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ORIGINAL ARTICLE

Male Health

Testicular fine-needle aspiration for the assessment of intratesticular hormone concentrations

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Measurement of intratesticular sex steroid concentrations in men informs both the development of male hormonal contraceptives and the understanding of male infertility. Given the challenges of using invasive techniques to measure testicular hormone physiology, our group has used a minimally-invasive fine-needle aspiration technique to measure intratesticular hormones in normal healthy men. Herein, we present a *post-hoc* analysis of the safety and efficacy of testicular fine-needle aspiration (FNA) completed as part of six clinical trials. From 2001 through 2011, a total of 404 procedures were conducted among 163 research volunteers, 85.9% of which were successful in obtaining sufficient fluid for the measurement of intratesticular steroid concentrations. Pain was the most common side effect, with 36.8% of procedures associated with moderate procedural pain and 4.7% with severe procedural pain. Postprocedural pain was uncommon and abated within a few days. Mild local bruising occurred with 14.9% of procedures. Two serious adverse events (0.5%) required surgical intervention. The risk of an adverse event was not associated with age, body mass index, testicular size, or the volume of fluid aspirated. Testicular FNA to obtain fluid for measurement of intratesticular steroid concentrations frequently causes mild to moderate procedural pain, but serious adverse events occur rarely. Testicular FNA has been instrumental for defining human intratesticular hormone physiology and is a minimally-invasive, safe, effective method for obtaining fluid for research on testicular physiology and pathology.

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INTRODUCTION

Intratesticular testosterone (ITT) is essential for spermatogenesis, but research into the physiology of the human intratesticular environment has been slow because traditional methods of obtaining intratesticular samples are invasive, technically difficult, and confounded by the acute effects of anesthesia on steroidogenesis. Three techniques have been described for the measurement of intratesticular testosterone. Measuring steroid concentrations from the effluent veins of the testes is minimally-invasive, but it is technically challenging to cannulate the testicular veins, and the steroid concentrations do not accurately reflect the intratesticular hormone concentrations due to dilution in the effluent flow.[1,2] Open testicular biopsy has been used for decades for assessment of intratesticular steroid concentrations in infertile men, but is too invasive for evaluation of normal healthy men and requires general anesthesia which might profoundly affect the results.[3,4] Testicular fine-needle aspiration (FNA) was first developed for its role in the evaluation of male infertility in 1965,[5] and became more widely used as a diagnostic procedure in the 1990s.[6] In 2001, Jarow and colleagues adapted this minimally invasive percutaneous FNA technique for the measurement of intratesticular hormones in normal, healthy men.[7]

Since the procedure was described in 2001, six clinical trials of male testicular hormone physiology have been completed at our institution.[8–13] This technique has been instrumental in defining human intratesticular hormone concentrations and the effect of hormone manipulation across the blood-testis barrier. More information regarding the relationship between intratesticular testosterone and spermatogenesis is needed to inform both the understanding and treatment of male infertility and the development of male hormonal contraceptives.

As a relatively new approach to studying the intratesticular hormonal environment in normal men, little information exists as to the safety and efficacy of this procedure for both clinical and experimental purposes. We present our institutional experience using percutaneous intratesticular FNA including the success rate of obtaining adequate specimens for analysis and safety data.

METHODS

Fine-needle aspiration procedure

All testicular aspiration procedures were completed using the procedure described by Jarow *et al.*[7] Subjects were placed in the supine position and draped with a sterile cloth. The skin over the spermatic cord was cleansed with alcohol on both sides. A spermatic cord block was then performed with 10 ml of 1% buffered lidocaine. After adequate anesthesia was obtained, the skin overlying the testis was cleaned with

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betadine 3 times. A 19-gauge butterfly needle with tubing attached to a 10-ml syringe via stopcock was inserted into the superior anterior portion of the testis (**Figure 1**). Negative pressure was then applied using the syringe and stopcock, and the needle and testis were held firmly in place until the testicular fluid was drawn into the tubing. The tubing was then clamped at both ends with a hemostat, and the needle was removed from the testis. Gentle pressure was applied to the testis with a 2 × 2 gauze to obtain hemostasis while the tubing was placed on ice until processing.

