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Live Birth Rate of Fresh Embryo Transfer with GnRH Agonist Long Protocol is Higher Than Frozen Embryo Transfer

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Abstract

Background: Along with progress in embryo cryopreservation, especially in vitrification has made freeze all strategy more acceptable. Some studies found comparable or higher live birth rate with frozen embryo transfer (FET) than with fresh embryo transfer（ET）in gonadotropin releasing hormone antagonist (GnRH-ant) protocol. But there were no reports about live birth rate differences between fresh ET and FET with gonadotropin releasing hormone agonist (GnRH-a) long protocol. The aim of this study is to analyze whether patients benefit from freeze all strategy in GnRH-a protocol from real-world data.

Methods: This is a retrospective cohort study, in which women undergoing fresh ET or FET with GnRH-a long protocol at Chongqing Reproductive and Genetics Institute from January 2016 to December 2018 were evaluated. The primary outcome was live birth rate. The secondary outcomes were implantation rate, clinical pregnancy rate, pregnancy loss and ectopic pregnancy rate.

Results: A total of 7,814 patients met inclusion criteria, implementing 5,216 fresh ET cycles and 2,598 FET cycles, respectively. The demographic characteristics of the patients were significantly different between two groups, except BMI. After controlling for a broad range of potential confounders (including age, infertility duration, BMI, AMH, no. of oocytes retrieved and no. of available embryos), multivariate logistic regression analysis demonstrated that there was no significant difference in terms of clinical pregnancy rate, ectopic pregnancy rate and pregnancy loss rate between two groups (all $P>0.05$). However, the implantation rate and live birth rate of fresh ET group were significantly higher than FET group ($P<0.001$ and $P=0.012$, respectively).

Conclusion: Compared to FET, fresh ET following GnRH-a long protocol could lead to higher implantation rate and live birth rate in infertile patients underwent *in vitro* fertilization (IVF). The freeze all strategy should be individualized and made with caution especially with GnRH-a long protocol.

Backgound

Gonadotropin-releasing hormone agonist (GnRH-a) has been used since the early 1980s and plays an important role in controlled ovarian stimulation (COS) (1). Currently, GnRH antagonist (GnRH-ant) protocol is widely used due to its shorter treatment time, fewer injections and lower ovarian hyperstimulation syndrome (OHSS) rate than GnRH-a protocol (2). However, the standard GnRH-a long protocol is still one of the key down regulation protocol in China, due to its steady and higher clinical pregnancy rate in fresh embryo transfer (ET) in vitro fertilization (IVF) patients (3). The selection of ovarian stimulation protocol is mainly determined by weighing the balance between chance of pregnancy and risk of OHSS (4).

The fresh ET is preferred in most IVF centers when patients have available embryos, lower OHSS risk, and no other negative factors. However, increasing evidence indicates that the supra physiologic condition caused by COS may influence the endometrial and uterine environments and lead to adverse outcomes of pregnancy (5). Therefore, implementation of vitrification and subsequent increase of success rates made

frozen embryo transfer (FET) become a very effective approach to avoid the above problem in assisted reproductive technology (ART) treatment (6). Since the development of vitrification technology, FET is suggested more efficacy and safety. It is not only used in hyper-responders to reduce OHSS rate or in pre-implantation genetic testing (PGT) patients, but also proved to improve the reproductive outcomes of IVF treatment (7).

Several studies suggested a significantly higher live birth rate and better perinatal outcomes in FET cycles compared with fresh ET cycles (8–10). A multicenter randomized controlled trial (RCT) study has reported that among infertile women with the polycystic ovary syndrome, FET was associated with a higher live birth rate after first transfer than was fresh ET (10). Some studies even support the hypothesis of so called freeze-all strategy in IVF, in which all embryos will be frozen and no fresh transfer conducted, to optimize success rates (11, 12). However, several other studies demonstrated no significant differences in obstetrical and neonatal complications and live birth rate between transfer of fresh or frozen embryos in women without polycystic ovary syndrome (13, 14). Specially, a new systematic review and meta-analysis indicated that there were no differences in LBR by the use of FET in preference to fresh ET in the overall (non-PGT) population undergoing IVF (15).

