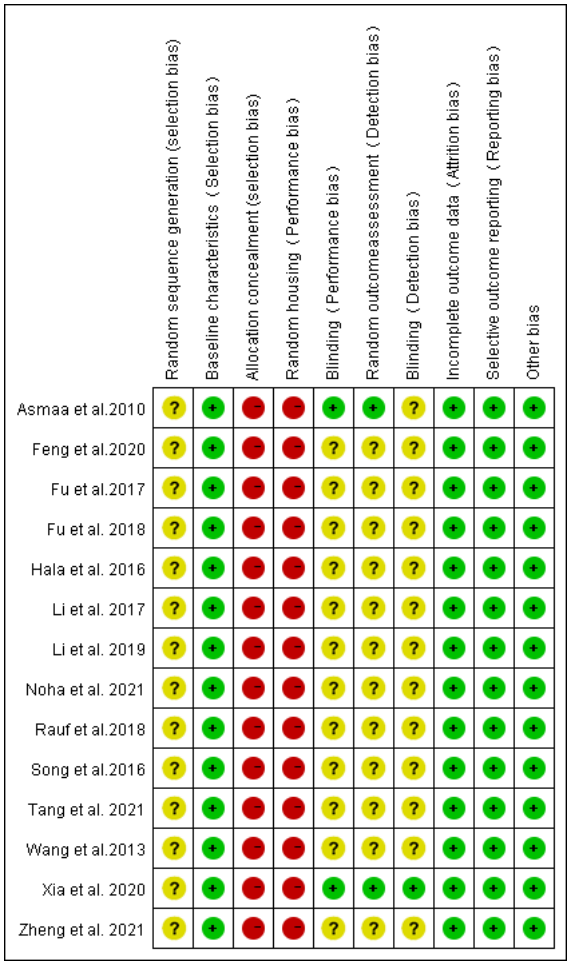

Figure 3

Risk of bias in individual study

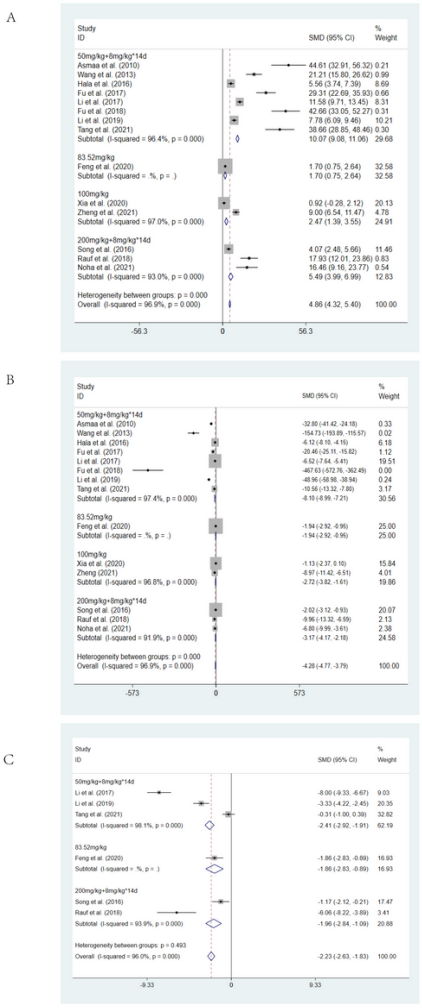

Figure 4

Forest plot of sex hormone concentration: (A) FSH; (B) E_2; (C) AMH

