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ORIGINAL ARTICLE

Male Reproduction

# Round spermatid injection into human oocytes: a systematic review and meta-analysis

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Many azoospermic men do not possess mature spermatozoa at the time of surgical sperm extraction. This study is a systematic review and meta-analysis evaluating outcomes following round spermatid injection (ROSI), a technique which utilizes immature precursors of spermatozoa for fertilization. An electronic search was performed to identify relevant articles published through October 2018. Human cohort studies in English involving male patients who had round spermatids identified and used for fertilization with human oocytes were included. Fertilization rate, pregnancy rate, and resultant delivery rate were assessed following ROSI. Meta-analysis outcomes were analyzed using a random-effects model. Data were extracted from 22 studies involving 1099 couples and 4218 embryo transfers. The fertilization rate after ROSI was 38.7% (95% confidence interval [CI]: 31.5%–46.3%), while the pregnancy rate was 3.7% (95% CI: 3.2%–4.4%). The resultant delivery rate was low, with 4.3% of embryo transfers resulting in a delivery (95% CI: 2.3%–7.7%). The pregnancy rate per couple was 13.4% (95% CI: 6.8%–19.1%) and the resultant delivery rate per couple was 8.1% (95% CI: 6.1%–14.4%). ROSI has resulted in clinical pregnancies and live births, but success rates are considerably lower than those achieved with mature spermatozoa. While this technique may be a feasible alternative for men with azoospermia who decline other options, couples should be aware that the odds of a successful delivery are greatly diminished and the prognosis is relatively poor.

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**Keywords:** azoospermia; *in vitro* fertilization; infertility; spermatozoa; testis

## INTRODUCTION

In the general population, azoospermia, or the lack of spermatozoa in the ejaculate, occurs in approximately 1% of men.<sup>1</sup> Among infertile men evaluated at urology clinics, approximately 10%–20% of patients are found to have azoospermia.<sup>2</sup> Men with azoospermia are commonly referred to male fertility specialists who may recommend surgical sperm extraction procedures in order to obtain spermatozoa for use in fertilization. Unfortunately, even with these extraction procedures, many men do not possess mature spermatozoa which can be successfully isolated.

A recent retrospective cohort study published in 2016 by Cissen *et al.*<sup>3</sup> reported that only 43.7% of men with nonobstructive azoospermia had successful initial testicular sperm extraction (TESE) procedures, with success defined as the presence of spermatozoa in the surgical sample. Similarly, a retrospective cohort study published by Vloeberghs *et al.*<sup>4</sup> in 2015 reported that only 40.5% of men with nonobstructive azoospermia had mature sperm successfully retrieved at the time of their first TESE procedure. Of the proportion of men who are able to undergo successful TESE procedures, only a small percentage of patients with nonobstructive azoospermia go on to become biologic fathers. In a study evaluating 714 men with nonobstructive azoospermia undergoing TESE, only one in seven

men (13.4%) eventually fathered a biologic child.<sup>4</sup> When compared to standard TESE, many publications have reported slightly more encouraging success rates when microscopic testicular sperm extraction (micro-TESE) is performed for nonobstructive azoospermia, with sperm isolated in approximately 60% of surgical samples.<sup>5</sup> However, the azoospermic population remains challenging from a clinical perspective due to a relative paucity of effective treatment modalities.

When no mature spermatozoa are obtained from a surgical sperm sample, men are left with limited options. The use of a sperm donor or adoption should be discussed when a patient has neither spermatozoa nor late-stage spermatids isolated from the testicular tissue.<sup>1</sup> However, it has been suggested that round spermatids, which are immature precursors to mature spermatozoa, can be successfully injected into human oocytes and used in the place of mature spermatozoa in the cases of last resort.<sup>1,6</sup> Among men with nonobstructive azoospermia where neither mature spermatozoa nor late-stage spermatids were isolated from testicular samples, it has been reported that approximately 30% of patients will possess round spermatids in their surgical samples.<sup>7</sup>

In both animal and human models, successful births have been reported using the technique of round spermatid injection (ROSI). Although many reports describe ROSI as inefficient and of no real

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clinical value, more recent data have suggested that ROSI may be a feasible, albeit last resort, alternative in patients who decline donor sperm and adoption. Importantly, ROSI appears to result in offspring without any unusual physical, mental, or epigenetic problems.<sup>1,6</sup> A publication by Tanaka *et al.*<sup>6</sup> in 2018 reported the births of ninety babies following ROSI and followed the offspring for 2 years to track physical and cognitive development. No significant differences were observed between offspring achieved via ROSI compared to spontaneous conceptions over the 2-year observational period.<sup>6</sup>

The purpose of this systematic review and meta-analysis is to evaluate clinical outcomes following ROSI using human oocytes. Specifically, this study seeks to objectively assess fertilization rate, pregnancy rate, and resultant delivery rate following ROSI based on the available literature.

