

# Laser-assisted selection of immotile spermatozoa has no effect on obstetric and neonatal outcomes of TESA-ICSI pregnancies

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## Research Article

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# Abstract

## Background

Azoospermic patients have benefited from both epididymal and testicular spermatozoa intracytoplasmic sperm injection (ICSI) treatment and lasers have been used to identify viable but immotile spermatozoa before the procedure. However, there are limited studies on the safety of laser-assisted selection of immotile spermatozoa. The aim of this study was to investigate the impact of laser-assisted selection of immotile spermatozoa on the obstetric and neonatal outcomes after ICSI.

## Methods

A retrospective comparative study was conducted on patients who underwent ICSI treatment with testicular spermatozoa in our Reproductive Medicine Unit from June 2014 to June 2018. The 132 cycles were divided into two groups according to whether laser-assisted selection of spermatozoa was used.

## Results

Compared with control group, no significant differences were found in the pregnancy, implantation, miscarriage and live birth rates in the laser group in either fresh or frozen transfer cycles. The cumulative live birth rate in the laser group was 69.70%, which was slightly higher than in the control group (60.61%), but this was not statistically different. There were no differences in the average gestational age, premature birth rate, neonatal birth weight and the malformation rate between the laser and control groups ( $P > 0.05$ ). In addition, the obstetric outcome between the two groups were not different ( $P > 0.05$ ).

## Conclusions

No negative effect on perinatal and neonatal outcomes was seen by using laser-assisted selection of immotile spermatozoa for TESA-ICSI. This study endorses the use of laser-assisted selection of viable spermatozoa for ICSI cycles.

## Background

Azoospermia is observed in approximately 1% of the population and in up to 15% of infertile men [1]. Such patients usually require surgery in order to obtain sperm, but the motility of spermatozoa obtained by surgery is usually very poor or even completely immotile [2, 3]. Embryologists often face a dilemma, not knowing how to choose spermatozoa for intracytoplasmic sperm injection (ICSI) when encountering immotile spermatozoa. As is well known, the precondition for a successful fertilization is that a sperm must enter an egg. Studies have shown that an oocyte injected with a live, immotile sperm can also

become successfully fertilized [4, 5], suggesting that successful fertilization is based on the injection of a live sperm rather than just a motile sperm.

Therefore, the question of how to select a live sperm from a number of immotile spermatozoa on the day of ovum acquisition for ICSI is critical. Although the injection of completely immotile spermatozoa either from ejaculates or testicular biopsies can result in successful pregnancies and healthy babies [5, 6], there are many reports that the fertilization and embryo utilization rates are significantly lower when ICSI is performed with immotile spermatozoa compared to motile ones [7, 8]. In addition, in clinical practice, the goal of many infertility clinics is to obtain motile sperm for testicular surgery. Hence, when presented with immotile spermatozoa, clinicians will often choose to discard these and re-operate in order to ensure the availability of motile sperm. This re-operation surgery is invasive for patients. Thus, laboratory methods which can distinguish between viable but immotile and dead spermatozoa are a necessity in order to provide the most convenient and cost effective treatment for patients.

Many approaches have been developed for detecting the viability of immotile sperm, and these include hypo-osmotic swelling (HOS) tests [9], use of chemicals for induction of tail movement [3] and laser [10, 11]. Studies have shown that the use of laser-assisted selection of viable but immotile spermatozoa for ICSI can provide better merits than conventional ICSI using immotile sperm methods [12–14]. ICSI with viable but immotile spermatozoa selected by laser assessment could result in similar fertilization and embryo cleavage rates when compared with use of motile testicular spermatozoa [15]. However, very little data has been published on whether lasers can affect perinatal and neonatal outcomes of ICSI patients. Thus, the objective of this retrospective study was to evaluate whether the use of laser-assisted selection of viable but immotile testicular spermatozoa for ICSI affects the eventual pregnancy outcome.

## Patients And Methods

### Patients

A retrospective study was carried out from June 2014 to June 2018. The inclusion criteria was those patients using motile or immotile testicular spermatozoa in order to perform ICSI treatment. The exclusion criteria were as follows: (1) no transplantable embryos available, including abnormal fertilization, no cleavage embryos or embryo degeneration on day 3 and (2) those embryo transfers performed at the day 1 and 2 zygotic stages. The patients were divided into a motile testicular spermatozoa ICSI group (control) and a group who had laser-assisted selection of viable but immotile testicular spermatozoa (laser group) according to whether motile spermatozoa were observed after *in vitro* cultures of 2~4 hours.

