Knowledge gaps in male infertility: a reproductive endocrinology and infertility perspective

Scott J. Morin[1,2], Richard T. Scott[1,2]

[1]IVI RMA New Jersey, Basking Ridge, NJ, USA; [2]Sidney Kimmel Medical College, Thomas Jefferson University, Philadelphia, PA, USA

Contributions: (I) Conception and design: None; (II) Administrative support: All authors; (III) Provision of study materials or patients: None; (IV) Collection and assembly of data: None; (V) Data analysis and interpretation: All authors; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Scott J. Morin, MD. IVI RMA Global New Jersey, Basking Ridge, NJ, USA; Sidney Kimmel Medical College, Thomas Jefferson University, Philadelphia, PA, USA. Email: smorin@rmanj.com.

Abstract: Reproductive research has moved forward at a remarkable pace. Some of these advances are the result of a separation between male and female specialties, allowing focused study in specific areas of the field. However, the different training programs between male and female fertility specialists has created an environment in which some discoveries are not put in the greater context of clinical care. At times, interventions have been measured against surrogate markers of outcome that may not impact the most meaningful outcome for patients—the delivery of a healthy neonate. For example, medical and surgical interventions that use changes in semen parameters may have a limited impact on the likelihood of achieving a live birth due to the limitations inherent in the semen analysis for predicting outcomes. Other commonly used tests, such as sperm DNA fragmentation assays provide promising biological plausibility to account for subfertility of some male partners. However, until well defined thresholds for predicting outcomes in different treatment scenarios are available, changes in sperm DNA fragmentation testing is not an adequate outcome for measuring the utility of interventions. The biggest limitation for these tests remains their analysis of bulk semen. Tests allowing interrogation of the reproductive competence of a given sperm, while allowing that sperm to be used in assisted reproductive technology procedures remain elusive. Progress toward reaching this end (whether by hyaluronic acid binding, IMSI, or Ramen spectroscopy) is underway, but much remains to be learned. Achieving testing and capture of individual sperm would better facilitate studies that measure the most meaningful outcome for patients and providers—the delivery of a healthy baby.

Keywords: Reproductive endocrinology and infertility (REI); infertility; assisted reproductive technologies (ART)

Introduction

The practice of fertility specialists is unique in that two patients are engaged in treatment at a time. Given the training paradigms in place, male and female care is often fragmented between reproductive urologists and reproductive endocrinologists (RE). While greater degrees of subspecialization have allowed more focused study in reproductive medicine and likely have accelerated discovery in certain areas of the field, this arrangement has also undoubtedly led to inefficiencies in studying the reproductive process. Indeed, RE approaches couples from a female-dominant mindset. Similarly, urologists often have limited experience with the subsequent processes in assisted reproduction (such as embryo culture and transfer).

This natural gap between male and female focus created by our training system has generated challenges in the fertility literature. Rather than studying the process as a whole, many studies (in both male and female patients) have relied on surrogate outcome markers that are more familiar

S284

Morin and Scott. Knowledge gaps in male infertility: an REI perspective

to the scope of care provided by the clinician scientists organizing the study. For instance, while treatments that improve semen parameters or fertilization efficiency may indeed indicate some improvement in the reproductive function of men, the current literature often falls short of assessing whether a meaningful increase occurred in the likelihood of achieving the ultimate goal—the delivery of a healthy baby.

The field of reproductive medicine moves so quickly that endpoints that were previously considered significant are quickly made irrelevant by advancing knowledge. As a result, there is a great need to recalibrate the goal of each diagnostic test or treatment approach to ensure that it maintains relevance in the current era of treatment. For instance, a treatment that improves the number of day three embryos available for transfer appears a major boon in fertility treatment. However, this outcome may be less important in an era of blastocyst culture, preimplantation genetic testing, and single embryo transfer. The new focus in assisted reproduction should be on improving the efficiency of the treatment process to optimize the gametes used in treatment so that better selection opportunities are available. In other words, the major questions in reproductive medicine are no longer based solely on surrogate endpoints such as improving sperm yield, but rather on markers that truly maximize the chance of identifying the one embryo that is most likely to lead to the live birth of a healthy, singleton neonate.

This review will discuss the many major achievements in male fertility research. However, it will also attempt to identify areas where knowledge gaps persist. This commentary does not intend to highlight shortcomings in male reproductive research, but rather to serve as a guide to the mindset of the RE with respect to male treatment options. It also provides one view on how research findings should be regularly reviewed to ensure that they continue to remain relevant as treatment paradigms shift over time. Hopefully, this approach will help foster an era of collaboration that will benefit our patients and help them achieve their goals in building a healthy family.