## MATERIALS AND METHODS

### Study design

This systematic review and meta-analysis was performed in accordance with the Preferred Reporting Items of Systematic Reviews and Meta-analyses (PRISMA) guidelines.<sup>8</sup> Institutional review board approval was not required for this study. This systematic review was registered in the PROSPERO database (registration No. CRD42019139171). Given the observational nature of potential studies, this meta-analysis was performed in accordance with the Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines.

### Literature search

This review evaluated cohort studies which investigated the impact of ROSI on embryology and pregnancy outcomes. A systematic review of the literature was conducted in May 2019 with the use of MEDLINE, EMBASE, CENTRAL, and ClinicalTrials.gov electronic databases. These databases were searched for publications reporting clinical outcomes for human subjects undergoing ROSI between January 1, 1990, and October 1, 2018. Only studies published in English were included. The following keywords were used: “round spermatid injection” or “ROSI” in combination with “fertilization”, “pregnancy”, “delivery”, and “birth”. The Medical Subject Headings (MeSH) terms which were utilized to identify publications included the following: “azoospermia/etiology”, “azoospermia/pathology”, “azoospermia/physiopathology”, “fertilization *in vitro*”, “infertility, male/etiology”, “infertility, male/pathology”, “infertility, male/physiopathology”, “infertility, male/therapy”, “male”, “microdissection”, “sperm injections, intracytoplasmic”, “sperm retrieval”, “spermatids/pathology”, and “spermatogenesis/physiology”.

### Study selection

A study was included in the systematic review if: (1) the publication reported male patients undergoing ROSI; (2) the couple underwent embryo transfer using fresh and/or frozen embryos obtained from the ROSI procedure; and (3) the publication reported pregnancy outcomes (including fertilization rate, pregnancy rate per cycle, resultant delivery rate per cycle, pregnancy rate per couple, and/or resultant delivery rate per couple).

The following were considered exclusion criteria: (1) noncohort studies (reviews, case reports, meta-analyses, opinions, book chapters, *etc.*); (2) duplicate studies; (3) nonhuman studies involving gametes from animals; and (4) studies which failed to perform an intervention (fertilization or embryo transfer) using ROSI. Language restrictions were applied, and only publications in the English language were taken into consideration.

The following definitions were used in this study. Fertilization rate was defined as the number of two pronuclei (2PN) stage embryos

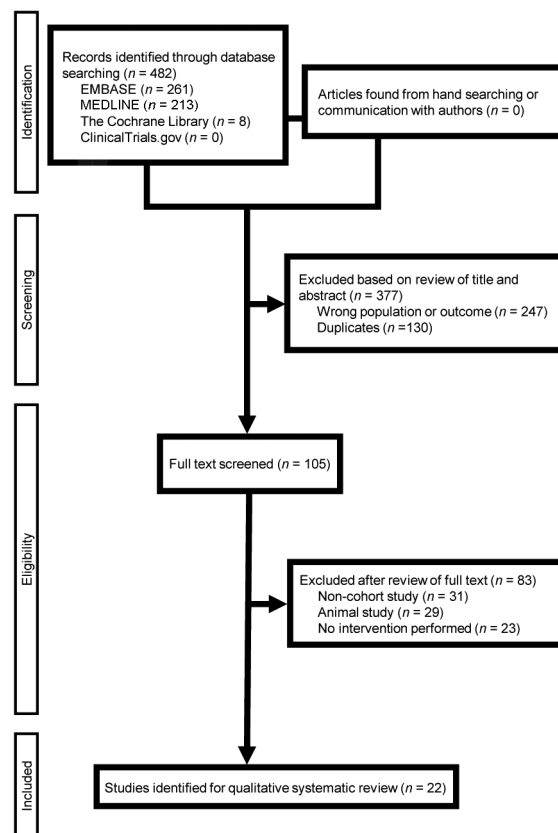
obtained divided by the number of metaphase II (MII) oocytes injected using the ROSI technique. Pregnancy rate was defined as the number of pregnancies reported based on serum or ultrasound findings divided by the number of fresh and frozen embryos transferred. Resultant delivery rate was defined as the number of pregnancies resulting in delivery divided by the number of fresh and frozen embryos transferred. Pregnancy rate per couple was defined as the number of pregnancies reported based on serum or ultrasound findings divided by the number of male patients undergoing ROSI.

