Original Investigations

Early-cleavage versus blastocyst stage embryo transfer: a prospective comparative study

İnal and İnal Early-cleavage versus blastocyst transfer

Hasan Ali İnal, Zeynep Öztürk İnal

Departmant of Reproductive Endocrinology, Konya Training and Research Hospital, Konya, Turkey

Address for Correspondence: Hasan Ali İnal
Phone: +90 332 323 67 09 e-mail: dr.hasanaliinal@yahoo.com **ORCID:** orcid.org/0000-0002-8361-7908

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Abstract

Objective: To evaluate whether or not embryo transfer day has an effect on the rates of clinical pregnancy (CPR) and live birth (LBR) in in vitro fertilization-intracytoplasmic sperm injection (IVF-ICSI) treatment.

Material and Methods: A total of 757 patients who had undergone IVF-ICSI treatment between 2012 and 2017 were included in this study. The participants were stratified according to embryo transfer day as Group 1 (day 2 transfer; n= 43), Group 2 (day 3 transfer; n=633), and Group 3 (day 5 (blastocyst) transfer; n=81). Basal parameters and IVF-ICSI outcomes were compared between the groups.

Results: Groups 1 and 2 patients were older, had a higher BMI, worse responder rate, lower antral follicle count, lower peak E_2 levels, and less endometrial thickness, and required an increased total gonadotropin dose than the other transfer day groups. In addition, the number of oocytes and MII oocytes, fertilization rate, and 2 PN were statistically different between the groups and the CPR (19.5% vs 36.9% vs 39.0%, respectively) and LBR (14.6% vs 30.4% vs 35.1%, respectively) were lower in group 1 than groups 2 and 3 ($p<0.05$). The grade I embryos were significantly higher in groups 1 and 2 with clinical pregnancy positive (OR=4.444; 95% CI 0.876-22.536; p=0.001 and OR=1.756; 95% CI 1.234-2.500; $p<0.001$) and live birth (OR=5.021; 95% CI 0.787-31.768; p=0.001 and OR=1.676; 95% CI 1.154-2.433; p=0.007).

Conclusions: The data suggest that an earlier embryo transfer day has a negative effect on the clinical pregnancy rate. Older primary infertile women should not postpone their desire to have a baby because they are poor responders, and it should be explained that the chances of successful treatment are lower.

Keywords: Assisted reproductive techniques, embryo transfer day, ovulation induction, clinical pregnancy rate

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Introduction

The last stage of in vitro fertilization-intracytoplasmic (IVF-ICSI) treatment is the transfer of embryos which have been formed through the fertilization of oocytes with sperm following controlled ovarian stimulation into the endometrium (1,2). Embryo transfer (ET) is an important steps in assisted reproductive technology, and although day 2 or day 3 transfer of early-cleavage embryos is widely clinically preferred, blastocyst ET is gaining attention because it provides better synchronization between embryo and endometrium as well as high-quality embryo presentation (3,4).

Early-cleavage ET of four- or eight-cell embryos on day 2 or 3, respectively, may be advantageous to embryonic survival in terms of requiring less in vitro time (5,6). There are two key reasons for the widespread adoption of this form of ET: First, the development of the embryos is slower, and second, embryos placed in the endometrium at this stage are more likely to survive (2). However, as a result of accelerating advances in blastocyst culture over recent decades, ET has shifted from the early-cleavage period to this later stage (7). A number of existing studies report that the synchronization between embryo and endometrium in the blastocyst stage increases implantation success and, consequently, the rates of clinical pregnancy (CPR) and live birth (LBR) (8,9). There are also studies that report no appreciable difference (2,10). The aim of this study, therefore, was to interpret whether or not embryo transfer day has an effect on CPR and LBR in IVF-ICSI treatment.

Materials and Method

Study participants and data collection

This prospective study was carried out at Necmettin Erbakan University Faculty of Medicine, IVF Unit. Outcomes of 757 fresh ICSI cycles were reviewed between January 2012 and December 2017. Inclusion criteria were participants aged 20–44 years, body mass index (BMI) between 18 and 35 kg/m^2, regular menstrual cycles, no uterine abnormalities in the ultrasound, and normal baseline hormonal levels. Participants were excluded from the study if they were $\geq$45 years, BMI $\geq$35 kg/m^2, any significant illness or metabolic disorders. The ethical board approval was given from the institutional review board (2012/57). Written and oral informed aggrement was given from the participants.

