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# Does using testicular sperm retrieval rather than ejaculated spermatozoa improve reproductive outcomes in couples with previous ART failure and poor ovarian response? A case-controlled study

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## SUMMARY

The objective of this study was to assess whether testicular-retrieved spermatozoa improve reproductive outcomes compared to fresh ejaculate in women with poor ovarian response and a history of previous ART failure. The study was performed as a retrospective case–control study at a university-based reproductive center in Montreal, Quebec, Canada. Eighteen poor-responder patients were matched 3 : 1 with 54 controls. Poor responders were defined as those with ≤3 oocytes retrieved at oocyte pickup. Cases were identified as poor responders, and only those with previous IVF failure(s) as an indication for testicular-retrieved spermatozoa were included. Controls were age and cycle attempt number matched. All patients were included only once. From January 1, 2009 to December 31, 2015, all patients and controls underwent an IVF cycle using ICSI with either testicular spermatozoa or ejaculated spermatozoa, respectively. Outcomes included live birth rate, pregnancy rate, miscarriage rate, oocyte number, and embryo transfer (ET) day. The results showed live birth rates, pregnancy rates, and miscarriage rates were similar. There were fewer day 2 ETs (8.5% vs. 48.6%, $p = 0.01$) and more day 5 blastocyst transfers (25.0% vs. 5.4%, $p = 0.05$) in the testicular sperm retrieval group compared to controls and thus an overall suggestion of better embryo quality in the testicular sperm group. Overall, however, the use of testicular sperm retrieval appears to add little. Women with poor ovarian response typically have a poor prognosis with respect to live birth rates, and this is further supported in this study. The suggestion of better embryo quality in the testicular-retrieved sperm group would need to be further assessed in a larger multicentered study.

## INTRODUCTION

Traditionally, testicular-retrieved spermatozoa have been used in cases of azoospermia whether obstructive or non-obstructive in etiology (Stahl *et al.*, 2011). More recently, there has been some evidence that using testicular spermatozoa may result in improved pregnancy rates in patients with poor sperm parameters or previous ART failures (Ben-Ami *et al.*, 2013; Esteves *et al.*, 2015).

However, testicular sperm retrieval does carry risk for the male patient—it is an invasive surgical procedure with potential complications including but not limited to bleeding, infection, and irreversible testicular tissue damage (Gnoth *et al.*, 2014). We therefore need to be cautious when recommending this procedure to patients to ensure the potential benefits outweigh the risks.

Typically, women with poor ovarian response are excluded from studies looking at testicular spermatozoa and no reports in the literature were identified examining the use of testicular in couples with repeated IVF failures and with diminished female ovarian response. One study found abnormal sperm DNA fragmentation had a negative effect on reproductive outcomes in women with reduced ovarian reserve; however, this examined only ejaculated spermatozoa (Jin *et al.*, 2015).

Couples using testicular spermatozoa for repeated IVF failures with ejaculated spermatozoa in female partners who were poor responders pose an interesting challenge for the fertility provider as both female and male factors may play a role in their previous IVF failures. A decision about employing the use of surgically retrieved spermatozoa therefore needs to be considered in the

context of the poor ovarian response of the female partner as well as the risks associated with testicular sperm retrieval and the uncertainty that testicular-retrieved spermatozoa will provide any additional benefit to the use of fresh ejaculated spermatozoa.

The objective of this study was to determine whether the use of testicular-retrieved spermatozoa improves reproductive outcomes compared to fresh ejaculated spermatozoa in women with diminished ovarian response and a history of previous ART failure.

## MATERIALS AND METHODS

The electronic medical record database at a university-based reproductive center was used for data collection for this case–control study. All ART cycles using testicular spermatozoa for fertilization were identified from January 1, 2009 to December 31, 2015. Eight hundred and two cycles were found, and charts were reviewed. Poor-responder patients, as per the Bologna criteria (Ferraretti *et al.*, 2011), were defined as those with ≤3 oocytes retrieved at oocyte pickup. One hundred and fifteen patients met this cutoff. These cases were then analyzed based on indication for surgical sperm retrieval: obstructive azoospermia, non-obstructive azoospermia, and repeated ART failures. There were 18 patients who underwent 18 cycles that were identified as using testicular spermatozoa for previous IVF failure(s) with ejaculated spermatozoa. Female age-matched (within 12 months) and cycle attempt number-matched controls using ICSI with ejaculated spermatozoa for fertilization were identified for all cases in a 3 : 1 ratio. There was one 37-year-old patient on her 7th cycle and one 29-year-old patient undergoing her 3rd cycle, each of whom had only one age- and cycle-matched control available, and the two additional matched controls were age matched but undergoing their 6th cycles and 2nd cycles, respectively. All controls were also poor-responder patients using the same Bologna criteria (Ferraretti *et al.*, 2011).