All citations were reviewed by two trained reviewers (BMH and TPK) to identify potentially relevant studies. Titles and abstracts were reviewed, and the full-text articles were retrieved if they were relevant or if there was uncertainty about a publication's inclusion criteria based on the title and abstract. Discrepancies in inclusion were resolved by a consensus of authors. A flow diagram for study selection is presented in **Figure 1**.

The technique utilized for round spermatid identification was based on microscopy in all included publications. However, the specific visual criteria which were employed to identify round spermatids varied slightly between studies. A standardized set of criteria for round spermatid identification was not available prior to the study by Tanaka *et al.*<sup>1</sup> published in 2015. Earlier studies which independently generated visual selection criteria were included in the current review and meta-analysis.

### Data collection

Data extraction was performed in a systematic manner using a structured data extraction template by two trained investigators (BMH and TPK). Discrepancies in inclusion were resolved by a consensus of authors. The following data were extracted for each study



**Figure 1:** Flow diagram of studies identified in the systematic review.

included in the systematic review and meta-analysis: authors, year of publication, journal of publication, country of origin, population studied, number of male patients undergoing ROSI, number of MII oocytes injected with round spermatids, number of oocytes fertilized via ROSI, number of fresh embryo transfers performed following ROSI, number of frozen embryo transfers which took place after ROSI, number of pregnancies observed, and number of resultant deliveries.

### Quality assessment

The risk of bias was assessed within studies by using the criteria outlined in the Cochrane Handbook for Systematic Reviews of Interventions by two trained investigators (BMH and TPK).<sup>9</sup> Methodologic quality assessment of studies was made for potential risk of bias with the use of the Newcastle–Ottawa scale for observational studies (Table 1).<sup>10</sup>

### Data analyses

The data analysis was performed with the use of R 3.4.1 using the package Metaprop (Microsoft, Redmond, WA, USA). Higgins  $I^2$  statistic was used to assess heterogeneity between studies. Because of high heterogeneity ( $I^2 > 40\%$  for fertilization rate, clinical pregnancy rate, and resultant delivery rate), a random-effects model was applied to analyze raw data from each study and obtain pooled means and 95% confidence intervals (CIs) for each of the outcomes of interest.

## RESULTS

### Study characteristics

A flow diagram of the systematic review is shown in Figure 1. A total of 22 publications were included for analysis which included 1099 couples and 4218 embryo transfers. Of the 22 publications included in the analysis, two were retrospective cohort studies<sup>11,12</sup> and 20 were prospective cohort studies.<sup>1,6,13–31</sup> A qualitative analysis of the characteristics of the included studies is presented in Table 2. Year of publication for included studies ranged from 1996 to 2018.

There were five studies from Turkey,<sup>18,24,27,28,31</sup> two studies from Japan,<sup>6,17</sup> three studies from Italy,<sup>15,16,23</sup> two studies from France,<sup>13,25</sup> two studies from Portugal,<sup>12,30</sup> two studies from Israel,<sup>11,19</sup> one study from Belgium,<sup>14</sup> one study from Iran,<sup>29</sup> one study from Germany,<sup>22</sup> one study from China,<sup>26</sup> one study from Jordan,<sup>21</sup> and one study from Spain.<sup>20</sup> There were no studies performed in the USA. Based on the inclusion criteria of the systematic review, all studies performed ROSI using human gametes. Two studies reported pregnancy outcomes with frozen embryo transfers following ROSI,<sup>6,12</sup> and the remaining publications reported pregnancy outcomes with fresh embryo transfers following ROSI.

### Fertilization rate after ROSI

A random-effects model was utilized to evaluate oocyte fertilization rate after ROSI. A forest plot was constructed to demonstrate the pooled analysis of the included studies (Figure 2). The overall fertilization rate (2PN) per MII oocyte injected was 38.7% (95% CI: 31.5%–46.3%). There was a high degree of heterogeneity noted for the included studies, with a Higgins  $I^2$  of 97%. The number of successfully fertilized oocytes in the majority of studies was small, with only four studies reporting greater than 100 fertilized oocytes.<sup>6,21,24,28</sup>

### Pregnancy rate after ROSI

The pregnancy rate after ROSI was calculated for the 18 studies which reported pregnancy outcomes as well as the number of embryos transferred. A random-effects model was applied for the analysis of pregnancy rate. A positive pregnancy was based on either serum or sonographic findings.