Data were obtained for age, BMI (kg/m^2), smoking status, infertility period, cause of infertility, the baseline at day 3 for follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estradiol (E_2) levels, thyroid-stimulating hormone (TSH), prolactin, antral follicle count, stimulation parameters, IVF-ICSI outcomes, and CPR.

Ovarian stimulation and oocyte retrieval

Controlled ovulation stimulation was negotiated using the agonist (long luteal, gonadotropin-releasing hormone agonist (GnRHa) or microdose flare-up protocol) or the flexible gonadotropin-releasing hormone antagonist (GnRHant) protocol.

The GnRHa protocol: First, pituitary down-regulation was performed with a GnRH agonist. Then, the ovaries were stimulated by exogenous gonadotropins. The GnRH agonist leuprolide acetate (Lucrin; Abbott Cedex, Istanbul, Turkey) was administered subcutaneously daily from day 21 of the preceding luteal phase (0.5 mg/day, sc) until menstruation, and then the dose was decreased to 0.25 mg/day until ovulation was triggered. Recombinant FSH (Puregon; Organon, Oss, the Netherlands, or Gonal F; Serono, Istanbul, Turkey) was used for stimulation. The initial gonadotropin dose used was individualized according to the patient's age, baseline serum FSH concentration on day 3, body mass index, and previous response to ovarian stimulation. The starting regimen was fixed for the first 3 days (100–225 IU recombinant FSH/day). Thereafter, the dose of gonadotropin was adjusted according to the individual ovarian responses, which were monitored by measuring serum estradiol levels and transvaginal ultrasonography (LOGIC 200 PRO, GENERAL ELECTRIC, Seoul, South

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Korea). Ovulation was triggered by the administration of 250 IU recombinant human chorionic gonadotropin (HCG) (Ovitrelle, Serono, Istanbul, Turkey) when at least two follicles reached 18 mm in diameter. Oocytes were retrieved 36 h after the HCG injection, and ICSI was performed for all IVF-ET patients.

Microdose flare-up protocol: Recombinant FSH (Puregon; Organon, Oss, the Netherlands, or Gonal F; Serono, Istanbul, Turkey) and the GnRH agonist leuprolide acetate daily together (Lucrin; Abbott Cedex, Istanbul, Turkey) were administered subcutaneously (0.5 mg/day, subcutaneously for 5 days) on day 3 of a withdrawal bleed following at least 3 weeks of oral contraceptive use. The initial gonadotropin dose used was individualized according to the patient's age, baseline serum FSH concentration on day 3, body mass index, and previous response to ovarian stimulation. The starting regimen was fixed for the first 3 days (100–225 IU recombinant FSH/day). Thereafter, the dose of gonadotropin was adjusted according to the individual ovarian responses, which were monitored by measuring serum estradiol levels and transvaginal ultrasonography (LOGIC 200 PRO, GENERAL ELECTRIC, Seoul, South Korea). Ovulation was triggered by the administration of 250 IU recombinant human chorionic gonadotropin (HCG) (Ovitrelle, Serono, Istanbul, Turkey) when at least two follicles reached 18 mm in diameter. Oocytes were retrieved 36 h after the HCG injection, and ICSI was performed for all IVF-ET patients.