However, the COS protocol in all the studies mentioned above or in studies included in the meta-analysis was GnRH-ant protocol. There has been no report regarding the comparison of differences between fresh ET and FET with GnRH-a protocol. The aim of this study was to evaluate the pregnancy outcomes of cryopreservation of all embryos and subsequent FET compared with fresh transfer using GnRH-a long protocol.

Methods

Patients

This is a retrospective cohort study, in which the infertile women undergoing fresh ET or FET at Chongqing Reproductive and Genetics Institute from January 2016 to December 2018 were evaluated. All patients underwent first IVF cycle with GnRH-a long protocol, and then had first transfer with two D3 embryos in the fresh ET or in the subsequent first FET cycles. Only patients with moderate or severe OHSS risk (16) for freeze-all cycles were included in FET cycles. Exclusion criteria were (1) patients' age > 34 years old; (2) patients with the thickness of endometrium on the day of embryo transplantation < 0.7 cm; (3) patients with available embryos < 2; (4) patients with blastocyst transfer or PGT cycles; and (5) chromosome abnormality and uterine malformation. All procedures of this study were approved by the Institutional Review Board of Chongqing Health Center for Women and Children. The requirement for patient informed consent was waived by the Institutional Review Board because of the retrospective cohort study involved existing data and records at the time of investigation, and did not retain personal identifiers in the collected information. The study patients were divided into two groups according to fresh ET cycles or FET cycles.

Gnrh-a Long Protocol

From mid-luteal phase in previous menses, GnRH-a (Triptorelin 0.1 mg/d or 0.05 mg/d, Decapeptyl Ferring, Germany) was used for pituitary down regulation. After 14–18 days of GnRH-a administration, if the level of estrogen < 50 pg/ml, luteinizing hormone < 5 mIU/ml and P < 1 ng/ml, 75–300 IU recombinant follicle stimulating hormone (rFSH) was administrated per day, depending on the patients' age, anti-müllerian hormone (AMH) level and antral follicle counts (AFC). The dose of GnRH-a was remained 0.05 mg/d, or decreased to 0.05 mg/d from 0.1 mg/d on the Gn initiative day and continued until ovulation induction. When leading follicles reached 18 mm in diameter, an injection of 250 μg of recombinant human chorionic gonadotropin (rhCG) (Merck Serono, Italy) was given, and oocyte retrieval was performed 36 h later.

Luteal-phase support was started on the day of oocyte retrieval and performed by combination of vaginal progesterone (Utrogestan 200 mg every 8 hours, Besins Healthcare, Spain or Crinone 90 mg/d, Merck Serono, UK) and oral progesterone (Duphaston 10 mg twice a day, Abbott Biologicals, Netherlands). In the fresh ET group, on day 3 of the embryo culture, two good or excellent quality embryos were transferred. In the FET group, embryos in case of a freeze-all policy were vitrified on day 2 or day 3 by vitrification system. For FET transfers artificial cycle with or without GnRH down-regulation or natural cycle were used for endometrium preparation. Luteal-phase support was started three days before FET and performed by combination of vaginal and oral progesterone. If pregnancy achieved, luteal phase support continued until 12 weeks of gestation in both groups.

Outcomes And Definitions

The primary outcome was live birth rate. The secondary outcomes were implantation rate, clinical pregnancy rate, pregnancy loss and ectopic pregnancy rate. Live birth was defined as delivery of any neonate after 28 weeks of gestation (17). Implantation was defined as the detection of an intrauterine gestational sac using ultrasonography. Clinical pregnancy was defined as a viable pregnancy with a fetal heart activity under ultrasonography. Ectopic pregnancy was defined as the detection of a gestational sac outside the uterus. Pregnancy loss was defined as the spontaneous loss of the embryo or fetus before 28 weeks of gestation (17).

Statistical analysis

Data were carried out using the Statistical Package for the Social Science software (version 20, SPSS Inc, Chicago. IL, USA). Analysis was done by two-sample t test for continuous variables. Categorical variables were analyzed by Chi-square test and Fisher's exact test. Multivariate logistic regression analysis was performed to assess the effect of FET on LBR. P < 0.05 was considered statistically significant.