A forest plot was constructed to demonstrate the pooled analysis of the included studies (Figure 3). The overall pregnancy rate per embryo transferred was 3.7% (95% CI: 3.2%–4.4%). There was a high degree of heterogeneity noted for the included studies, with a Higgins  $I^2$  of 72%. However, the degree of heterogeneity between studies was slightly

**Table 1: Methodologic quality assessment of the included studies on ROSI and its effect on clinical outcomes**

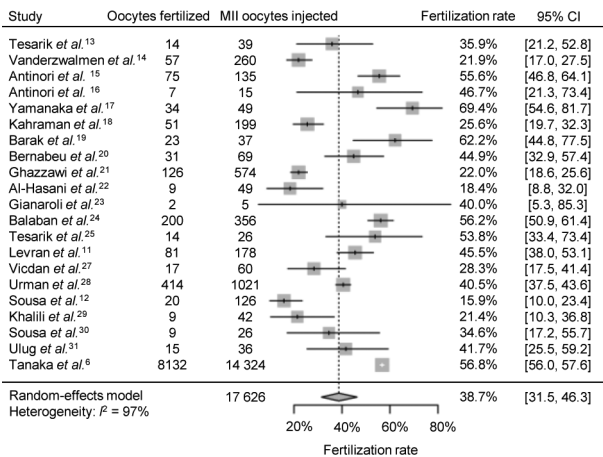
Author	Year of publication	Study design	Quality of evidence score <sup>a</sup>	Selection	Comparability	Outcome
Tesarik <i>et al.</i> <sup>13</sup>	1996	Prospective cohort study	6	***		***
Vanderzwalmen <i>et al.</i> <sup>14</sup>	1997	Prospective cohort study	6	***		***
Antinori <i>et al.</i> <sup>15</sup>	1997	Prospective cohort study	5	***		**
Antinori <i>et al.</i> <sup>16</sup>	1997	Prospective cohort study	3	**		*
Yamanaka <i>et al.</i> <sup>17</sup>	1997	Prospective cohort study	6	***		***
Kahraman <i>et al.</i> <sup>18</sup>	1998	Prospective cohort study	6	***		***
Barak <i>et al.</i> <sup>19</sup>	1998	Prospective cohort study	6	***		***
Bernabeu <i>et al.</i> <sup>20</sup>	1998	Prospective cohort study	6	***		***
Ghazzawi <i>et al.</i> <sup>21</sup>	1999	Prospective cohort study	8	****	*	***
Al-Hasani <i>et al.</i> <sup>22</sup>	1999	Prospective cohort study	6	***		***
Gianaroli <i>et al.</i> <sup>23</sup>	1999	Prospective cohort study	7	****	*	**
Balaban <i>et al.</i> <sup>24</sup>	2000	Prospective cohort study	4	**		**
Tesarik <i>et al.</i> <sup>25</sup>	2000	Prospective cohort study	6	****	*	*
Levrان <i>et al.</i> <sup>11</sup>	2000	Retrospective cohort study	8	****	*	***
Ng <i>et al.</i> <sup>26</sup>	2000	Prospective cohort study	5	**	*	**
Vicdan <i>et al.</i> <sup>27</sup>	2001	Prospective cohort study	8	****	*	***
Urman <i>et al.</i> <sup>28</sup>	2002	Prospective cohort study	6	***		***
Sousa <i>et al.</i> <sup>12</sup>	2002	Retrospective cohort study	8	****	*	***
Khalili <i>et al.</i> <sup>29</sup>	2002	Prospective cohort study	6	***		***
Sousa <i>et al.</i> <sup>30</sup>	2002	Prospective cohort study	6	***		***
Ulug <i>et al.</i> <sup>31</sup>	2003	Prospective cohort study	7	***	*	***
Tanaka <i>et al.</i> <sup>6</sup>	2018	Prospective cohort study	7	***	*	***