All patients were included only once and, in the cases using testicular spermatozoa, the cycle reviewed was the patient's first cycle using testicular-retrieved spermatozoa.

Of 18 male patients, 16 underwent testicular sperm aspiration (TESA) using percutaneous aspiration of testicular tissue with a 14- to 18-gauge angiocatheter connected to a 10-mL syringe under local anesthesia. The remaining two patients underwent microdissection testicular sperm extraction (mTESE) performed as previously described in the literature (Schlegel, 1999). There was only one patient (who underwent mTESE) in which spermatozoa was not found. A variety of ovarian stimulation protocols were used, with the majority being the antagonist protocol, the microdose flare protocol, and the long agonist protocol. A total of four patients used either the midluteal patch protocol or a natural IVF cycle in both cases in controls.

Outcomes are live birth rate (LBR), number of oocytes retrieved, number of MII oocytes, number of 2PN embryos obtained, fertilization rate (number of 2PN embryos/number of MII oocytes), number of patients with no embryo transfer (ET) performed, number of patients with a day 2 ET, a day 3 ET, a day 5 ET, pregnancy rate (defined as positive quantitative bHCG per oocyte retrieval), and miscarriage rate.

Criteria for timing of embryo transfer are based on patient age and cycle number (i.e., first cycle vs. subsequent cycles). In patients ≤36 years old undergoing their first cycle, embryos are cultured to the blastocyst stage if there are two or more good-quality, 8-cell day 3 embryos. If not, a day 3 embryo transfer is performed. In subsequent cycles for patients ≤36 years old, if a blastocyst was obtained in the first cycle, the embryos are cultured to blastocyst. Otherwise, if no blastocysts were obtained, consideration for a day 3 embryo transfer is made in patients who had no blastocysts in their first cycle; however, if on day 3, the embryos appear to be of good quality, culturing to the blastocyst stage may be considered, following discussion. In patients >36 years old undergoing their first cycle, embryos are cultured to the blastocyst stage if there are four or more good-quality, 8-cell day 3 embryos. If not, a day 3 embryo transfer is performed. In subsequent cycles for patients >36 years old, if two or more blastocysts were obtained in the first cycle, the embryos are cultured to blastocyst (unless quality of previously obtained blastocysts was very poor).

Statistical analysis was performed using STATSDIRECT statistical software (version 3.0; StatsDirect Ltd., Cheshire, UK). Baseline characteristics of the two groups were compared using unpaired *t*-tests and Mann–Whitney *U*-tests. Outcomes were analyzed using unpaired *t*-tests and chi-squared tests.

Institutional ethics approval was obtained for retrospective chart review and analysis for the purposes of this study (IRB #15-388-MUHC). This study was conducted in accordance with the Declaration of Helsinki for Medical Research involving human subjects.

## RESULTS

Baseline characteristics including female and male age, attempt number, and male sperm parameters on ejaculate were similar between the two groups (see Table 1).

In the testicular sperm group, the majority of patients used an antagonist protocol [33.3% ($n$ = 6)] and microdose flare protocol [33.3% ($n$ = 6)], followed by the long agonist protocol, natural cycle IVF, and midluteal patch protocols for ovarian stimulation [16.7% ($n$ = 3); 11.1% ($n$ = 2); 5.6% ($n$ = 1), respectively]. In the control group, the majority of patients used a microdose flare protocol [46.3% ($n$ = 25)], followed by antagonist protocol, long agonist, and midluteal patch protocol [40.7% ($n$ = 22); 11.1% ($n$ = 6); 1.9% ($n$ = 1), respectively]. None of the patients in the control group used a natural IVF protocol for ovarian stimulation. TESA was used for surgical sperm retrieval in 88.9% and mTESE done in 11.1% of cases. Results of reproductive outcomes are shown in Table 2.