### Ovarian stimulation

Ovarian stimulation was performed using a routine protocol developed by our clinic. Briefly, all female patients were down-regulated by use of leuprolide acetate (Lupron; TAP Pharmaceuticals, Lake Forest, Illinois). Ovarian stimulation was achieved with the use of recombinant follicle stimulating hormone

(Gonal-F or Puregon; Merck Serono, Italy). When two or more follicles reached 18 mm in mean diameter, 5,000–10,000 IU human chorionic gonadotropin (hCG) (Serono, Switzerland; or Livzon, China) was administered. Oocytes were collected by follicular aspiration with the use of vaginal ultrasonography 36 hours after hCG administration.

### **Testicular sperm aspiration**

The patients were placed in the supine position and disinfected following routine procedures. Anesthesia was performed by using 2% lidocaine to block the spermatic cord. A 50-mL syringe containing 0.5mL of fertilization Quinn's 1020 medium (Sage, Trumbull, CT, USA) and a 16-gauge needle were used for aspiration of the seminiferous tubules. The tubules were independently minced using two sterile needles in a culture dish containing 2mL of Quinn's 1020 medium. Then, the processed samples were observed under high magnification ( $\times 200$  magnification). If no motile sperm were found either immediately or after 2~4 hours of culture in a 6% carbon dioxide incubator maintained at 37 °C, the sperm were considered to be immotile.

### **Laser selection of immotile spermatozoa**

Using a protocol based on the method of Aktan *et al* [10], the tips of immotile sperm were targeted with a laser beam of approximately 200 $\mu$ J with an irradiation time of about 2 ms (RI Saturn 5<sup>TM</sup> Laser System, UK). Those spermatozoa which presented with curling of the tails after the laser shot, were regarded as viable, while others which did not respond in this way were considered to be non-viable. The viable but immotile sperm selected were subsequently used for ICSI injections.

### **ICSI**

All ICSI procedures were performed 39–40 hours after hCG administration. After injection with hCG, oocytes were transferred to culture dishes and kept in fertilization medium (Quinn Advantage medium, ART-1020) supplemented with 10% Quinn Advantage serum protein substitute (SPS, ART-3010; Sage) until pro-nucleation was observed.

### **Fertilization checking, embryo culture and transfer**

Fertilization was confirmed after observation of two pro-nuclei and two distinct polar bodies at 16–18h after ICSI. Quinn's medium was used for culture of embryos. Zygotes displaying two pro-nuclei were transferred to the cleavage culture media (Quinn Advantage medium, ART-1026) supplemented with 10% SPS for further culture. The day of ICSI manipulation was considered as day 0. All of the day 3 embryos from patients with good prognoses or the surplus day 3 embryos after transfer or vitrification were delayed in culture until days 5 or 6. Blastocyst culture media (Quinn Advantage medium, ART-1029) supplemented with 10% SPS was used for this. Fresh embryo transfers were performed on day 3 at the cleavage stage or on day 5 (the blastocyst stage). Frozen-thawed embryo transfers were performed after endometrium transformation on day 3 for cleavage stage embryos or on day 5 for blastocyst transfers,

respectively. The serum levels of hCG were measured on day 14 after transfers. Clinical pregnancies were confirmed by transvaginal ultrasonography imaging of the presence of one or more gestational sacs within the uterine cavity after 28 days of transfer.

### **Embryo vitrification, thawing and transplantation**

Vitrification and thawing were performed according to the methods established in our clinic and described previously [16]. The common modality for frozen-thawed embryo transfers was the natural or hormone replacement cycles after endometrial preparation.

### **Follow up and evaluation indexes**

The main outcome measures consisted to be the cumulative live birth rate as well as the obstetric and neonatal outcomes. The cumulative live birth rate was calculated by dividing the number of live births over a period for each egg collection cycle (including both fresh and frozen embryo transfer cycles and when the number of live births was greater than or equal to 2 in one ovum acquisition cycle was considered to be one) by the total number of ovum acquisition cycles. The data from patients undergoing frozen-thawed cycles were included from up to December 2019.