Results

During the recruitment period, 15,772 women were assessed for eligibility. Finally, a total of 7,814 patients underwent first IVF cycle met study inclusion criteria, including 5,216 fresh ET and 2,598 FET cycles (Fig. 1). The demographic characteristics were presented in Table 1. The age of patients was significantly older in fresh ET group than FET group (29.55 ± 2.86 vs. 28.96 ± 2.99 years, P < 0.001). The infertility duration of fresh ET group was significantly longer than FET group (4.64 ± 3.07 vs. 4.43 ± 2.88 years, P = 0.004). There were significantly less previously nulliparous women in the fresh ET group (48.4% vs. 51.0%, P = 0.044). The basal FSH level of fresh ET group was significantly higher than that of FET group (5.64 ± 0.03 versus 5.04 ± 0.03, P < 0.001), while AMH level was significantly lower in fresh ET group (3.26 ± 2.54 vs. 5.58 ± 3.64, P < 0.001). There was no significant difference with regard to BMI between fresh ET and FET groups. Primary causes of infertility were significantly different between two groups, which was mainly due to ratio of ovulatory obstacle (6.5% vs. 13.4%, P < 0.001).

Table 1
Baseline characteristics in fresh ET and FET groups.

Characteristics	Fresh ET (n = 5216)	FET (n = 2598)	P
Age at egg retrieval (year)	29.55 ± 2.86	28.96 ± 2.99	< 0.001
BMI (kg/m^2)	21.80 ± 2.74	21.74 ± 2.91	0.322
Infertility duration (year)	4.64 ± 3.07	4.43 ± 2.88	0.004
AMH (ng/ml)	3.26 ± 2.54	5.58 ± 3.64	< 0.001
Basal FSH (mIU/ml)	5.64 ± 0.03	5.04 ± 0.03	< 0.001
Primary infertility, n (%)	2522 (48.4)	1326 (51.0)	0.044
Primary cause of infertility			< 0.001
Pelvic and tubal factor, n (%)	4236 (70.2)	2040 (61.5)	
Male, n (%)	645 (12.4)	401 (15.4)	
Unexplained, n (%)	234 (4.5)	125 (4.8)	
Ovulatory obstacle, n (%)	37 (6.5)	14 (13.4)	
Endometriosis, n (%)	60 (6.4)	18 (4.8)	

Note: Presented as n (%) for categoric variables and mean SD for continuous variables. BMI body mass index; AMH, Anti-Mullerian Hormone; FSH, Follicle-Stimulating Hormone; FET frozen embryo transfer; fresh ET, fresh embryo transfer.

There was no significant difference between the fresh ET and FET groups with regard to the duration of ovarian stimulation. The total gonadotropin dose in fresh ET group was significantly higher than FET group (2274.50 ± 740.08 IU vs. 1938.81 ± 652.88 IU, P < 0.001). Estradiol level on hCG trigger day in fresh ET group was significantly lower than the FET group (3235.65 ± 1196.11 vs. 4480.45 ± 789.60 pg/ml, P < 0.001). Number of oocytes retrieved and available embryos in fresh ET group was significantly less than

the FET group (10.49 ± 3.77 vs. 17.97 ± 5.47 and 4.00 ± 2.03 vs. 5.44 ± 2.69, P < 0.001). With regard to the insemination method, there was significant difference between the two groups by using IVF and ICSI (P < 0.001). (Table 2)

Table 2
Treatment characteristics in fresh ET and FET groups

Parameters	Fresh ET (n = 5216)	FET (n = 2598)	P
Ovarian stimulation (days)	10.90 ± 1.30	10.85 ± 1.28	0.109
Gonadotropin dose (IU)	2274.50 ± 740.08	1938.81 ± 652.88	< 0.001
Estradiol level on hCG trigger day (pg/ml)	3235.65 ± 1196.11	4480.45 ± 789.60	< 0.001
No. of oocytes retrieved	10.49 ± 3.77	17.97 ± 5.47	< 0.001
Insemination method			< 0.001
IVF, n (%)	4376 (83.9)	840 (16.1)	
ICSI, n (%)	2095 (80.6)	503 (19.4)	
No. of available embryos	4.00 ± 2.03	5.44 ± 2.69	< 0.001
Note: Presented as mean SD for continuous variables. ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilization; FET frozen embryo transfer; fresh ET, fresh embryo transfer.			