**Table 1** Baseline characteristics (mean ± standard deviation is shown when data are normally distributed, and median ± IQR is shown when data are not normally distributed)

| | Testicular sperm group ($n$ = 18) | Control group ($n$ = 54) | $p$-value |
|---|---|---|---|
| Mean female age | 38.0 (±3.79) | 38.1 (±3.67) | 0.95 |
| Mean male age | 42.9 (±5.59) | 40.6 (±5.83) | 0.14 |
| Mean IVF attempt number | 3.8 (±1.42) | 3.8 (±1.36) | 0.84 |
| Median basal semen volume | 2.5 (±1.5) | 2.1 (±2) | 0.89 |
| Mean sperm concentration | 29.6 (±47.60) | 48.9 (±58.11) | 0.21 |
| Median total sperm motility | 25.5% (±0.41) | 47% (±0.56) | 0.30 |
| Median normal morphology by Kruger strict criteria | 3% (±1.3) | 4% (±0.12) | 1.00 |

**Table 2** Reproductive outcomes (mean ± standard deviation is shown when data are normally distributed, and percentages are shown when two independent proportions are examined)

| | Testicular sperm group ($n$ = 18) | Control group ($n$ = 54) | $p$-value |
|---|---|---|---|
| Mean oocytes retrieved | 2.2 (±0.86) | 2.0 (±0.75) | 0.54 |
| Mean MII | 1.8 (±0.79) | 1.6 (±0.74) | 0.21 |
| Mean 2PN | 1.2 (±0.99) | 1.0 (0.80) | 0.58 |
| Fertilization rate | 54.7% | 50.9% | 0.71 |
| No ET | 33.3% | 31.5% | 0.88 |
| Day 2 ET | 8.3% | 48.6% | 0.01* |
| Day 3 ET | 66.7% | 45.9% | 0.21 |
| Day 5 ET | 25.0% | 5.4% | 0.05* |
| Pregnancy rate | 22.2% | 16.7% | 0.60 |
| Miscarriage rate | 25.0% | 33.3% | 0.76 |
| LBR | 16.7% | 11.1% | 0.54 |

*Statistical significance.

These results show fewer day 2 ETs and more blastocyst transfers in the testicular sperm retrieval group compared to controls, thus a suggestion of better embryo quality in the testicular sperm group. However, the other reproductive outcomes between the two groups were similar.

## DISCUSSION

To our knowledge, this is the first study to examine the use of testicular-retrieved spermatozoa in couples with repeated IVF failure when diminished ovarian response is also present. There has been a growing interest in the recent literature to favor the use of testicular spermatozoa over ejaculated spermatozoa in couples experiencing previous failure with ICSI, particularly in the context of subnormal semen quality (Ben-Ami *et al.*, 2013; Esteves *et al.*, 2015) and/or increased sperm DNA fragmentation (Esteves *et al.*, 2015). It has been proposed that spermatozoa retrieved from the testis have less oxidative damage, rendering better sperm chromatin structure integrity that can potentially lead to superior outcomes with ICSI compared to the use of fresh ejaculate (Abhyankar *et al.*, 2016). Studies suggesting the use of testicular spermatozoa for repeated IVF failures are limited in the literature and some show conflicting results, although most do favor the use of testicular spermatozoa over fresh ejaculate (Ben-Ami *et al.*, 2013; Negri *et al.*, 2013; Alrabeeah *et al.*, 2014). Combining testicular spermatozoa with a poor responder female partner in cases of recurrent IVF failure has not yet been studied specifically.

We intentionally chose not to compare the outcomes of the testicular sperm group with the outcomes of their previous failed cycles as we did not want to artificially increase the potential benefit of testicular sperm extraction. Instead, we chose to use age-matched and cycle-matched controls from the same center within the same study period to allow a fairer comparison of the impact of testicular sperm retrieval on reproductive outcomes.