The GnRHant protocol: The pituitary down-regulation was achieved and maintained using the flexible GnRHant protocol. Recombinant human FSH (r-FSH; Gonal-F, Merck-Serono, or Puregon, MSD) or human menopausal gonadotropin (hMG; Menogon or Menopur; Ferring) was used for COH. The initial gonadotropin dose used for ovarian stimulation was individualized according to the patient's age, baseline serum FSH concentrations on day 3, BMI, and previous response to ovarian stimulation. The starting regimen was fixed for the first three days (150–225 IU rec FSH/day), and thereafter, the gonadotropin dose was adjusted according to the individual's ovarian response. Serial estrogen levels and two-dimensional follicle measurements by transvaginal ultrasonography (LOGIC 200 PRO, GENERAL ELECTRIC, Seoul, South Korea) were performed. A daily dose of 0.25mg of GnRHant (Cetrotide, Merck-Serono, or Orgalutran, MSD) was initiated when the leading follicle diameter was ≥13 mm or the serum E_2 level reached ≥ 300 pg/ml. When at least two dominant follicles reached dimensions of 18 mm or greater in diameter, hCG (250 µg, Ovitrell, Merck-Serono) was administered, and oocytes were retrieved 36 hours after the hCG injection. ICSI was then applied in accordance with our clinical procedures.

Embryo grading and ET procedure

Embryos were classified according to a simplified system based on Veeck's morphological criteria: Grade I embryos have equal-sized blastomeres and no cytoplasmic fragmentation, grade II embryos have blastomeres of equal size and minor cytoplasmic fragmentation covering ≤10% of the preembryo surface, grade III embryos have blastomeres of distinctly unequal size and variable fragmentation, grade IV embryos have blastomeres of equal or unequal size and moderate-to-significant cytoplasmic fragmentation covering >10% of the preembryo surface, and grade V embryos have few blastomeres of any size and severe fragmentation covering ≥50% of the preembryo surface. None of the embryos were classified as grade V in this study. Blastocyst quality was categorized as excellent (AA), good (AB, BA, BB), fair (BC, CB) or poor (CC), on the basis of trophectoderm and inner-cell-mass quality scores (11). The highest quality embryos were selected for embryo transfer on days 2, 3, and 5 after fertilization. The number of embryos transferred (two or fewer per patient) complied with national regulations in Turkey.

Two senior physicians performed the ETs accompained ultrasonographic appearance (Logiq 200 Pro, General Electric, Seoul, South Korea) using an embryo transfer catheter system. A

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sterile speculum was introduced to the vagina in the lithotomy position and the vagina and the cervix were cleared using sterile cotton swabs.
An embryologist loaded the embryos into a soft transfer catheter which was advanced to the ET physician who deposited the embryos approximately 10 mm from the uterine fundus under USG. The catheter was gently removed after 5 seconds. In cases of ET with external guidance, an initial catheter with inner sheath was inserted into the external cervical os, and then advanced through the cervical canal and internal os to 10 mm of the uterine fundus using USG. The internal sheath was withdrawn, and a second catheter loaded with embryos was introduced in its place and advanced to approximately 10 mm from the uterine fundus where the embryos were deposited. Difficult transfers required the use of a stylet in addition to this form of external guidance.
All catheters were immediately checked for retained embryos, blood, and the patient remained in the Trendelenburg position for about 10 minutes. Patients in whom tenaculum were excluded from the study. Luteal phase support was provided with progesterone in the form of Crinone 8% gel (Serono, Istanbul, Turkey) at a daily dose of 90 mg. Baseline parameters and IVF-ICSI outcomes were compared between the groups. The subjects were categorized according to embryo transfer day as Group 1 (day 2 transfer; n= 43), Group 2 (day 3 transfer; n=633), and Group 3 (day 5 (blastocyst) transfer; n=81). Basal parameters, clinical and laboratory IVF-ICSI outcomes, and pregnancy rates were compared between the groups.

Statistical analysis

The statistical analyses were performed using SPSS 15.0 for Windows (SPSS, Chicago, IL, USA). The Shapiro-Wilk test was used for examining the continuous variables with normal and non-normal distributions. The one-way analysis of variance (ANOVA) for normally distributed variables and Kruskal-Wallis test for not-normally distributed variables were used to compare groups. Categorical data were examined by Pearson's chi-square test, and Fisher's exact test was applied if the expected frequency was less than 5 in >20% of all cells. The continuous variables were presented as the mean±standard deviation (SD) and the categorical variables were demonstrated as the number of cases and percentages. The Bonferroni-adjustment was used to control the type I errors for all possible multiple comparisons. Logistic regression analyses were used to evaluate the factors thought to affect CPR and LBR. A $p<0.05$ value was established as statistical significant.