Evolution of fertility treatment paradigms and implications for current research

Reproductive medicine training has experienced major shifts in focus since the first programs were introduced in the middle of the 20th century. Initially, the majority of research and education programs in the endocrinology

of reproduction emerged within departments of internal medicine (1). At this time, disorders of the hypothalamic-pituitary-gonadal axes between men and women were often treated by the same clinicians. When treatment options were limited, there was less separation in both study and clinical care between male and female patients.

In the 1970s, the subspecialty of reproductive endocrinology and infertility (REI) was officially recognized by the American Board of Obstetrics and Gynecology, and fellowship programs designed for gynecologists were introduced (2). The early days of REI training benefited substantially from a number of highly productive researchers and educators from within obstetrics and gynecology departments, such Howard and Georgeanna Seeger Jones at Johns Hopkins (and later Eastern Virginia Medical School), Samuel Yen and Robert Jaffe at the University of California, and Leon Speroff at Yale (and later Oregon Health Sciences University). These luminaries propelled the field of REI down a path that was tilted toward a female focus, given their training in obstetrics and gynecology.

Due to its emergence from within the field of internal medicine, the early days of REI training maintained a focus on the endocrinology of reproduction. Much time was spent on designing and testing hormonal assays and describing the physiologic basis for disorders such as precocious puberty, primary amenorrhea, or ambiguous genitalia. This training was supplemented with advanced surgical training in microsurgery and the development of laparoscopy. RE was thus counted on to understand the physiologic basis for endocrinopathies related to reproduction and to perform surgical procedures on female patients, such as tubal reanastomosis or fimbrioplasty to optimize a patient's chances at fertility (3).

The advent of in vitro fertilization (IVF) created a sweeping shift in the focus of reproductive medicine (4). The focus of research and clinical care moved from hormonal assays and surgery to understanding gamete biology and cell culture. This major advancement also helped to bring the treatment of male and female patients closer together again as both male and female gametes were closely studied to better understand the physiologic requirements of fertilization and preimplantation embryo development.

However, physicians were rarely responsible for the most significant advances in embryo culture techniques. Indeed, the field of human IVF benefited substantially from classically trained embryologists from the animal science world (5). While REs and male reproductive

specialists were closely involved with many of the treatment paradigms that allowed for improvement in clinical IVF success, such as gonadotropin stimulation protocols, their clinical training rarely translated into major contributions in clinical embryology, such as extended embryo culture or cryobiology. These advances came from the basic sciences. Thus, the clinical world gradually became separated from the assisted reproductive technology (ART) laboratory.

As ART evolved, robust research programs in male reproduction also emerged from within urology departments with a focus on improving treatment options for male infertility. Up to this point, both male and female partners were primarily treated by one physician. This was primarily a RE. Things began to change upon the initial reports of successful surgical sperm retrieval. This advancement combined with options for micromanipulation revolutionized treatment for couples with limited options previously (6,7). However, the surgical nature of these high profile advances in male infertility meant that male infertility treatment became entrenched as the purview of urologists. Training programs grew to promote research in male reproductive and sexual function and to train specialists in microsurgical techniques for sperm retrieval.

With greater subspecialization of the study of reproductive physiology and practice of fertility medicine between female specialists (largely REs), laboratorians (embryologists and andrologists), and male specialists (largely urologists) comes a greater risk of losing a comprehensive view of the couple under treatment. For example, the natural inclination of REs is to focus solely on optimizing the number of oocytes retrieved following ovarian stimulation—rather than determining whether those oocytes exhibit optimal developmental potential. Similarly, much of the current male fertility literature examines measurable outcomes, such as rate of sperm retrieval in a surgical case.

This current reality has led to some debate regarding whether combined training programs in male and female infertility, whereby one clinician assumes the majority of care for a couple seeking pregnancy are optimal (3,8). While this would be a drastic change in the training paradigm at play in the United States and abroad (9), a simpler solution would be an increase in collaboration between REs and male reproductive specialists so that the most relevant endpoints are being examined. The most obvious setting for this collaboration would be within an academic medical center with access to both male and female reproductive specialists. Much progress has already been made in optimizing care through collaboration between the REI and Urology departments in many centers. Furthermore, some

of the largest private fertility clinics have recently hired full time male specialists. This approach allows all care of a given couple to be done in one setting and increases research collaboration between REIs and urologists.

Research areas for future focus

Semen analysis

The semen analysis is the most widely utilized tool to evaluate male fertility. It provides information on the function of accessory sex glands and the efficiency of spermatogenesis. However, there has been vigorous debate regarding the utility of the standard semen analysis in helping clinicians counsel patients regarding their likelihood of achieving pregnancy. In truth, semen characteristics that discriminate between fertile and infertile men are poorly defined. Indeed, abnormalities in individual parameters, such as morphology, have not been consistently found to correlate with pregnancy rates (10). For these reasons and others, the semen analysis remains limited as a counseling tool.