The clinical outcomes in fresh ET and FET groups were shown in Table 3. After adjusting for potential confounders (including age, infertility duration, BMI, AMH, No. of oocytes retrieved and no. of available embryos), multivariate logistic regression analysis demonstrated that there was no significant difference in terms of clinical pregnancy rate (65.8% vs. 66.9%, P = 0.683), ectopic pregnancy rate (2.9% vs. 4.2%, P = 0.297) and pregnancy loss rate (9.2% vs. 12.4%, P = 0.072) between fresh ET group and FET groups. However, the implantation rate and live birth rate of fresh ET group were significantly higher than FET group (48.6% vs. 47.0%, P < 0.001 and 57.1% vs. 54.8%, P = 0.012, respectively).

Table 3
Clinical outcomes in fresh ET and FET groups after matching.

Outcomes	Fresh ET (n = 5216)	FET (n = 2598)	95% CI	P
Live birth, n (%)	2978 (57.1%)	1425 (54.8%)	1.037–1.332	0.012
Implantation, n (%)	5066 (48.6%)	2433 (47.0%)	1.103–1.315	< 0.001
Clinical Pregnancy, n (%)	3431 (65.8%)	1737 (66.9%)	0.918–1.139	0.683
Ectopic pregnancy, n (%)	153 (2.9%)	109 (4.2%)	0.601–1.168	0.297
Pregnancy loss, n (%)	316 (9.2%)	215 (12.4%)	0.997–1.066	0.072

Note: Presented as n (%) for categoric variables. P value was adjusted for age, infertility duration, BMI, AMH, No. of oocytes retrieved and No. of available embryos. BMI body mass index; AMH, Anti-Mullerian Hormone.

Discussion

The progress of embryo cryopreservation, particularly in vitrification, has made freeze-all strategy more acceptable. However, the freeze all strategy is still controversial due to unclear advantages or disadvantages. In this study, we analyzed whether patients benefited from freeze all strategy in comparison with fresh ET cycles in GnRH-a long protocol. The reason we chosen the first ET cycle was that the first ET usually selected the best-quality embryo, whereas the embryo quality of the second FET cycle may differ from the fresh ET. Simultaneously, patients whose age > 34 years, endometrium thickness < 0.7 cm, blastocyst transfer and PGT cycles were excluded, due to these confounding factors were extremely unbalanced between the two groups. The characteristics of the patients including age, infertility duration, AMH level, basal FSH level, primary infertility and primary cause of infertility were still significantly different between two groups (Table 1). Especially, the younger age and higher AMH level in FET group, which might indicate that the function of ovarian reserve of the FET group was better than the fresh ET group. As expected, the outcomes of COS of the FET group were better, including less gonadotropin dose, more oocytes retrieved and more available embryos (Table 2).

It is still controversial about the efficacy of GnRH-a and GnRH-ant protocols with fresh ET. Several studies suggested that the pregnancy rate, ongoing pregnancy rate and live birth rate of fresh transfer cycles were lower in the GnRH-ant protocol than in the GnRH-a protocol (3, 18), whereas other studies showed no significant difference (2, 19). Furthermore, implantation is one of the most important steps to achieve live birth, therefore it is considered as an important indicator to the efficacy of the treatment. Implantation relies on embryo quality and endometrial receptivity (20). Hershko *et al* have conducted a randomized trial showed there was no difference in embryo quality between GnRH-a and GnRH-ant group (21). Hernandez *et al* have reported GnRH-ant may disrupt an auto/paracrine loop, that is essential for the mitotic programme of the endometrial epithelial cells, leading to decrease of pregnancy rates and an increase of abortion rates (22). Rackow *et al* found HOXA10 (an essential regulator of endometrial receptivity) expression was significantly decreased in endometrial stromal cells in GnRH-ant-treated