In this study, the results are similar overall between the testicular-retrieved sperm group and case-matched controls, though the numbers of cases of controls are too small to conclude this with certainty. Both pregnancy rates and LBRs are low in this poor prognosis population whether ejaculated or testicular-retrieved spermatozoa were used. However, there is a suggestion of better embryo quality (as determined by more day 5 ET and less day 2 ET) in the testicular sperm population. Although the LBR rate is higher in the testicular sperm group (16.7%) compared with the ejaculated sperm group (11.1%), this does not achieve statistical significance in large part because of a small number of cases. Assuming an overall pregnancy rate in poor responders of 11% with an odds ratio of 1.5, sample size calculations show that 644 cases of repeated IVF failure associated with poor ovarian response using testicular-retrieved spermatozoa and 1932 controls (for 3 : 1 matching) would need to be studied to provide us with statistical significance of 5% and 80% power. It is unlikely that this number would be achieved in a single center.

The major weakness of the study is the small sample size. In addition, although the cases were matched with controls based on age and cycle attempt number, there may be other confounding variables present, such as underlying female diagnosis and previous parity, which were not included in the analysis. In addition, cases were matched only with respect to female age, and although there was no statistically significant difference in male age between cases and controls, matching was not specifically performed for male age between groups. Due to the overall low LBRs and pregnancy rates in this population, larger numbers are needed to assess whether a significant difference exists between testicular and ejaculated spermatozoa. Generally, it is difficult to recruit large numbers of patients fitting these criteria given that they represent only a small subset of patients undergoing infertility treatment and, given their relatively poor prognosis, they may be less likely to undergo multiple IVF cycles. As mentioned, a larger sample size would be needed to show a statistically significant, and clinically significant, effect on important reproductive outcomes.

Although embryo quality may be better, LBRs are still very low. This is important when counseling patients in these challenging clinical situations: poor ovarian response, male factor infertility, and previous ART failures. Ensuring realistic expectations of an overall lower LBR to these patients is paramount in order for them to fully understand their prognosis. Although there were no complications from testicular sperm retrieval in our study, there are potential significant risks (as discussed) that are associated with surgical sperm retrieval and reasonable expectations of having a healthy take-home baby need to be discussed in detail with the couple. Even with the potential modest results of improved embryo quality with the use of testicular spermatozoa, the overall pregnancy rates are low and more studies are needed to confirm, or refute, this possible effect.

## CONCLUSIONS

Patients with poor ovarian response pose a challenge for clinicians as there is little evidence that, when using autologous oocytes, any specific intervention (including use of testicular spermatozoa) significantly improves pregnancy and live birth rates. The results of this study may be helpful to couples when counseling them about prognosis and associated potential risks of surgical sperm retrieval in order to provide them with realistic expectations when faced also with poor ovarian reserve. More research in a poor-responder female patient population using testicular spermatozoa for fertilization is needed.

This is the first study to evaluate the use of testicular spermatozoa in couples with repeated ART failure with poor ovarian response. LBRs in this population are low and likely multifactorial and driven particularly because of poor oocyte

 

quality. Testicular spermatozoa could improve LBR, but a much larger prospective randomized study ($n > 1000$) would be needed to conclusively answer this question, as even small improvements in the LBR in this population are highly appreciated. Even within our own institution where more than 800 IVF cycles using testicular spermatozoa were reviewed, only 18 couples fit the criteria of having poor ovarian response with repeated IVF failure(s). Our aim is to have this study that acts as a starting point for a potential larger multicentered study, which may be needed to better answer this challenging clinical question.

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## DISCLOSURES

The authors have no potential conflicts to disclose.

## AUTHORS' CONTRIBUTIONS

(i) Gilman AR contributed to research design, acquisition and interpretation of data; drafted the manuscript and revised it critically; and approved the submitted and final versions of the manuscript. (ii) Younes G contributed to research design and acquisition of data, revised the manuscript critically, and approved the submitted and final versions of the manuscript. (iii) Tannus S contributed to research design, revised the manuscript critically, and approved the submitted and final versions of the manuscript. (iv) Son WY contributed to research design and acquisition of data, revised the manuscript critically, and approved the submitted and final versions of the manuscript. (v) Chan P contributed to research design and acquisition of data, revised the manuscript critically, and approved the submitted and final versions of the manuscript. (vi) Buckett W contributed to research design, acquisition of data, analysis and interpretation of data; revised the manuscript critically, and approved the submitted and final versions of the manuscript.

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