First, the normal variability in parameters between ejaculates makes projecting the fertility of a man exceedingly difficult based on one or two specimens (11). One elegant study demonstrated within-subject variability in sperm concentration of 26.8% over a 10-week period (12). This variability is the rationale behind the World Health Organization's recommendation that multiple semen analyses be performed to truly gauge a man's reproductive function (13). This is particularly important in light of the fact that sperm concentration and motility tend to be improved in the second test in men with a previous abnormal specimen (14). These patients would typically be those recruited to research studies given their apparent suboptimal reproductive function. This variability must be kept in mind when studies utilize improvements in semen parameters as the primary outcome.

A second limitation of using the semen analysis in the research setting is evidence of high intra- and inter-observer variability in reporting parameters from the same specimen (15). One study described inter-laboratory coefficients of variation of 70% for sperm morphology, 40% for vitality, 34% for sperm concentration, and 20% for total motile sperm count (16). This level of variability reflects the technical complexity of performing semen analysis, as discrepancies are seen even in laboratories that comply with rigorous quality control programs (17). Given the variability between labs, regardless of quality control accreditation,

tau.amegroups.com   *Transl Androl Urol* 2018;7(Suppl 3):S283-S291

S286

Morin and Scott. Knowledge gaps in male infertility: an REI perspective

differences in semen analysis results between facilities may not be interpretable or reflective of a clinical difference.

Furthermore, morphology testing has been demonstrated to provide little to no prognostic value. Despite a significant focus in andrology labs in demonstrating technician proficiency in morphologic testing, this data point is not predictive of pregnancy rates following IUI (18) or IVF (10). Thus, even when an adequate quality control mechanism produces consistent results within and between technologists, a test that does not prognosticate pregnancy has very limited value.

Perhaps the greatest limitation of the semen analysis in the context of ART is its limited capacity to analyze sperm function. Indeed, as many as 30% of men in infertile couples have no abnormalities on semen analysis (19). Many of these men end up in IVF and still demonstrate either poor fertilization or limited embryo development, suggesting compromised sperm function despite normal parameters. This raises the important point that a comprehensive male evaluation (in addition to a semen analysis) is essential. However, many adjunct tests of sperm function have also been developed in an effort to interrogate sperm cell membrane integrity (hypo-osmotic swelling test), acrosome function (ARIC), zona binding (hemizona assay), interaction with the oolemma (hamster zona-free ovum test) or autoimmunity (sperm antibody testing) (20). However, the predictive value of each test is either extremely limited or poorly defined (21). Sperm antibodies, for instance, do not correlate well with spontaneous, IVF, or ICSI pregnancy rates (22,23). Thus, the proper place of adjunctive sperm function testing in evaluating infertile couples is still not clear.

These significant challenges suggest that studies designed to measure semen parameters alone before and after an intervention provide limited information on the most important issue to both patient and clinician—the delivery of a healthy neonate. For example, studies on hormonal manipulation of the male hypothalamic-pituitary-gonadal axis in oligozoospermic males should seek not to only improve semen analysis parameters, but also to demonstrate improved delivery rates (24). The salient, ultimate research question is whether or not these patients may be able to avoid more invasive, expensive treatment (IVF) by achieving adequate pregnancy rates as a result of their improved spermatogenic function. In contrast, if a couple is utilizing IVF and ICSI for any other reason (when only one sperm is needed per oocyte), studies should not only evaluate for improvements in semen parameters, but also determine whether these improvements improve delivery rates. In both situations, a comprehensive

view of the treatment pathway planned for each couple is helpful in defining the research questions needed.

Sperm DNA fragmentation assays

Assays for sperm chromatin integrity are the most widely utilized and best studied adjunctive diagnostics in male infertility. A high level of sperm DNA damage is more common in infertile men than fertile men. Furthermore, the ASRM Practice Committee considers the available evidence strong enough to report that sperm DNA fragmentation (SDF) is associated with recurrent pregnancy loss and may contribute to additional reproductive dysfunction (25). However, many questions regarding DNA fragmentation testing remain.