### **Statistical Analysis**

Statistical analysis was performed with the use of the Student t test and chi-squared analysis.  $P < 0.05$  was considered to be statistically significant.

## **Results**

The final data analysis included 132 ICSI treatment cycles of which there were 33 and 99 cycles in the laser and control groups, respectively (Fig. 1). The main characteristics of the female patients and the male infertility factors relating to the reasons for undergoing testicular sperm aspiration are shown in Tables 1 and 2, respectively.

Table 1  
Characteristics of the female patients in this study.

	Laser group	Control group	P value
Cycles	33	99	
Women's age (y)	30.88 ± 5.52	30.97 ± 5.53	0.935
Duration of infertility (y)	4.70 ± 2.90	4.64 ± 3.81	0.565
Maternal body mass index (kg/m <sup>2</sup> )	20.96 ± 2.40	21.66 ± 3.31	0.265
Baseline FSH (IU/L)	6.69 ± 1.38	7.50 ± 2.95	0.132
Baseline LH (IU/L)	5.35 ± 2.94	5.45 ± 2.60	0.855
Endometrial thickness on the day of embryo transfer (mm)	11.74 ± 2.74	11.62 ± 2.20	0.827

Table 2  
Male infertility factors that led to the use of testicular sperm aspiration.

	Laser group	Control group	P value
Cycles	33	99	
Men's age (y)	33.91 ± 6.87	33.64 ± 5.82	0.824
Obstructive azoospermia n (%)	21 (63.64%)	66 (66.67%)	0.833
Non-obstructive azoospermia n (%)	1 (3.03%)	9 (9.09%)	0.450
Severe oligospermia n (%)	3 (9.09%)	3 (3.03%)	0.165
Occult spermia n (%)	2 (6.06%)	0	0.061
Failure of masturbation n (%)	3 (9.09%)	5 (5.05%)	0.412
Anejaculation n (%)	3 (9.09%)	8 (8.08%)	1.000
Congenital absence of vas deferens n (%)	0	2 (2.02%)	1.000
Spermatogenic dysfunction n (%)	0	1 (1.01%)	1.000
Retrograde ejaculation n (%)	0	4 (4.04%)	0.572
Y chromosome microdeletion (SY127 in AZFb region) n (%)	0	1 (1.01%)	1.000

No differences were found between the fertilization, cleavage and top embryo rates on day 3 in these two groups. The fresh clinical pregnancy rate (64.00 vs 45.33%), fresh implantation rate (45.95 vs 34.86%),

fresh miscarriage rate (12.50 vs 11.76%) and fresh live birth rate (56.00 vs 38.67%) were not statistically different between the laser and control groups (Table 3).

Table 3  
Comparison of embryo cultures and pregnancy outcomes between the laser and control groups.

	Laser group	Control group	P value
Cycles	33	99	
Mean no. of oocytes retrieved	14.36 ± 7.80	12.82 ± 6.67	0.272
Fertilization rate	78.17 % (283/362)	80.48 % (763/948)	0.352
Cleavage rate	95.76% (271/283)	96.59 % (737/763)	0.523
Top embryos rate on day 3	44.65% (121/271)	43.14% (318/737)	0.670
Fresh transfer cycles	25	75	
No. of fresh embryos transferred	1.48 ± 0.59	1.45 ± 0.53	0.832
Fresh clinical pregnancy rate	64.00 % (16/25)	45.33% (34/75)	0.165
Fresh implantation rate	45.95 % (17/37)	34.86 % (38/109)	0.244
Fresh miscarriage rate	12.50 % (2/16)	11.76% (4/34)	1.000
Fresh live birth rate	56.00 % (14/25)	38.67 % (29/75)	0.163
Frozen transfer cycles	24	85	
Frozen clinical pregnancy rate	58.33% (14/24)	49.41% (42/85)	0.494
Frozen implantation rate	53.57 % (15/28)	46.24 % (43/93)	0.524
Frozen live birth rate	41.67% (10/24)	40.00% (34/85)	1.000
Cumulative live birth rate	69.70% (23/33)	60.61% (60/99)	0.409

Up to December 2019, 24 cycles in the laser group and 85 cycles in the control group were from frozen-thawed embryos. There were no statistically differences in the frozen clinical pregnancy rate (58.33 vs 49.41%), frozen implantation rate (53.57 vs 46.24%) and frozen live birth rate (41.67 vs 40.00%) between the laser and control groups. Furthermore, the cumulative live birth rate of the laser group was higher than that of the control group (69.70 vs 60.61%), but there was not significantly different ( $P > 0.05$ ) (Table 3).