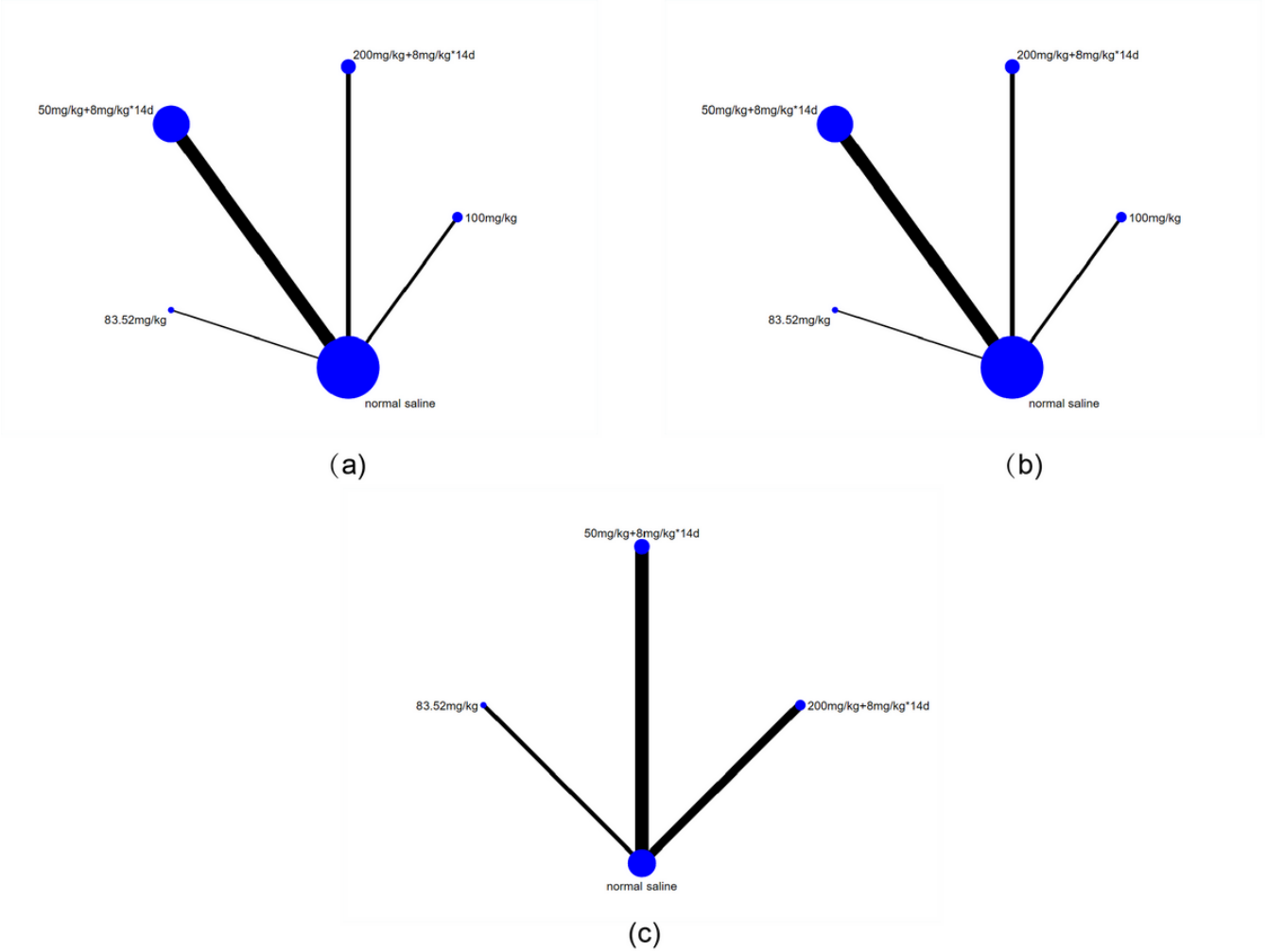

Figure 5

Network evidence plot hormone: (a) FSH; (b) E_2; (c) AMH

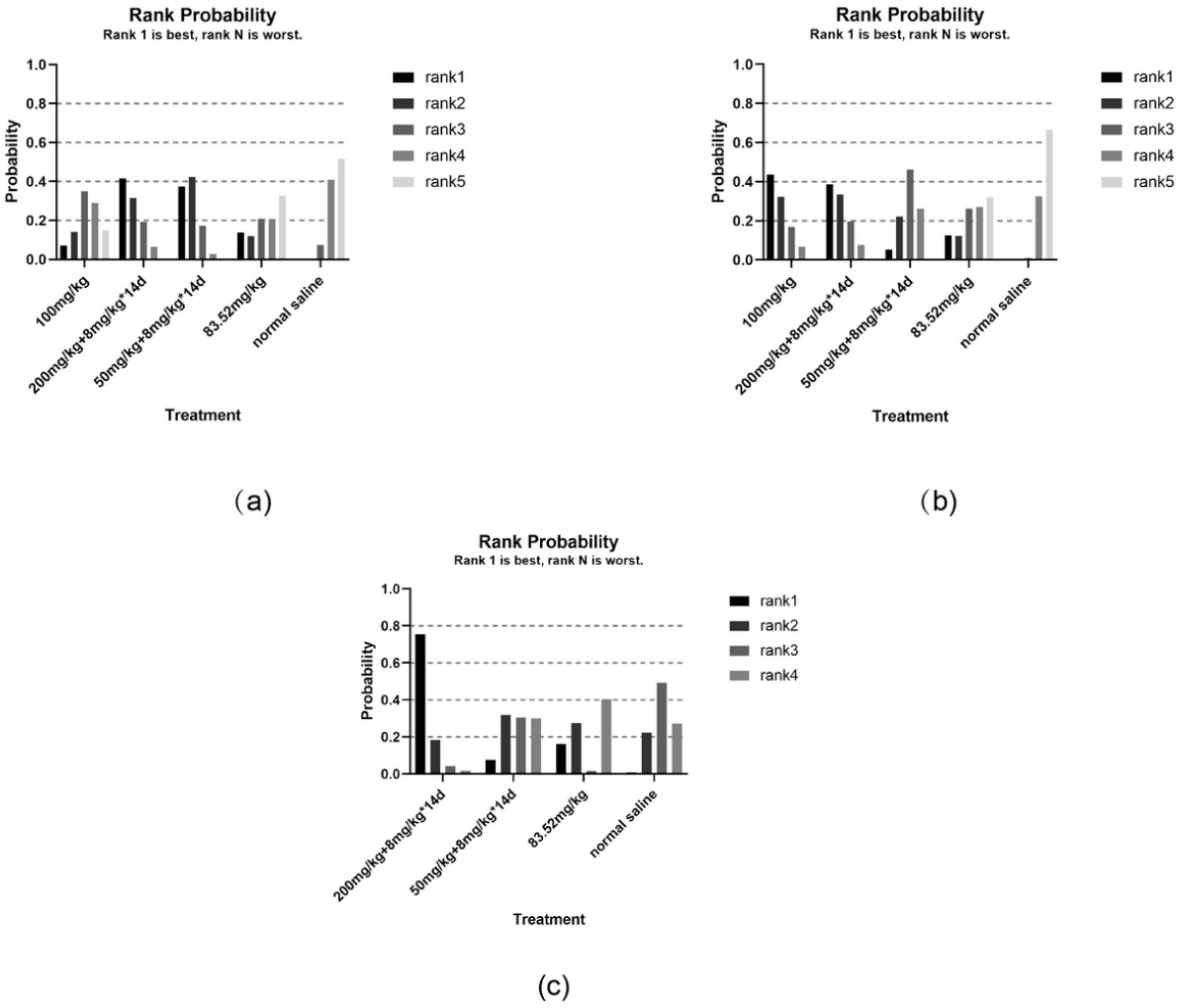

Figure 6

Rank probability plot: (a) FSH; (b) E_2; (c) AMH

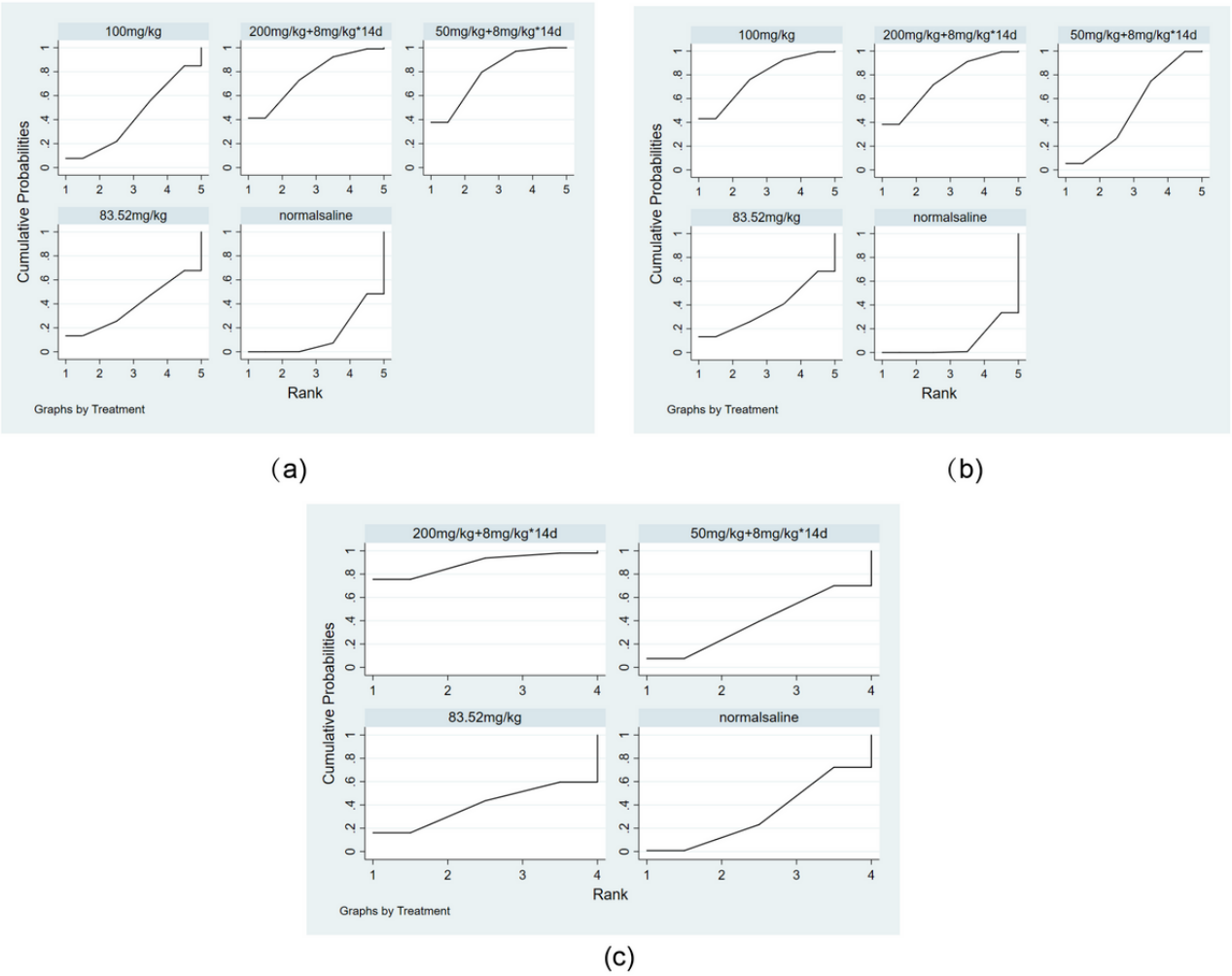