The greatest limitation in the current literature is the lack of uniformity of thresholds for labeling DNA fragmentation as abnormal. Different studies using the same assay have labeled samples with >7%, >15%, or >30% as having "elevated" levels of fragmentation (26-28). This inconsistency has sprung from the lack of rigor in the foundational papers in statistically defining threshold values (29). Studies have also been too small and have failed to adequately control for female factors to provide reliable estimates of sensitivity and specificity for delivery at each potential threshold of abnormality (30). Furthermore, there are multiple different commercially available assays (31) but little information is available regarding whether sperm DNA fragmentation levels correlate between the different assays. Assessing reproducibility and determining clear thresholds for abnormality are difficult tasks for any biomarker of infertility, but these are essential in order to determine the ideal candidate for which a test can convey new and clinically useful information; sperm DNA fragmentation has not yet achieved this goal.

In fact, while current available evidence suggests that high levels of DNA fragmentation are associated with a reduction in natural fertility, knowledge regarding the manner in which these test results impact the likelihood of success in various types of treatment is much more limited. Studies analyzing the impact of SDF on the likelihood of success in intrauterine insemination (IUI) cycles are conflicting. One prospective cohort study demonstrated that in controlled ovarian hyperstimulation (COH) cycles coupled with IUI, the SDF level correlated with pregnancy rates after controlling for a number of female prognostic factors (32). However, another study demonstrated no correlation between SDF levels and pregnancy rates in 100 analyzed cycles (33).

There is better evidence that high SDF decreases IVF success (median PPV calculated at various thresholds for IVF failure of 77%) (34), when conventional insemination is used. However, most studies included in this meta-analysis were small and don't include control groups so the value of this information is less certain. Similar results have been reported in studies utilizing ICSI (35). However, a recent meta-analysis suggested that utilization of ICSI overcomes any reduction in pregnancy rates seen with high levels of SDF (at various thresholds) (34).

Thus, while the available data provide evidence of a biological link between levels of SDF and reduced fertility, both female and male specialists currently find themselves confronting the question of when to order SDF tests and the counseling dilemmas of how to interpret the results. Further work is needed.

Varicocele

Large descriptive studies demonstrate that varicoceles are significantly more common in men presenting for infertility evaluation compared to those with proven fertility (36). However, it is has been difficult to untangle how varicoceles impact a couple's likelihood of achieving pregnancy. Men with varicoceles are at greater risk for abnormal semen parameters (37). Furthermore, there is consensus that varicocelectomy treatment of a varicocele is indicated if the male partner has abnormal semen parameters and the female partner has a normal work-up (38), as treatment of a varicocele in these couples, specifically young couples with the luxury of time, appears to improve the likelihood of achieving spontaneous pregnancy (39,40).

However, there is debate on whether treatment of an asymptomatic varicocele in a couple destined for IVF/ICSI has any influence on the likelihood of success. Most studies that have evaluated the success of varicocele treatment have reported on improvement in semen parameters alone. One study reported a mean improvement in sperm concentration of 12 million/mL and an 11% increase in motility (41). However, while improvement in semen parameters suggests return of normal spermatogenic function, the semen analysis has many limitations that are documented above. Furthermore, semen parameters have been shown to have very little impact on the success of IVF/ICSI (42). The ASRM Practice Committee has suggested that varicocelectomy is typically not indicated when IVF/ICSI is already planned for female indications (38). However, a recent meta-analysis provided robust evidence that

varicocelectomy prior to IVF improved live birth rates when oligozoospermia is present in men with varicoceles (43). Furthermore, in men with non-obstructive azoospermia, varicocelectomy has been demonstrated to increase the likelihood sperm returning to the ejaculate following repair, though reported success rates vary widely (44,45). Thus, a varicocelectomy may allow IVF or ICSI without the need for surgical sperm retrieval. However, many men still require surgical sperm retrieval even after varicocelectomy (46), raising the question of varicocelectomy's clinical- and cost-effectiveness for these patients. The additional cost of varicocele repair may be at least partially obviated by the possibility of sufficient sperm returning to the ejaculate to justify attempts at IUI in some men (47). However, more data are certainly needed.

Thus, more information is needed regarding specific male patient characteristics that are associated with significant improvement following surgical treatment of a varicocele. This information is important for counseling couples regarding the timing of varicocelectomy and subsequent reproductive techniques, as it typically takes 3 to 6 months before improvement in spermatogenic function can be expected following varicocelectomy (48). Future research to determine this predictive information would be helpful as not all patients have the luxury of time, depending on the results of the female work-up.

Sperm selection for IVF/ICSI

Perhaps the biggest limitation in current laboratory assessments of male fertility is that semen is primarily evaluated in bulk. No test is available currently that can evaluate a single sperm and isolate it for use according to the results of a given analysis. Thus, even when men have evidence of spermatogenic dysfunction (either in a semen analysis, DNA fragmentation assay, or sperm functional assays), there is no opportunity to identify those individual sperm with greatest reproductive competence in a given specimen. A diagnostic test that could provide information about the functionality of a particular sperm without rendering it unusable would be highly valuable to clinicians and patients.