Table 4 shows the neonatal outcomes after ICSI treatment. A total of 94 babies consisting of 6 twin babies in the laser group and 8 twin babies in the control group were born. No significant differences with respect to method of delivery were seen. In addition, no differences were observed in the mean gestational age ( $38.26 \pm 1.28$  vs  $38.37 \pm 1.35$ ), preterm delivery rate (11.11 vs 7.46%), mean birth weight at delivery ( $2894.82 \pm 623.32$  vs  $3101.34 \pm 435.04$ ) and malformation rate (0.00 vs 1.49%) between the

laser and control groups, respectively ( $P > 0.05$  in all cases). With respect to obstetric outcomes between the laser and control groups, no significant differences were seen ( $P > 0.05$ ) (Table 5).

Table 4  
Comparison of neonatal outcomes between the laser and control groups

	Laser group	Control group	P value
Cumulative live birth babies	27	67	
Gestational weeks at delivery	38.26 $\pm$ 1.28	38.37 $\pm$ 1.35	0.987
Preterm delivery (< 37 weeks)	11.11% (3/27)	7.46% (5/67)	0.685
Birth weight (grams)	2894.82 $\pm$ 623.32	3101.34 $\pm$ 435.04	0.071
Birth weight < 2500 grams	14.80% (4/27)	8.96% (6/67)	0.465
Birth weight > 4000 grams	3.70% (1/27)	1.49% (1/67)	0.494
Malformation rate	0	1.49 % (1/67)	1.000

Table 5  
Comparison of obstetric outcomes between the laser and control groups.

	Laser group	Control group	P value
Total deliveries cycles	24	63	
Cesarean delivery	45.83% (11/24)	52.38% (33/63)	0.637
gestational hypertension	8.33% (2/24)	3.17% (2/63)	0.304
Gestational Diabetes	12.5 % (3/24)	6.35 % (4/63)	0.389
Premature rupture of membranes	8.33% (2/24)	6.35 % (4/63)	0.666
Placenta previa	0.00% (0/24)	1.59% (1/63)	1.000
Postpartum hemorrhage	0.00 % (0/24)	3.17% (2/63)	1.000
Fetal distress	16.67% (4/24)	6.35% (4/63)	0.208
Premature birth	12.50 % (3/24)	9.52% (6/63)	0.702
Low birth weight	16.67 % (4/24)	12.70 % (8/63)	0.730

## Discussion

Our study was undertaken to investigate the effectiveness and safety of lasers for the identification of viable but immotile spermatozoa in TESA-ICSI cycles. We found that there were no statistical differences in embryo development and pregnancy outcomes between the laser and control groups. Furthermore, we also confirmed that there were no negative effects on obstetric and neonatal outcomes by using laser



assisted selection of viable but immotile spermatozoa. This is the first study to focus on the obstetric and neonatal outcomes after ICSI using immotile spermatozoa selected by laser technology.

Azoospermia males have previously benefited from the retrieval of spermatozoa by using testicular sperm aspiration, testicular sperm extraction and micro-testicular sperm extraction surgery. However, frequently use of these procedures can result in a few motile sperm and in some cases only immotile spermatozoa are retrieved [8]. The embryologist is often struggling to find enough viable spermatozoa for the ICSI protocol. The selection of a suitable spermatozoon for ICSI is an essential prerequisite in order to achieve fertilization and optimal pregnancy rates. There is still no consensus on how to select viable but immotile spermatozoa for ICSI. The selection of the most suitable spermatozoon is often dependent on the experience of the embryologist. In addition, any chemicals or other parameters used during the selection process may have adverse effects on the development of the embryo as well as the outcome of the pregnancy. Thus, a quick, easy and safe technique for selection of a suitable candidate spermatozoon for ICSI would be welcomed by both the clinician and the patient.