Results

A total of 51 patients were excluded from the study, specifically those with age $\geq$45 (n=19), BMI $\geq$35kg/m^2 (n=14), systemic disease (n=9), endocrine or metabolic disorders (n=6), and concomitant medication (n=3). The remaining 757 participants were classified into the three ET groups and their outcomes analyzed (Figure 1).
A comparison of the sociodemographic and stimulation characteristics of the participants is provided in Table 1. No differences were noticed between the groups in smoking status, infertility period, cause of infertility, baseline FSH, LH, E_2, TSH, prolactin levels, duration of stimulation, stimulation protocol, progesterone levels, and endometrial thickness on hCG administration ($p>0.05$). Groups 1 and 2 patients were older, had a higher BMI, worse responder rate, lower antral follicle count, lower peak E_2 levels, and less endometrial thickness, and required an increased total gonadotropin dose than the other transfer day groups.
The laboratory and reproductive outcomes of the participants are summarized in Table 2. While the embryo transfer technique was comparable between the groups ($p>0.05$), the numbers of oocytes retrieved, MII oocytes, 2 PN, fertilization rate, and the rate of grade I embryo per woman decreased in groups 1 and 2 ($p<0.05$). The CPR (19.5% vs 36.9% vs 39.0%, respectively) and LBR (14.6% vs 30.4% vs 35.1%, respectively) were lower in group 1 than the other groups ($p<0.05$).

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Logistic regression analysis of the factors thought to affect CPR and LBR are given in Table 3. The grade I embryos were significantly higher in groups 1 and 2 with clinical pregnancy positive (OR=4.444; 95% CI 0.876-22.536; p=0.001 and OR=1.756; 95% CI 1.234-2.500; p<0.001) and live birth (OR=5.021; 95% CI 0.787-31.768; p=0.001 and OR=1.676; 95% CI 1.154-2.433; p=0.007).

Discussion

We found that Groups 1 and 2 patients were older, had a higher BMI, worse responder rate, lower antral follicle count, lower peak E_2 levels, less endometrial thickness, and required an increased total gonadotropin dose than the other transfer day groups. In addition, the number of oocytes and MII oocytes, 2 PN, fertilization rate, and grade I embryos were statistically different between the groups and the CPR were lower in group 1 than groups 2 and 3.

Conventional early-cleavage ET on day 2 or 3 is thought to be the most suitable approach in terms of intrauterine microenvironment for the survival of embryos formed by the sperm injection of oocytes following controlled ovarian stimulation in IVF-ICSI treatment (12). With this form of ET, embryos will spend less time in vitro (2). Two reasons why early-cleavage stage ET is widely accepted in IVF-ICSI treatment are the low embryonic growth rate in the culture environment and their survival rate once placed in the uterus (13). In the selection of embryos to be transferred in the early-cleavage stage, the number of blastomeres, fragmentation rate, and morphological appearance are assessed, and genomic activation and gene transcription are limited according to the blastocyst. As such, it is possible to overlook chromosomal anomalies (14,15).

Rapid developments in blastocyst culture over the last two decades have prompted a shift to day 5 or day 6 ET in many clinics (2), although the debate about the perinatal outcomes of either approach continues. Whilst improved success has been reported in blastocyst over early-cleavage ET in the literature (8,9), a significant difference was not found in a number of other studies (2,10). Since cell compaction and genomic activation are beyond the control of maternal RNA by the 5th day, culture media are enriched by the addition of organic and inorganic material to ensure the longer survival of the embryos (16).