cycles compared with GnRH-a-treated cycles or natural cycle (23). Ruan *et al* found GnRH agonist, may partially restore the endometrial physiological secretion and improve uterine receptivity in mice (24). A comparative proteomic analysis demonstrated endometrial receptivity was more strongly impaired by GnRH-ant than GnRH-a treatments (25). The results of the above studies (22–25) indicated that the endometrial receptivity of GnRH-a protocol might be better than GnRH-ant protocol in fresh ET cycles. As we know, FET has become increasingly common in many countries (8). It has been hypothesized that FET may provide a more physiologic uterine environment for embryo implantation than fresh ET (26). Furthermore, the elective freezing embryos also can reduce the risk of OHSS, which is an iatrogenic, serious, and potentially life-threatening complication in COS treatment (27, 28). However, it should be noted to take care the damage of embryo by freezing and thawing, which was associated with ice crystal formation, increased of salt concentrations and cryoprotectant agents toxicity caused by cryopreservation (29–31). Tachataki *et al* demonstrated that cryopreservation affected the normal pattern of gene expression during human pre-implantation development (32). Therefore, when the uterine environment benefits from FET are less than the damages of freezing and thawing to embryos, the live birth rate will be finally lower for the FET compared to fresh ET. Moreover, freeze all strategy and additional frozen cycles will increase time to live birth and treatment costs for infertile patients (33).

There are controversial opinions regarding the ectopic pregnancy rate and pregnancy loss rate between fresh ET and FET cycles in different studies. Most researchers thought the supra physiologic hormonal levels could confer a higher ectopic pregnancy rate with fresh ET cycles (34, 35). However, Xiao *et al* found no significant difference in ectopic pregnancy rate between fresh ET and FET (36). Chen *et al* found there was higher pregnancy loss rate in the fresh ET group compared to FET group in ovulatory women (13). However, Heather *et al* found a higher first trimester pregnancy loss risk after FET compared with fresh ET among women younger than 38 years old (37). In this cohort study, the ectopic pregnancy rate and pregnancy loss rate were higher in the FET group, but after adjusting for potential confounders, multivariate logistic regression analysis showed no significant difference between two groups, while that implantation rate and live birth rate in the fresh ET group was significantly higher than FET group although the younger age and higher AMH level in FET group.

This study has its inherent limitation as a retrospective analysis. The characteristics of patients were unbalanced. Although we adjusted the confounders and used multivariate logistic regression analysis, some potential confounders still might be ignored. In conclusion, compared to FET, fresh ET following GnRH-a long protocol tended to increase live birth rate in patients undergoing their first ART cycle. This study suggest that freeze all strategies should be individualized and made with caution especially for GnRH-a long protocol in clinical practice. A well-designed, multicenter, prospective RCT is still required to further support these results.

Conclusion

Compared to FET, fresh ET following GnRH-a long protocol could lead to higher implantation rate and live birth rate in infertile patients underwent IVFF. The freeze all strategy should be individualized and made

with caution especially with GnRH-a long protocol.

Abbreviations

FET
Frozen Embryo Transfer
ET
Embryo Transfer
GnRH-ant
Gonadotropin Releasing Hormone antagonist
GnRH-a
Gonadotropin Releasing Hormone agonist
IVF
In Vitro Fertilization
ICSI
Intracytoplasmic Sperm Injection
BMI
Body Mass Index
AMH
Anti-Müllerian Hormone
COS
Controlled Ovarian Stimulation
OHSS
Ovarian Hyperstimulation Syndrome
ART
Assisted Reproductive Technology
PGT
Pre-implantation Genetic Testing
RCT
Randomized Controlled Trial
FSH
Follicle Stimulating Hormone
AFC
Antral Follicle Counts
rhCG
recombinant human Chorionic Gonadotropin

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee Review Board of Chongqing Health Center for Women and Children (No. 2019-1208) for retrospective analysis and clinical data reporting.

Consent for publication

Not applicable.

Availability of data and material

The datasets generated for this study are available on request to the corresponding author.

Competing interests

All the authors declare that we have no competing interests.