In recent years, laser has been widely used in the field of assisted reproductive technology, including assisted hatching [17, 18], embryo biopsies [19] and sperm immobilization [20]. The clinical use of sperm selection with a laser has recently gained more attention. The ability of laser technology to identify viable spermatozoa was first reported by Anta *et al.* [10]. Using this technique, they achieved higher fertilization and cleavage rates in cases with fresh testicular spermatozoa as well as in cases with ejaculated sperm. Successful pregnancies were obtained by several groups using laser-assisted selection of viable spermatozoa before ICSI [21, 22]. In addition, it was reported that using laser-assisted selection of immotile testicular spermatozoa and polarization microscopy for selection of oocytes significantly increased the fertilization rate of the testicular sperm extraction (TESE ) ICSI program [23].

Previous studies were mainly focused on the fertilization rate, whereas systematic research on clinical and neonatal outcomes after laser-assisted selection of immotile sperm is not well documented. In our study, it was gratifying to find that there were no significant differences in the fertilization and cleavage rates as well as achieving a high quality of day 3 embryos rate between the laser and control groups. Furthermore, the clinical pregnancy, implantation and live birth rates were not significantly different in both the fresh and frozen-thawed transfer cycles between the two groups. The cumulative live birth rate of laser group was slightly higher than that of control group although this did not reach statistical significance.

The safety of babies born from assisted reproductive technology treatment is always a concern. A number of studies have reported that in-vitro fertilization (IVF) or ICSI-conceived offspring, even if they are singleton pregnancies, are associated with low birth weights and preterm deliveries [24, 25]. Meta-analysis studies have also concluded that children conceived from IVF and ICSI can present with an increased risk for congenital malformations compared with those naturally conceived, although these risks did not differ between IVF and ICSI [26–28]. Concerns with respect to the safety of using immotile spermatozoa for ICSI have arisen mainly as some assisted conception methods have used chemical

substances to select viable spermatozoa [29, 30]. The exact biochemical effects of these compounds on human spermatozoa and embryos are not well demonstrated.

Studies have confirmed that laser-assisted operation in embryo hatching, embryo biopsies and sperm immobilization did not appear to increase the risk of adverse neonatal outcomes [31–33]. Laser beams used for determination of spermatozoa viability is generally based on their protein activities and the integrity of the tail membranes. In theory, a single laser shot applied to the far end of the flagellum of a viable spermatozoon should not cause any adverse effects on its genetic material [34]. Moreover, using a laser beam to select spermatozoa does not require the use of any chemical substances to either induce spermatozoa motility or cause spermatozoa flagellum curling. Laser-assisted selection of viable but immotile spermatozoa can be directly used for ICSI in a petri dish, and the sperm can be injected immediately into the oocyte. Consequently, no accompanying side-effects are expected. In our study, no statistically significant differences in adverse obstetric and neonatal outcomes were found when the laser group was compared to the control group. These results will provide encouraging evidence for embryologists to select viable but immotile spermatozoa by using laser technology.

There are some limitations in this study. Firstly, the sample size was too small to divide subgroups for independent analysis of the use of fresh and frozen-thawed spermatozoa. However, no statistical differences were found in the fertilization and good quality embryo rates between the frozen-thawed immotile spermatozoa group and the routine fresh immotile spermatozoa ICSI group [35]. Larger samples are needed to compare the clinical outcomes of these two subgroups. Secondly, only neonatal outcome was collected and the study would benefit from long term follow-up for children beyond neonatal stage. These two failings will be addressed in our future studies.

In conclusion, there was no statistical increase in the risk of obstetric and neonatal outcomes in the TESA ICSI treatment following laser-assisted selection of viable but immotile spermatozoa. Patients presented with viable but immotile spermatozoa will benefit from laser application in the ICSI program.

## Abbreviations

TESA: testicular sperm aspiration; ICSI: intracytoplasmic sperm injection; HOS: hypo-osmotic swelling; hCG: human chorionic gonadotropin; SPS: serum protein substitute; TESE: testicular sperm extraction; IVF: in-vitro fertilization.

## Declarations

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## Authors' contributions

HC and CW participated in the design of the study, performed the statistical analysis and drafted the manuscript. HZ carried out the controlled ovarian stimulation. JS performed the ICSI procedure. XG enrolled the male patients. KX, ZW and GH helped to perform the laboratory operations. XD performed the ovum acquisition operations and embryo transfers. RL read and revised the manuscript. All authors read and approved the final manuscript.