Blastocyst ET has two potential advantages over an early-cleavage approach in that this later stage physiologically overlaps better with the intrauterine microenvironment and it allows the more accurate selection of the embryos that are most likely to survive (17). In early-cleavage ET, the intrauterine microenvironment has been seen to stress the embryos and reduce implantation success (18). In addition, uterine contractility is lower in the blastocyst period, and so expulsion rates of the transferred embryos are reduced (19). Considering the possibility of embryonic arrest in the blastocyst stage, embryologists must conduct careful evaluation and the most suitable embryos should be left to day 5 or day 6 for ET. Otherwise, the IVF-ICSI cycle may be need to be canceled because of the likelihood of developmental cessation (20). One study randomized 243 IVF-ICSI cycles across day 2, day 3, and blastocyst ET, and while there was no difference between the groups in terms of CPR, the miscarriage rate was higher in the blastocyst transfer patients (4). Elsewhere, although transfers of blastocyst embryos have returned higher live birth rates as compared to early-cleavage ET, no significant difference was observed in terms of cumulative pregnancy rates (2). According to a meta-analysis of 13 randomized controlled studies, blastocyst ET partially increases CPR and live birth rate and causes no change in multiple pregnancy or miscarriage rates as compared to the early-cleavage approach (2). However, findings regarding cumulative pregnancy rates are insufficient, and more data is needed to clarify this issue.

In the present study, the mean age and BMI of the early-cleavage group were higher than the blastocyst ET patients. Ovarian reserve rates were lower, and so the number of oocytes retrieved, MII oocytes, 2 PN and grade I embryos, and the fertilization rate were all lower in this group. Early-cleavage ET therefore had to be applied in this group as the quality of

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embryos developed had parameters that would adversely affect the success of the IVF-ICSI treatment.

The strong point of the current study consist of its prospective arrangement, the adequate number of subjects in each group, and the prototypical sample from central Turkey. The results can be generalized to most of the country's population. However, the potential limitations of the study are that it was conducted in a tertiary care institution and that the cumulative CPR was not evaluated because no frozen ETs were included.

Conclusion

The results show that an earlier embryo transfer day has a negative impact on CPR. Older infertile women should not postpone their desire to have a baby because they are poor responders, and it should be explained that the chances of successful treatment are lower. Further studies with more participants are needed to clarify this situation.

Conflict of Interest: No conflict of interest is declared by the authors.
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Table 1. Demographic and stimulation characteristics of the patients							
		Day 2 transfer Group 1 (n=43)	**Day 3 transfer Group 2 (n=633)**	**Day 5 transfer Group 3 (n=81)**	**p**		
					1 vs 2	**1 vs 3**	**2 vs 3**
Age (years)		32.12±5.65	29.68±4.52	28.36±4.33	0.003	<0.001	0.046
Age>40 (years) (%)		4.5%	1.6%	1.3%	0.323		
BMI (kg/m^2)		27.76±4.31	25.89±4.53	25.21±4.86	0.030	0.011	0.433
Smoking rate (%)		4.9%	7.9%	3.9%	0.324		
Duration of infertility (years)		7.21±4.45	5.97±3.40	5.95±3.48	0.085		
Etiology of infertility (%)	**Male factor**	26.8%	37.5%	35.1%			
	Tubal factor	2.4%	2.2%	1.3%	0.012	<0.001	0.021
	Unexplained	26.9%	39.1%	55.8%			
	Poor responder	43.9%	21.2%	7.8%			
Baseline-FSH (IU/mL)		7.41±2.87	7.08±2.28	6.57±1.97	0.109		
Baseline-LH (IU/mL)		5.13±2.39	5.53±2.87	6.20±3.37	0.119		
Baseline-Estradiol (pg/mL)		40.82±16.94	44.22±15.95	46.55±18.74	0.189		
Antral follicle count		5.35±2.37	6.51±2.50	7.78±2.21	0.026	0.001	0.045
TSH (µIU/mL)		2.44±0.96	2.17±1.13	2.17±1.09	0.317		
Prolactin (ng/mL)		16.02±7.90	16.09±8.62	18.69±12.21	0.058		
Stimulation protocol (%)	**Long**	26.8%	19.1%	26.0%			
	Antagonist	73.2%	80.1%	74.0%		0.327	
	Microdose	0.0%	0.8%	0.0%			
Duration of stimulation (days)		10.12±1.40	9.74±1.54	9.78±1.61	0.304		
Gonadotropin dose (IU)		2567.68±1193.01	1948.55±834.94	1708.05±829.89	0.006	<0.001	0.043
Estradiol levels on day hCG (pg/mL)		1499.49±691.37	1903.98±1199.97	2741.39±1265.31	0.003	<0.001	<0.001
Progesterone levels on day hCG (pg/mL)		0.85±0.43	0.81±0.38	0.91±0.40	0.078		
Endometrial thickness on day hCG (mm)		9.80±1.77	10.22±1.66	10.44±1.74	0.151		
Endometrial thickness on transfer day (mm)		9.64±1.55	1070±1.88	10.91±2.26	0.007	0.017	0.744
BMI: Body mass index, FSH: Follicle stimulating hormone, LH: Luteinizing hormone, TSH: Thyroid stimulating hormone, hCG: Human chorionic gonadotropine, p<0.05 is statistically significant							