At present, the only assays that attempt to isolate the most reproductively competent sperm after standard washing techniques have limited clinical data to support their use. Intracytoplasmic morphologically selected sperm injection (IMSI) allows morphology evaluation at high powered magnification (at least 6,000×) to help laboratorians

S288

Morin and Scott. Knowledge gaps in male infertility: an REI perspective

detect subtle abnormalities that would otherwise be missed at standard magnification levels (49). While initial results suggested improvement in clinical pregnancy rates, the methodological rigor of the trials is limited. Four of the nine prospective trials that evaluated clinical pregnancy rate as an outcome featured higher oocyte yields and higher numbers of embryos transferred in the IMSI group compared to the ICSI group, suggesting that the IMSI groups included patients with a better prognosis (50). The only study with live birth as the primary outcome did not demonstrate a difference between IMSI and ICSI (51). Furthermore, some have called into question whether a segment of the morphologic characteristics that would be labeled as abnormal in IMSI may actually represent normal physiologic process (such as vacuole presence) (52).

The next best-studied technique for sperm selection is the hyaluronic acid (HA) binding assay. This test is based on the premise that membrane alterations that occur during normal spermiogenesis result in the appearance of HA binding sites (53). Early reports suggested that HA-bound sperm featured lower rates of both aneuploidy and markers associated with apoptosis (54). A subsequent clinical trial utilizing ICSI reported improvements in implantation rate among embryos derived from oocytes injected with HA bound sperm, though it is notable that the implantation rates in this study were low overall (10.3% control *vs.* 17.1% study group, P<0.05) (55). With such low implantation rates, it is difficult to know if this improvement would be replicated in contemporary ART practices, which typically have higher implantation rates than those in this study. In addition, the largest prospective trial on HA binding sperm selection for ICSI was cancelled prior to reaching the planned sample size due to funding reasons; however, the available data demonstrated a trend toward improvements in clinical pregnancy rate (50.8% *vs.* 37.9%, P>0.05) and a significant decrease in pregnancy loss (3.3% *vs.* 15.1%, P=0.02) (56). However, once again, the practice patterns in this study still raise questions regarding the applicability to modern practice. Embryos were transferred on days 2, 3, and 5 in this study and as many as 7 blastocysts were transferred in one transfer cycle in one patient. In current practice, most IVF clinics perform predominantly blastocyst transfers, with the occasional day 3 transfer. Furthermore, most clinics will not transfer more than two embryos in any cycle. However, the data regarding HA-binding sperm selection are sufficiently encouraging that further study is certainly warranted.

Advanced sperm diagnostics as a supplement to semen analysis

Given that the semen analysis is extremely limited in its ability to classify men as either fertile or infertile and treatments such as endocrine manipulation or surgical correction of varicocele have only demonstrated inconsistent improvement in meaningful outcomes, new efforts at describing the mechanisms underlying idiopathic male infertility may require more powerful tools to uncover the mechanisms at play. One promising new technique is the evaluation of sperm epigenetics. A recent elegant study performed genome-wide sperm DNA methylation analysis and identified the top 100 most differentially methylated CpG sites in 127 samples. Using a machine learning approach, the authors were able predict the fertility status of the men from which samples were derived with a positive predictive value of 99% (57). More sensitive tools, such as this, may prove more valuable that current techniques in providing data to use when counseling men about their fertility status.

Like most other sperm assays, epigenetic profiles are performed on bulk semen and thus employ averages among millions of sperm to create a picture of the overall methylation pattern in a given ejaculate. However, there is evidence that methylation levels differ between individual sperm in the same ejaculate (58). Thus, the ability to isolate individual sperm with the desired molecular characteristics would prove extremely valuable for the predictive value of each diagnostic assay and would allow selection of sperm with greater reproductive competence.

Sperm sorting

All current diagnostics that rely on pooling multiple sperm from an ejaculate to provide a profile of the reproductive competence of a given patient would be made significantly more valuable if techniques for sperm sorting were available. It appears likely that spermatogonial stem cells are differentially impacted by pathologies that limit male fertility (59). The selfish spermatogonial stem cell theory suggests that the volume of *de novo* mutations vary among sperm. Epigenetic modifications also vary within a single ejaculate. Therefore, even subfertile men who produce a greater proportion of compromised sperm likely also produce many reproductively competent sperm. If these could be isolated and used clinically, it is very likely that normal embryogenesis and improved pregnancy outcomes

could occur in IVF cycles with or without ICSI.