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## Availability of data and materials

Please contact author for data requests.

## Ethics approval and consent to participate

The study was approved by the Ethics Committee of Guangxi Maternal and Child Health Hospital. All patients signed informed consents regarding ART.

## Consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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Figures

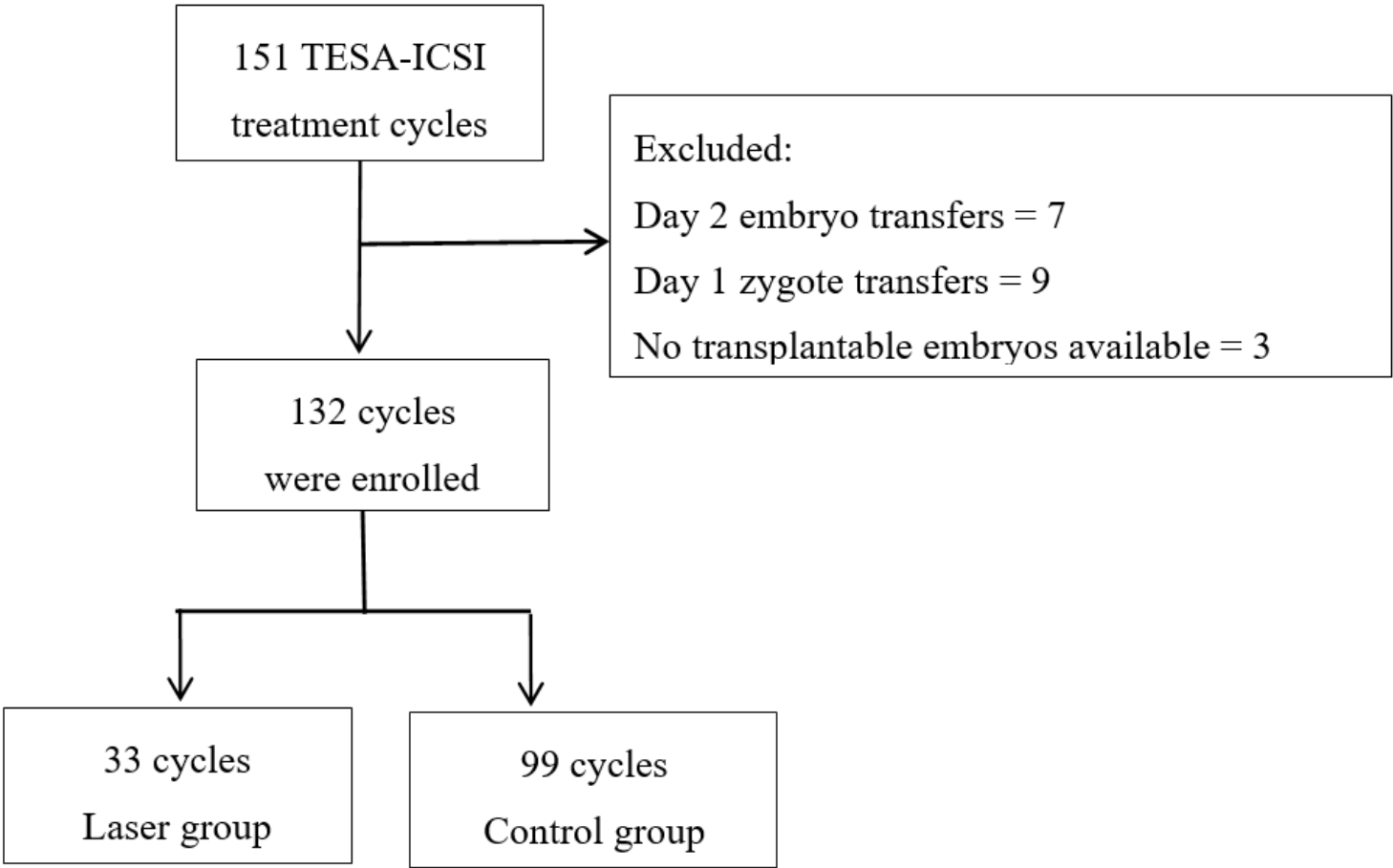


Figure 1

Flow chart of ICSI treatment cycles included in this study.