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Table 2. Laboratory and reproductive outcome parameters of the patients							
		Day 2 transfer Group 1 (n=43)	**Day 3 transfer Group 2 (n=633)**	**Day 5 transfer Group 3 (n=81)**	**p**		
					1 vs 2	**1 vs 3**	**2 vs 3**
Number of oocytes retrieved		5.71±3.91	9.23±5.36	13.55±5.66	<0.001		
Number of MII oocytes		3.95±2.46	7.18±4.11	11.19±5.05	<0.001		
2 Pronucleus		2.07±1.27	4.69±3.04	8.03±3.56	<0.001		
Fertilization rate (%)		61.17±26.99	68.59±24.01	74.36±19.30	0.129	0.012	0.043
Grade I embryo (%)		34.1%	64.7%	97.4	<0.001		
The embryo transfer technique (%)	**Easy transfer with a soft catheter**	24.4%	21.2%	15.6%	0.704		
	After external guidance transfer	68.3%	72.2%	75.3%			
	Difficult transfer with a stylet	7.3%	6.6%	9.1%			
Clinical pregnancy rate (%)		19.5%	36.9%	39.0%	0.039	0.028	0.710
Live birth rate (%)		14.6%	30.4%	35.1%	0.033	0.021	0.434
BMI: Body mass index, p<0.05 is statistically significant							

Table 3. Logistic regression analysis of the factors thought to affect clinical pregnancy and live birth rates							
		Clinical pregnancy			**Live birth rate**		
		Odds Ratio	**95% Confidence Interval**	**p**	**Odds Ratio**	**95% Confidence Interval**	**p**
Age (years)	**Day 2 transfer**	0.985	0.857-1.133	0.834	0.970	0.828-1.137	0.709
	Day 3 transfer	0.941	0.906-1.107	0.057	0.956	0.919-1.033	0.382
	Day 5 transfer	0.929	0.829-1.040	0.399	0.948	0.846-1.062	0.354
Grade I embryo (%)	**Day 2 transfer**	4.444	0.876-22.536	0.001	5.021	0.787-31.768	0.001
	Day 3 transfer	1.756	1.234-2.500	<0.001	1.676	1.154-2.433	0.007

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	Day 5 transfer	-	0.0-0.0	0.999	-	0.0-0.0	0.999
Number of oocytes retrieved	**Day 2 transfer**	1.052	0.856-1.266	0.606	1.123	0.924-1.364	0.244
	Day 3 transfer	1.044	0.913-1.076	0.156	1.048	0.916-1.082	0.103
	Day 5 transfer	1.088	0.998-1.186	0.055	1.084	0.994-1.181	0.068
Number of MII oocytes	**Day 2 transfer**	1.010	0.738-1.383	0.750	1.104	0.796-1.532	0.554
	Day 3 transfer	1.076	0.934-1.120	0.341	1.087	0.943-1.133	0.059
	Day 5 transfer	1.070	0.979-1.181	0.131	1.069	0.973-1.174	0.165
Gonadotropin dose (IU)	**Day 2 transfer**	0.956	0.906-1.010	0.445	0.954	0.886-1.114	0.377
	Day 3 transfer	0.974	0.979-1.105	0.820	0.904	0.896-1.046	0.092
	Day 5 transfer	0.990	0.909-1.055	0.725	0.924	0.909-1.011	0.187
P<0.05 is statistically significant							