Given the exciting possibilities, developing technologies for sperm separation is an active area of research. There are a number of microfluidics devices that have been reported to sort sperm according to various characteristics. Some of these technologies rely on the physiology of sperm movement in the female reproductive tract: such as chemoattractant or thermotaxis-driven microfluidics (60,61). Other devices attempt to interrogate the molecular characteristics of single sperm without destroying them (62,63)—one particularly interesting technology is the utilization of Raman spectroscopy to evaluate the molecular characteristics of sperm (64).

While further research is needed, combination of Raman spectroscopy with microfluidics devices could permit temporary trapping of individual sperm to allow for molecular analysis and then shunting of favorable sperm to different channels for clinical use. Clinical application likely is still far off, but microfluidics may allow advanced diagnostics to be used in concert with sperm selection. Microfluidics may be especially useful in cases where sperm sorting is necessary (and tedious), such as searching following micro-TESE. Development and validation of a useful tool such as this would require collaboration between not only urologists and REs, but also andrologists, molecular biologists, and engineers. Team-based approaches are the reality in science in the 21st century, and are imperative for a future that improves clinical outcomes.

Conclusions

Greater subspecialization and division between male and female sub-specialists has led to significant advances in knowledge and treatment options for infertility patients. In many ways, the field of reproductive medicine should be proud of the outstanding improvements in clinical care that are reflected by the increasing success and safety of our treatments. However, sequestration also brings a risk of missing how each facet of care fits together. In truth, it has become much more difficult to maintain a wide perspective of infertility treatment and collaboration now requires careful effort as we are often separated from our colleagues.

This review only presents a small selection of topics that may benefit from greater collaboration. Our field would benefit from a periodic review of whether or not the treatment that we assume is evidence-based should still be recommended as the ground beneath us continues to move. Finally, the ultimate goal shared by both male and female

subspecialists (improving the rate of live birth for couples presenting with infertility) must remain at the forefront for both laboratory scientists and clinicians, as we continue to evaluate our current treatment paradigms and seek new methods of diagnosis and therapy for these patients.

Acknowledgements

Footnote

References

1. The Endocrine Society. History of the Endocrine Society. Available online: www.endo-society.org/about/history/cfm. Accessed February 22, 2015.
2. Gambone JC, Segars JH, Cedars M, et al. Fellowhship training and board certification in reproductive endocrinology and infertility. Fertil Steril 2015;104:3-7.
3. Schlaff WD. Who are we? A perspective on the reproductive endocrinologist and infertility specialist in the 21st century. Fertil Steril 2014;101:1510-1.
4. Barnhart KT, DeCherney AH. Are reproductive endocrinologists still gynecologists. Fertil Steril 2015;104:24-5.
5. Biggers JD. Pioneering mammalian embryo culture. In: Bavister BD. editor. The Mammalian Preimplantation Embryo. Boston, MA: Springer, 1987.
6. Temple-Smith PD, Southwick GJ, Yates CA, et al. Human pregnancy by in vitro fertilization (IVF) using sperm aspirated from the epididymis. J In Vitro Fert Embryo Transf 1985;2:119.
7. Palermo G, Joris H, Devroey P, et al. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. Lancet 1992;340:17-8.
8. Sigman M. Is it about business, education, or patient care? Fertil Steril 2014;101:1512-3.
9. de Ziegler D, de Ziegler N, Sean S, et al. Training in reproductive endocrinology and infertility and assisted reproductive technologies: options and worldwide needs. Fertil Steril 2015;104:16-23.
10. Hotaling JM, Smith SJ, Rosen M, et al. The relationship between isolated teratozoospermia and clinical pregnancy after in vitro fertilization with or without intracytoplasmic

sperm injection: a systematic review and meta-analysis. Fertil Steril 2011;95:1141-45.

11. Poland ML, Moghissi KS, Giblin PT, et al. Variation of semen measures within normal men. Fertil Steril 1985;44:396-400.

12. Alvarez C, Castilla JA, Martinez L, et al. Biological variation of seminal parameters in healthy subjects. Hum Reprod 2003;18:2082-8.

13. World Health Organization. WHO laboratory manual for the examination and processing of human semen. 5th ed. Geneva: World Health Organization, 2010.

14. Baker HW, Kovacs GT. Spontaneous improvement in semen quality: regression towards the mean. Int J Androl 1985;8:421-6.

15. Barroso G, Mercan R, Ozgur K, et al. Intra- and inter-laboratory variability in the assessment of sperm morphology by strict criteria: impact of semen preparation, staining techniques and manual versus computerized analysis. Hum Reprod 1999;14:2036-40.

16. Alvarez C, Castilla JA, Ramírez JP, et al. External quality control program for semen analysis: Spanish experience. J Assist Reprod Genet 2005;22:379-87.