Data acquisition

Data gathered for this *post-hoc* analysis of the safety and efficacy of testicular FNA for measurement of intratesticular hormones in men come from retrospective chart review including all subjects from six completed investigational studies that included testicular FNA.[8–13] The studies were conducted from 2001 through 2011. Information abstracted from the study chart included age, weight, BMI, and testicular volume as well as the volume of fluid aspirated during the procedure. In addition, the charts were reviewed for any complications associated with the procedure including the level of procedural pain, vasovagal reaction, postprocedural pain, minor bleeding, swelling, and infection.

In all but the earliest study,[8] data for immediate postprocedure pain, bruising, and minor adverse events were recorded systematically for 328 aspirations. Men were asked to rate their pain during the aspiration and immediately after the procedure on a scale of 0–10. For simplicity of presentation, these scores are shown as mild (pain scores of 0–3), moderate (4–6), or severe pain (7–10). Data from the earliest study (n = 75 aspirations) were not included in the analysis of minor adverse events due to incomplete recording but were included in the analysis of sample volumes and serious adverse events.

All subjects provided written informed consent prior to any study procedures, and the University of Washington Institutional Review Board approved all studies, including this current analysis. All trials after September 2008 were posted on www.clinicaltrials.gov including NCT 00756561, NCT 00839319, NCT 01215292.

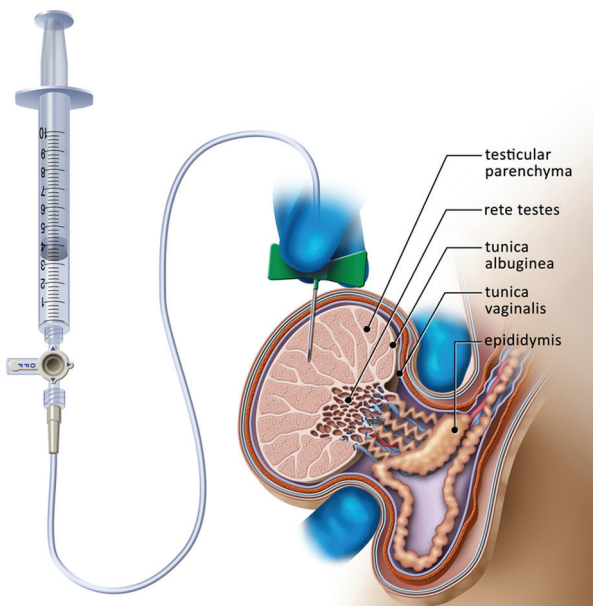

Figure 1: Testicular fine-needle aspiration technique for the collection of intratesticular fluid.

Statistical analysis

We report the median and interquartile ranges for the volume of fluid samples obtained. For the comparisons of characteristics between the groups, a Kruskal–Wallis analysis of variance (ANOVA) with a Wilcoxon-rank sum *post-hoc* test was used without correction for multiple comparisons. To determine if any characteristics were significantly associated with the risk of complications, logistic regression was performed using age, body mass index (BMI), study group, testicular volume, and volume of testicular fluid aspirated as covariates. Statistical analysis was performed using STATA (STATA Corp LP, College Park, TX, USA). $P < 0.05$ was considered statistically significant.

RESULTS

Subjects

A total of 163 men participated in six research studies over an 8-year period. Studies were designed as either male hormonal contraceptive studies or male hormonal physiology studies. All subjects were young, healthy men with normal baseline gonadal function and no history of testicular surgery. The baseline characteristics of the subjects and the type of study are presented in **Table 1**. There were only minor differences in baseline characteristics between the six groups. The men enrolled in the two male hormonal contraceptive studies[9,10] were significantly older and had greater median body mass indices and smaller pretreatment testicular volumes than the men enrolled in the physiology studies ($P < 0.05$ for all three comparisons).

Aspirations

Four hundred and four procedures were performed in 163 men. One hundred and sixty-two of the men consented to and underwent two or more fine-needle aspirations as a part of a study protocol. One subject withdrew consent to undergo a second fine-needle aspiration due to severe pain and anxiety with the first procedure. The majority of procedures (347/404, 85.9%) resulted in the adequate fluid collection for hormonal analysis (≥2 μl). Of the 57 inadequate procedures, 20/404 (5.0%) obtained a "dry" aspiration (no fluid), and 44/404 (10.9%) yielded too little fluid for analysis (<2 μl). The proportion of men with a successful testicular aspiration did not vary over time between the studies or between investigators performing the procedure.