<sup>a</sup>Quality of evidence assessed by means of the Newcastle–Ottawa scale for observational and nonrandomized studies, score 0–9. \*A point which is given when a study meets specific criteria in the categories of selection, comparability, or outcome, the sum of these points results in the total score for quality of evidence according to the Newcastle–Ottawa scale. ROSI: round spermatid injection

**Table 2: Qualitative analysis of studies included in the systematic review**

Author	Year	Journal	Country	Male patients undergoing ROSI (n)	MII oocytes injected via ROSI (n)	Oocytes fertilized via ROSI (n)	Frozen embryos transferred after ROSI (n)	Fresh embryos transferred after ROSI (n)	Pregnancies (n)	Resultant deliveries (n)
Tesarik <i>et al.</i> <sup>13</sup>	1996	<i>Hum Reprod</i>	France	7	39	14	0	12	2	2
Vanderzwalmen <i>et al.</i> <sup>14</sup>	1997	<i>Hum Reprod</i>	Belgium	32	260	57	0	7	1	1
Antinori <i>et al.</i> <sup>15</sup>	1997	<i>Hum Reprod</i>	Italy	19	135	75	0	56	2	NR
Antinori <i>et al.</i> <sup>16</sup>	1997	<i>Hum Reprod</i>	Italy	2	15	7	0	6	1	NR
Yamanaka <i>et al.</i> <sup>17</sup>	1997	<i>J Assist Reprod Genet</i>	Japan	9	49	34	0	24	0	0
Kahraman <i>et al.</i> <sup>18</sup>	1998	<i>Hum Reprod</i>	Turkey	20	199	51	0	32	1	0
Barak <i>et al.</i> <sup>19</sup>	1998	<i>Fertil Steril</i>	Israel	6	37	23	0	23	1	1
Bernabeu <i>et al.</i> <sup>20</sup>	1998	<i>Hum Reprod</i>	Spain	8	69	31	0	31	0	0
Ghazzawi <i>et al.</i> <sup>21</sup>	1999	<i>Hum Reprod</i>	Jordan	87	574	126	0	40	0	0
Al-Hasani <i>et al.</i> <sup>22</sup>	1999	<i>Hum Reprod</i>	Germany	2	49	9	0	9	0	0
Gianaroli <i>et al.</i> <sup>23</sup>	1999	<i>Hum Reprod</i>	Italy	1	5	2	0	2	1	1
Balaban <i>et al.</i> <sup>24</sup>	2000	<i>Hum Reprod</i>	Turkey	33	356	200	0	0	N/A	N/A
Tesarik <i>et al.</i> <sup>25</sup>	2000	<i>Fertil Steril</i>	France	5	26	14	0	0	N/A	N/A
Levrant <i>et al.</i> <sup>11</sup>	2000	<i>Fertil Steril</i>	Israel	17	178	81	0	48	0	0
Ng <i>et al.</i> <sup>26</sup>	2000	<i>Chin Med J</i>	China	14	NR	NR	NR	NR	0	0
Vicdan <i>et al.</i> <sup>27</sup>	2001	<i>J Assist Reprod Genet</i>	Turkey	6	60	17	0	5	0	0
Urman <i>et al.</i> <sup>28</sup>	2002	<i>Hum Reprod</i>	Turkey	58	1021	414	0	16	0	0
Sousa <i>et al.</i> <sup>12</sup>	2002	<i>Hum Reprod</i>	Portugal	33	126	20	3	6	0	0
Khalili <i>et al.</i> <sup>29</sup>	2002	<i>J Assist Reprod Genet</i>	Iran	7	42	9	0	6	0	0
Sousa <i>et al.</i> <sup>30</sup>	2002	<i>Hum Reprod</i>	Portugal	8	26	9	0	0	N/A	N/A
Ulug <i>et al.</i> <sup>31</sup>	2003	<i>Fertil Steril</i>	Turkey	4	36	15	0	10	0	0
Tanaka <i>et al.</i> <sup>6</sup>	2018	<i>Fertil Steril</i>	Japan	721	14 324	8132	791	3091	138	84
<b>Total</b>				<b>1099</b>	<b>17 626</b>	<b>9340</b>	<b>794</b>	<b>3424</b>	<b>147</b>	<b>89</b>