17. Cooper TG, Björndahl L, Vreeburg J, et al. Semen analysis and external quality control schemes for semen analysis need global standardization. Int J Androl 2002;25:306-11.

18. Kohn TP, Kohn JR, Ramasamy R. Effect of sperm morphology on pregnancy success via intrauterine insemination: a systematic review and meta-analysis. J Urol 2017;199:812-22.

19. Esteves SC. Clinical relevance of routine semen analysis and controversies surrounding the 2010 World Health Organization criteria for semen examination. Int Braz J Urol 2014;40:443-53.

20. Samplaski MK, Agarwal A, Sharma R, et al. New generation of diagnostic tests for infertility: review of specialized semen tests. Int J Urol 2010;17:839-47. Erratum in: Int J Urol 2011;18:262.

21. Kovac JR, Pastuszak AW, Lamb DJ. The use of genomics, proteomics, and metabolomics in identifying biomarkers of male infertility. Fertil Steril 2013;99:998-1007.

22. Zini A, Fahmy M, Belzile E, et al. Antisperm antibodies are not associated with pregnancy rates after IVF and ICSI: systematic review and meta-analysis. Hum Reprod 2011;26:1288-95.

23. Leushuis E, van der Steeg JW, Steures P, et al. Immunoglobulin G antisperm antibodies and prediction of spontaneous pregnancy. Fertil Steril 2009;92:1659-65.

24. Leifke E Nieschlag E. Male infertility treatment in the light of evidence-based medicine. Andrologia 1996;28:23-30.

25. Practice Committee of the American Society for Reproductive Medicine. Diagnostic evaluation of the infertile male: a committee opinion. Fertil Steril 2012;98:294-301.

26. Mehta A, Bolyakov A, Schlegel PN, et al. Higher pregnancy rates using testicular sperm in men with severe oligospermia. Fertil Steril 2015;104:1382-7.

27. Greco E, Scarselli F, Iacobelli M, et al. Efficient treatment of infertility due to sperm DNA damage by ICSI with testicular spermatozoa. Hum Reprod 2005;20:226-30.

28. Moskovtsev SI, Lecker I, Mullen JB, et al. Cause specific treatment in patients with high sperm DNA damage resulted in significant DNA improvement. Syst Biol Reprod Med 2009;55:109-15.

29. Lewis SEM. The place of sperm DNA fragmentation testing in current day fertility management. Middle East Fertil Soc J 2013;18:78-82.

30. Practice Committee of the American Society for Reproductive Medicine. The clinical utility of sperm DNA integrity testing: a guideline. Fertil Steril 2013;99:673-7.

31. Larson KL, DeJonge CJ, Barnes AM, et al. Sperm chromatin structure assay parameters as predictors of failed pregnancy following assisted reproductive techniques. Hum Reprod 2000;15:1717-22.

32. Duran EH, Morshedi M, Taylor S, et al. Sperm DNA quality predicts intrauterine insemination outcome: a prospective cohort study. Hum Reprod 2002;17:3122-8.

33. Muriel L, Meseguer M, Fernandez JL, et al. Value of the sperm chromatin dispersion test in predicting pregnancy outcome in intrauterine insemination: a blind prospective study. Hum Reprod 2006;21:738-44.

34. Zini A. Are sperm chromatin and DNA defects relevant in the clinic? Syst Biol Reprod Med 2011;57:78-85.

35. Collins JA, Barnhart KT, Schlegel PN. Do sperm DNA integrity tests predict pregnancy with in vitro fertilization? Fertil Steril 2008;89:823-31.

36. Nagler HM, Luntz RK, Martinis FG. Varicocele. In: Lipshultz LI, Howards SS. editors. Infertility in the male. St. Louis, MO: Mosby Year Book; 1997:336-59.

37. Chehval MJ, Purcell MH. Deterioration of semen parameters over time in men with untreated varicocele: evidence of progressive testicular damage. Fertil Steril 1992;57:174-77.

38. Practice Committee of the American Society for Reproductive Medicine. Report on varicocele and infertility: a committee opinion. Fertil Steril 2014;102:1556-60.

39. Madgar I, Weissenberg R, Lunenfeld B, et al. Controlled trial of high spermatic vein ligation for varicocele in infertile men. Fertil Steril 1995;63:120-4.

40. Abdel-Meguid TA, Al-Sayyad A, Tayib A, et al. Does varicocele repair improve male infertility? An evidence-based perspective from a randomized, controlled trial. Eur Urol 2011;59:455-61.

41. Baazeem A, Belzile E, Ciampi A, et al. Varicocele and male factor infertility treatment: a new meta-analysis and review of the role of varicocele repair. Eur Urol 2011;60:796-808.