The median (25th, 75th percentile) volume of intratesticular fluid obtained from the successful procedures was 10.8 (5.6, 21.9) μl in all studies combined. The volume of testicular fluid recovered did not significantly differ between studies, or within studies (either pre- or post-hormonal therapy). A total of seven investigators performed the fine-needle aspirations, but the majority of procedures (84.9%) were performed by two of the investigators.

Procedural pain

Of the 190 procedures in which intra-procedural pain was formally quantified to assess the effectiveness of the spermatic cord block, 57.9% were associated with no or mild testicular pain, 36.8% with moderate pain, and 4.7% with severe pain during the procedure (**Figure 2a**). Of the 328 procedures in which immediate and sustained postprocedural pain were recorded, subjects reported no immediate postprocedural pain after 75.0% of the aspirations, but 25.0% of the procedures were associated with some immediate postprocedure testicular pain (**Figure 2b**). However, the majority of the men did not require analgesia, and the pain generally abated within a few hours after the procedure. Only 2.1% of men reported pain that required oral

Table 1: Characteristics of the study subjects presented as median (25th, 75th percentiles)

Study	*Type of study*	*Number of men/FNAs*	*Age*	*Testicular volume*	*BMI*
Coviello *et al.*[10]	Male hormonal contraceptive	29/75	35 (27, 40)*	25 (19, 26)	27.2 (25.5, 28.3)*
Coviello *et al.*[8]	Male hormonal physiology	29/118	22 (19, 31)	25 (20, 26.5)	25.7 (22.9, 27.1)
Page *et al.*[9]	Male hormonal contraceptive	22/45	35 (32, 43)*	20 (15, 20)*	27.9 (23.5, 30.3)*
Roth *et al.*[11]	Male hormonal physiology	11/22	22 (20, 32)	25 (20, 25)	26.2 (23.4, 28.7)
Roth *et al.*[12]	Male hormonal physiology	38/76	22 (20, 25.5)	25 (20, 25)	24.1 (22.0, 25.8)
Roth *et al.*[13]	Male hormonal physiology	34/68	23 (21, 27)	25 (20, 25)	24.0 (22.3, 25.6)
All studies		163/404	25 (21, 32)	25 (20, 25)	25.4 (23.2, 27.9)

*$P<0.05$ compared to studies in references 8 and 11–13. BMI: body mass index; FNAs: fine-needle aspirations

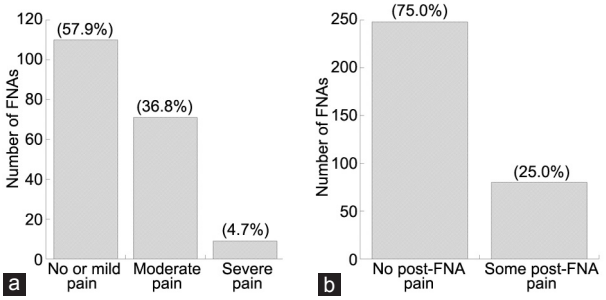

Figure 2: Intra-procedural (**a**) and postprocedural (**b**) pain during and after testicular fine-needle aspiration procedures. Intra-procedural typically resolved within a few minutes after the procedure, but postprocedure pain may last for up to a few days after the procedure. Intra-procedural and postprocedural pain was generally mild, and only 2.1% of men requested analgesics (acetaminophen alone [1.2%] or acetaminophen with opioids [0.9%]).

analgesics for 1–3 days after the procedure; 0.9% of men required prescription opioids (e.g., hydrocodone, oxycodone, or codeine) with acetaminophen.

Adverse events

Of the 328 procedures where we collected data on minor adverse events, 18.9% were associated with an adverse event other than pain, including minor scrotal bruising (14.9%), swelling (1.8%), mild vasovagal reactions during or after the procedure (0.9%), nausea or dizziness postprocedure (0.9%). No man experienced an infection as a consequence of the procedure.