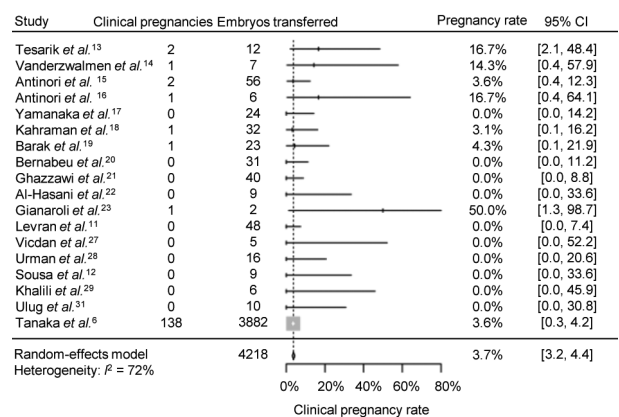
ROSI: round spermatid injection; MII: metaphase II; NR: not reported; N/A: not applicable



**Figure 2:** Oocyte fertilization rate after ROSI. ROSI: round spermatid injection; MII: metaphase II; CI: confidence interval.

less for pregnancy rate than the observed degree of heterogeneity for fertilization rate.

Interestingly, the majority of studies (10 out of 19, 52.6%) included in the assessment of pregnancy rate reported no pregnancies as a result of ROSI.<sup>11,12,17,20–22,27–29,31</sup> The only study with a reported pregnancy rate of greater than 20% was a publication which reported a 50% pregnancy rate after transferring only two embryos.<sup>23</sup> The vast majority of included publications reported a fairly low number of embryo transfers. Only



**Figure 3:** Pregnancy rate per embryo transferred after ROSI. ROSI: round spermatid injection; CI: confidence interval.

two out of 19 publications (10.5%) performed greater than 50 embryo transfers.<sup>6,15</sup> Of the included publications which performed relatively high numbers of embryo transfers, the overall pregnancy rates were low. The largest study by Tanaka *et al.*<sup>6</sup> in 2018 reported 138 pregnancies after 3882 embryo transfers, yielding a pregnancy rate of 3.6%. Most of the included publications (16 out of 18, 88.9%) solely performed fresh embryo transfers following ROSI.<sup>11,14–23,27–29,31,32</sup> Only two publications out of the 18 studies analyzed (11.1%) included a combination of both fresh and frozen embryo transfers.<sup>6,12</sup> Overall, 794 frozen embryos



created via ROSI were transferred, representing 18.8% of the 4218 total embryo transfers performed.

An additional analysis was performed to evaluate the secondary outcome of pregnancy rate per couple, defined as the number of pregnancies achieved per male patient undergoing ROSI. A random-effects model was applied for this portion of the analysis. The pregnancy rate per couple as reported in the included studies was 13.4% (95% CI: 6.8%–19.1%).

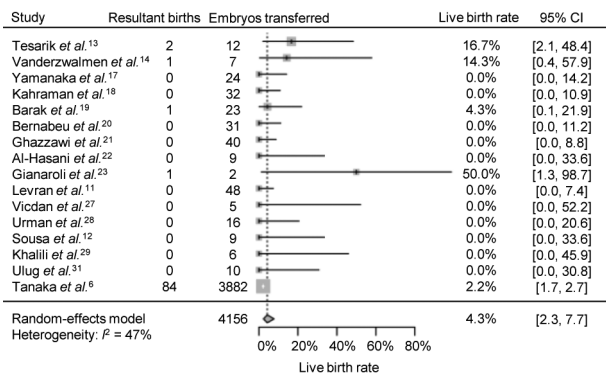
**Resultant delivery rate after ROSI**

The resultant delivery rate after ROSI was assessed for the 16 studies which reported delivery data. As stated previously, 18 publications were included in the analysis of pregnancy rate. However, two studies published results with ongoing pregnancies, so delivery data were not available for inclusion.<sup>15,16</sup> The resultant delivery rate was calculated as the number of pregnancies which resulted in delivery per embryo transferred. This outcome measure was selected because many publications did not specifically delineate singleton versus multifetal gestations, and it would be unreasonable to assume that all reported deliveries were singleton pregnancies, especially given the common practice of multiple embryo transfers during the time period of these publications. Therefore, the resultant delivery rate as opposed to birth rate was selected as the most appropriate outcome to assess.

A random-effects model was applied for analysis of the resultant delivery rate. A forest plot was constructed for the pooled analysis of the included studies (Figure 4). The resultant delivery rate per embryo transferred was 4.3% (95% CI: 2.3%–7.7%). The degree of heterogeneity noted for the included studies was lower than the degree of heterogeneity observed for both fertilization rate and pregnancy rate, with a Higgins *I*<sup>2</sup> of 47%.