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Author contributions

The present work was designed by HY. Data extraction and analysis were performed by XD and JY. LL, NY and LL participated in the data collection. GH and HY participated in revisions to the article. All authors have read and approved the final manuscript.

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Figures

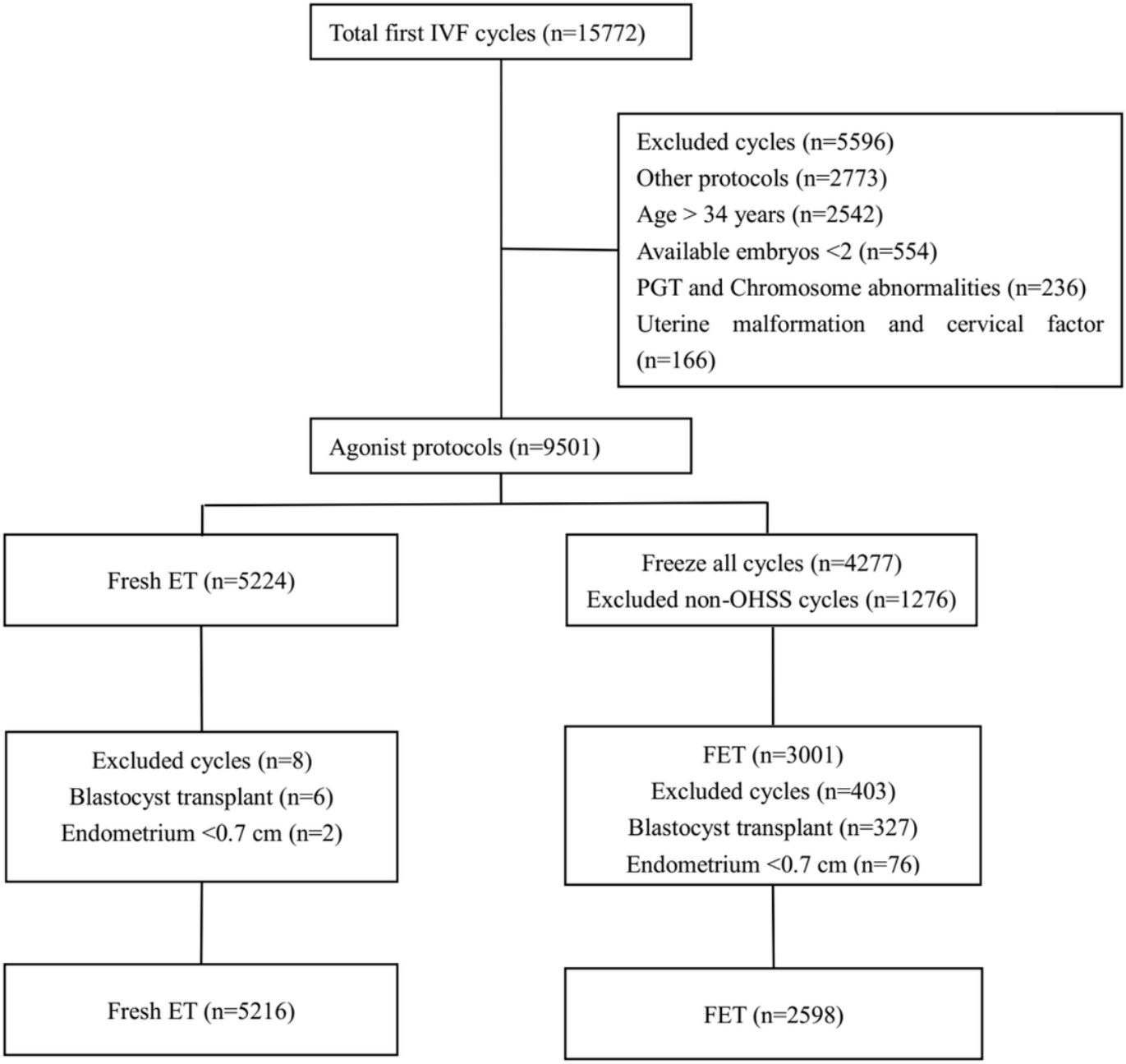

Figure 1

Flow chart of patient's allocation.