42. Nagy ZP, Liu J, Joris H, et al. The result of intracytoplasmic sperm injection is not related to any of the three basic sperm parameters. Hum Reprod 1995;10:1123-9.

43. Kirby EW, Wiener LE, Rajanahally S, et al. Undergoing varicocele repair before assisted reproduction improves pregnancy rate and live birth rate in azoospermic and oligospemric men with a varicocele: a systematic review and meta-analysis. Fertil Steril 2016;106:1338-43.

44. Matthews GJ, Matthews ED, Goldstein M. Induction of spermatogenesis and achievement of pregnancy after microsurgical varicocelectomy in men with azoospermia and severe oligoasthenospermia. Fertil Steril 1998;70:71-5.

45. Kim ED, Leibman BB, Grinblat DM, et al. Varicocele repair improves semen parametrs in azoospermic men with spermatogenic failure. J Urol 1999;162:737-40.

46. Schlegel PN and Kaufmann J. Role of varicocelectomy in men with nonobstructive azoospermia.Fertil Steril 2004;81:1585-8.

47. Dubin JM, Greer AB, Kohn TP, et al. Men with severe oligospermia appear to benefit from varicocele repair: a cost-effectiveness analysis of assisted reproductive technology. Urology 2018;111:99-103.

48. Al Bakri A, Lo K, Grober E, et al. Time for improvement in semen parameters after varicocelectomy. J Urol 2012;187:227-31.

49. Berkovitz A, Eltes F, Lederman H, et al. How to improve IVF-ICSI outcome by sperm selection. Reprod Biomed Online 2006;12:634-8.

50. Teixeira DM, Barbosa MA, Ferriani RA, et al. Regular (ICSI) versus ultra-high magnification (IMSI) sperm selection for assisted reproduction. Cochrane Database Syst Rev 2013;25:CD010167.

51. Balaban B, Yakin K, Alatas C, et al. Clinical outcome of intracytoplasmic injection of spermatozoa morphologically selected under high magnification: a prospective randomized study. Reprod Biomed Online 2011;22:472-6.

52. Setti AS, Figueira RC, Braga DP, et al. Gender incidence of intracytoplasmic morphologically selected sperm injection-derived embryos: a prospective randomized study. Reprod Biomed Online 2012;24:420-3.

53. Huszar G, Jakab A, Sakkas D, et al. Fertility testing and ICSI sperm selection by hyaluronic acid binding: clinical and genetic aspects. Reprod Biomed Online 2007;14:650-63.

54. Jakab A, Sakkas D, Delpiano E, et al. Intracytoplasmic sperm injection: a novel selection method for sperm with normal frequency of chromosomal aneuploidies. Fertil Steril 2005;84:1665-73.

55. Parmegiani L, Cognigni GE, Ciampaglia W, et al. Efficiency of hyaluronic acid (HA) sperm selection. J Assist Reprod Genet 2010;27:13-16.

56. Worrilow KC, Eid S, Woodhouse D, et al. Use of hyaluronan in the selection of sperm for intracytoplasmic sperm injection (ICSI): significant improvement in clinical outcomes – multicenter, double blinded and randomized controlled trial. Hum Reprod 2013;28:306-14.

57. Aston KI, Uren PJ, Jenkins TG, et al. Aberrant sperm DNA methylation predicts male fertility status and embryo quality. Fertil Steril 2015;104:1388-97.e1.

58. Jenkins TG, Aston KI, Trost C, et al. Intra-sample heterogeneity of sperm DNA methylation. Mol Hum Reprod 2015;21:313-9.

59. Maher GJ, Goriely A, Wilkie AO. Cellular evidence for selfish spermatogonial selection in aged human testes. Andrology 2014;2:304-14.

60. Xie L, Ma R, Han C, et al. Integration of sperm motility and chemotaxis screening with a microchannel-based device. Clin Chem 2010;56:1270-8.

61. Li Z, Liu W, Qiu T, et al. The construction of an interfacial valve-vased microfluidic chip for thermotaxis evaluation of human sperm. Biomicrofluidics 2014;8:024102.

62. Di Caprio G, Ferrara MA, Miccio L, et al. Holographic imaging of unlabeled sperm cells for semen analysis: a review. J Biophotonics 2015;8:779-89.

63. Nosrati R, Gong MM, San Gabriel MC, et al. Paper-based sperm DNA integrity analysis. Anal Methods 2016;8:6260-4.

64. Mallidis C, Sanchez V, Wistuba J, et al. Raman microspectroscopy: shining a new light on reproductive medicine. Hum Reprod Update 2014;20:403-14.