Of the included publications, 11 out of 16 studies (68.8%) reported no deliveries following ROSI and embryo transfer. Of the 87 deliveries which were reported after ROSI, 82 deliveries (94.3%) were described in a single recent publication.<sup>6</sup> This study was published in 2018, several years later than the rest of the included studies which were published between 1996 and 2003. From the remaining 15 publications which consisted of 274 embryo transfers, only five deliveries were reported in four publications.<sup>14,19,23,32</sup>

A secondary analysis was also performed to evaluate the resultant delivery rate per couple, defined as the number of pregnancies ending in delivery per male patient undergoing ROSI. A random-effects model was applied for this portion of the analysis. The resultant delivery rate per couple as reported in the included studies was 8.1% (95% CI: 6.1%–14.4%).



**Figure 4:** Resultant delivery rate per embryo transferred after ROSI. ROSI: round spermatid injection; CI: confidence interval.

**Assessment of publication bias**

The quality of the studies was evaluated based on the Newcastle–Ottawa scale for observational and nonrandomized studies (Table 1). The included studies had scores ranging from 3 to 8. In many studies, it was difficult to assess comparability due to the fact that controlling for confounders and identification of a control group was often lacking from included publications. In 13 studies, there was no adequate comparability of study groups.<sup>13–20,22,24,28–30</sup>

**DISCUSSION**

The current work demonstrates that since its inception, ROSI has resulted in numerous clinical pregnancies and live births. However, the majority of these successful pregnancies and live births have occurred only in recent years. Early attempts with ROSI were largely unsuccessful, likely due to the fact that laboratory conditions in the 1990s and early 2000s had not yet been optimized and a standard set of criteria to visually identify round spermatids had not been developed. Overall, while ROSI may be a feasible alternative for a select group of men with azoospermia who have no mature spermatozoa identified at the time of surgical sperm extraction, this technique remains substantially inferior to standard intracytoplasmic sperm injection (ICSI) with mature spermatozoa.

To our knowledge, no prior meta-analysis exists evaluating the clinical applicability of ROSI despite several publications reporting ROSI outcomes from as early as the 1990s to as recently as the 2010s. This systematic review and meta-analysis serves as the first comprehensive report of ROSI outcomes, and the specific outcome measures selected for analysis are felt to be strong indicators of the clinical utility of this technique.

This meta-analysis was limited by high degrees of heterogeneity among studies for all of the evaluated outcome measures. Additionally, the majority of publications examined small numbers of patients and embryos. The significance of data from smaller studies should not be ignored, although the larger studies included in this review have the statistical power to provide more meaningful outcomes. The most recent publication from 2018 reported outcomes from dramatically larger patient populations than the other studies. This study is also arguably more applicable to the modern fertility laboratory environment since publications from the 1990s and the early 2000s may not be representative of the current laboratory practice and technique.<sup>1,6</sup> It must also be noted that a standardized set of criteria for visual identification of round spermatids on microscopy was not available prior to 2015. Therefore, a limitation of the current work is that studies included which were published prior to 2015 likely used a variety of microscopic parameters to identify round spermatids. This may have resulted in higher rates of inaccurate identification of round spermatids in earlier studies.

Overall, with a significant proportion of studies failing to report successful deliveries, it can be difficult to draw conclusions regarding the generalized utility of this technique. Furthermore, the fact that a single study by Tanaka *et al.*<sup>6</sup> published in 2018 represents a substantial proportion of the embryos which were transferred introduces a certain degree of bias to the meta-analysis. While the findings of the Tanaka study are undoubtedly relevant, the inclusion of this study limits the overall external validity of the meta-analysis. It is unknown whether the findings of the Tanaka study are representative of the ROSI technique in a more general sense, particularly when ROSI is implemented in other laboratories. Because the overall results of any meta-analysis are skewed by studies with large numbers of patients, the inclusion of a single study which represents the majority of embryos transferred has

an undeniable impact on the findings of the meta-analysis. In spite of the fact that the more recent studies are larger and report improved outcomes compared to earlier studies, it is important to consider the recent publications in the context of the older publications because this allows for a more thorough understanding of the literature as a whole as it relates to ROSI.