Figure 7

Surface under the cumulative ranking curve plot: (a) FSH; (b) E_2; (c) AMH

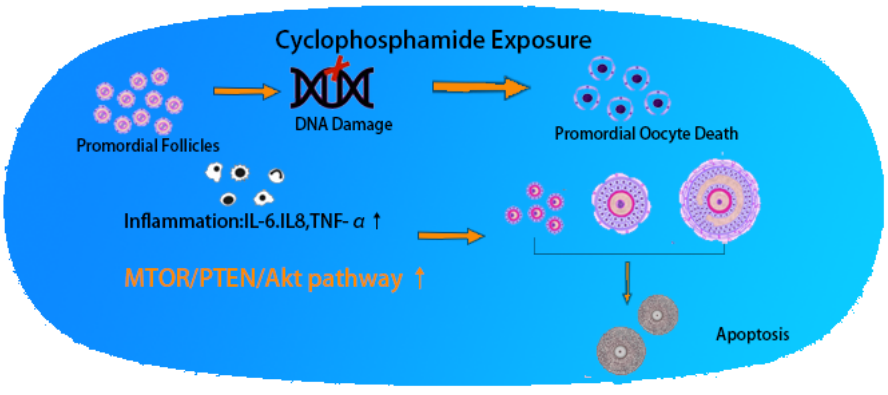

Figure 8

Schematic model of the oocyte apoptotic pathway induced by Cyclophosphamide exposure. Cyclophosphamide can damage the ovary by DNA breaks which lead to direct loss of primordial follicles. Inflammation and the upregulation of MTOR/PTEN/Akt pathway can cause growing follicles to undergo apoptosis.