Uncorrected Proof

Flowchart of the study

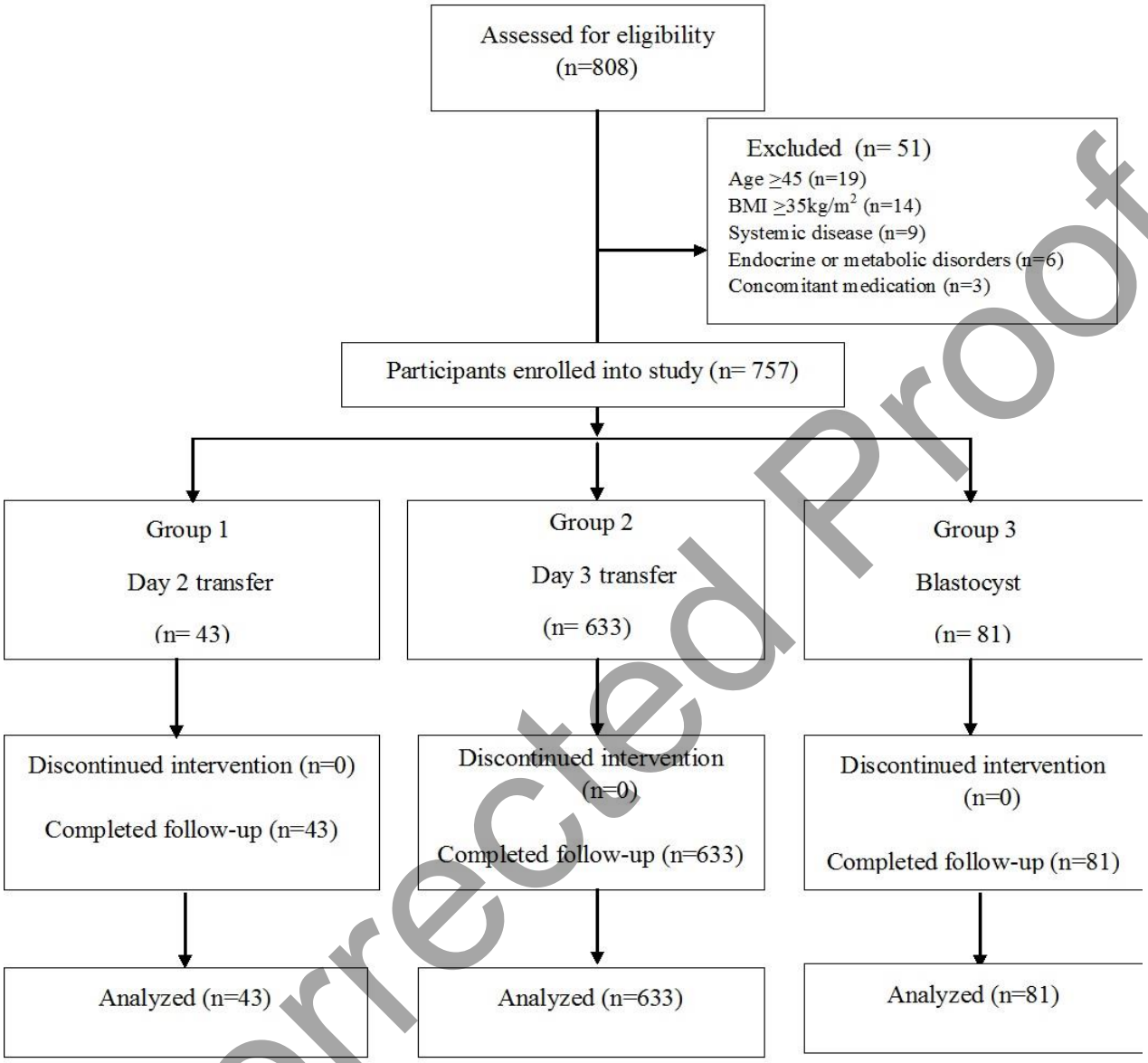

Fig 1. Enrollment and follow-up of the study subjects.

Uncorrected Proof