The lack of a formal control group within many studies represents another limitation of this meta-analysis. This impacted the quality scores for the included studies based on the Newcastle–Ottawa scale (Table 1). Comparing ROSI outcomes to embryology and pregnancy outcomes from mature spermatozoa within the same laboratory would allow for a direct comparison of fertilization rate, pregnancy rate, and delivery rate within the same clinical setting. However, the presence of a control group was rarely reported in the included publications.

This systematic review and meta-analysis reports evidence that the laboratory technique of ROSI can be utilized to achieve pregnancies and live births. However, success rates are considerably lower than those using mature spermatozoa. Based on preliminary data from the 2017 National Summary Report for the Society for Assisted Reproductive Technology (SART), the live birth rate for a woman <35 years old undergoing ICSI with autologous oocytes is 40.9% per oocyte retrieval cycle.<sup>33</sup> While not directly equivalent, the resultant delivery rate after ROSI of 4.5% per embryo transferred or 9.4% per couple represents a significantly worsened prognosis compared to more mainstream assisted reproductive technology procedures. With this in mind, one of the most significant limitations of the current study relates to the issue of reproducibility within the included publications. Although pregnancy rates and delivery rates in some studies achieved modest success, the majority of publications actually reported a 0% pregnancy rate and 0% delivery rate. These findings indicate that while, on an average, ROSI has resulted in some births, this was not the norm in most clinical settings. In order for ROSI to demonstrate clinical utility, a uniform level of success across multiple institutions must be achieved. At present, this has not yet been accomplished.

Despite the limitations of this study and the ROSI technique itself, ROSI may remain relevant as a potential treatment modality for men with azoospermia. This challenging patient population is one with limited clinical options, and when faced with the decision between adoption or donor sperm and ROSI, many couples may view ROSI as a reasonable alternative. The possibility of achieving a biological child using round spermatids may be something that patients are willing to consider when no mature spermatozoa are isolated. It must be emphasized that this technique should only be used in the select group of men with azoospermia who decline other options. Furthermore, couples should receive extensive counseling and must be aware that the odds of a successful delivery are greatly diminished and the prognosis is particularly poor. In current practice, ROSI is not being applied clinically in the USA. Based on the years of publication for many of the studies included in this analysis, it appears as though initial interest in this technique decreased when outcomes after ROSI were dismal. In the wake of recent Japanese publications which report more promising results and the reassuring findings regarding developmental outcomes of infants born following ROSI, a revitalized focus on ROSI's potential may be warranted.

If ROSI is to be used clinically, further studies are needed to determine how to optimize the ROSI technique to improve fertilization rates, maximize embryologic development following ROSI, and potentially improve pregnancy outcomes. Interventions such as preimplantation genetic testing (PGT) and the use of freeze-all cycles to achieve embryonic and uterine synchrony are commonly employed

to improve outcomes for infertile patients. These techniques, among others, may also play a role in the future of embryos created via ROSI. Developing an understanding of the frequency of aneuploidy among embryos which result from ROSI is also an important factor in determining the likelihood of success using this technique. Additionally, because many of the older studies evaluated in this meta-analysis were performed prior to the development of a consistent set of criteria by which to identify round spermatids, a useful future study may be to directly compare the outcomes obtained by Tanaka *et al.*<sup>1</sup> to the works performed prior to the Tanaka publications. This would allow for an analysis of how the development of visual identification criteria has dramatically improved success rates with ROSI. In summary, this meta-analysis demonstrates that reported outcomes following ROSI are poor, but if a revitalized focus on technique optimization and further scientific investigation lead to clinical improvements, a select group of azoospermic patients may benefit clinically from ROSI in future.

## AUTHOR CONTRIBUTIONS

BMH carried out the literature search, participated in the formal analysis and selection of relevant studies, and drafted the manuscript. TPK participated in the formal analysis and selection/review of studies, performed the statistical analyses, and generated the figures for the manuscript. AWP was involved with the conceptualization of the project, assisted in the statistical analysis, and helped draft the manuscript. RTS was involved with the conceptualization of the project, reviewed and edited the manuscript to ensure accuracy, and provided input regarding figures and tables. PJC was involved with carrying out the literature search, selecting relevant studies, and reviewing the manuscript. JMH generated the initial concept for the study, was involved with task delegation, assisted with the literature search, reviewed relevant studies for inclusion or exclusion, assisted with drafting the manuscript, and reviewed the final version of the manuscript. All authors read and approved the final manuscript.

## COMPETING INTERESTS

All authors declared no competing interests.

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