Two aspirations (0.5%) were associated with serious adverse events. In one subject shortly after the aspiration, a 2 cm hematoma was noted on the right testicle. During a basketball game approximately 4 months later, the subject sustained a traumatic injury to the scrotum that resulted in a testicular hematoma. One year after the testicular FNA and 8 months after the traumatic injury to the scrotum, the subject underwent a unilateral orchiectomy for persistent testodynia. Another subject developed a postprocedural hematocele that was initially stable. The hematocele had increased in size 8 days later following sexual intercourse and required operative drainage and ligation of a bleeding vessel. Follow-up included a normal testicular ultrasound and seminal fluid analysis 3 months after surgical intervention.

The risk of an adverse event increased nonsignificantly by 4.4% for every cubic centimeter increase in testicular volume ($P = 0.07$). Otherwise, no other variables including age, BMI, volume aspirated or study group demonstrated were associated with an increased risk for adverse events ($P > 0.10$).

DISCUSSION

Testicular FNA is a minimally-invasive, safe, effective method for obtaining fluid for research on testicular physiology and pathology that has been instrumental for defining human intratesticular hormone physiology. With experience from over 400 procedures, 85.9% of the testicular FNA procedures obtained a sufficient volume of fluid for steroid hormone analysis. The median volume of aspirate obtained exceeded 10 μl, sufficient for the measurement not only of ITT, but also other intratesticular steroids, such as dihydrotestosterone and estradiol that might also regulate spermatogenesis. By using this procedure in experimental physiology studies in men, we have defined the range of intratesticular sex steroid hormones in normal men, with testosterone concentrations 100–200 times greater than serum concentrations, estradiol concentrations nearly 100 times greater than serum, and dihydrotestosterone concentrations about 10–20 times greater than serum.[11,12] We have also shown that ITT concentrations decreased by 95% with maximal gonadotropin suppression, but do not reach concentrations lower than serum testosterone concentrations.[12] Importantly, for male infertility evaluation and treatment, we have shown that men can maintain normal serum testosterone concentrations in the setting of decreased ITT concentrations. This finding suggests that some otherwise eugonadal men might be subfertile due to low ITT concentrations; these men might have improved spermatogenesis with gonadotropin therapy to normalize ITT concentrations.[12] We have also shown that inhibitors of androgen steroidogenesis, such as ketoconazole, can further reduce ITT concentrations, potentially informing our development of male hormonal contraceptives.[13] As a whole, the body of knowledge obtained using the testicular FNA technique described here has advanced our understanding of male hormonal physiology.

Although few studies have directly assessed peri-procedural pain associated with testicular FNAs, local anesthesia has been evaluated for testicular sperm extraction and epididymal sperm extraction, and found to be highly effective for the majority of patients.[14] In our study, the majority of subjects reported no pain or mild pain during the procedure, with only 4.7% of procedures associated with severe postprocedural pain. Peri-procedural pain typically abated in a few minutes but occasionally lasted for a few hours. Significant postprocedure pain that persisted more than a few hours was uncommon, and only 2.1% required postprocedural oral analgesic for a few days, as compared to rates of >50% for open testicular biopsy and ~10% for percutaneous testicular biopsy.[15]

Similar to the complication rates for testicular FNA for nonobstructive azoospermia evaluation, adverse events are rare.[16] There were no cases of infection, suggesting that the risk of infection is very low and antibiotic prophylaxis is unnecessary. Of the men who did have adverse events, the majority of these were due to bruising over the scrotum or testis, thought to be related to mild superficial bleeding from the spermatic cord anesthesia.

Although the currently published safety data for percutaneous fine-needle mapping in sperm retrieval suggest that the procedure

is safe, our data set significantly increases the number of procedures reported.[16,17] Two men experienced serious adverse events that required surgical intervention. One of these men underwent unilateral orchidectomy after significant testicular trauma several months after his testicular aspiration. In this case, it was impossible to determine whether his adverse outcome was related to his testicular FNA. In the second case, the subject's bleeding occurred within days of his FNA and was more likely to be attributable to his FNA. It is worth noting that this subject used a nonsteroidal anti-inflammatory to treat his postprocedural pain despite verbal instructions to avoid such medications. It is possible that the anti-platelet effects of this medication might have contributed to the development of his hematocele.[18] Testicular hematomas have been reported previously with testicular biopsy and testicular aspirations performed for the management of male infertility, with the risk of bleeding from biopsy appearing slightly greater than the risk from FNA.[19]

In response to the serious adverse event that occurred, we developed a postprocedure information sheet that informed patients to avoid any aspirin and nonsteroidal anti-inflammatory drugs and to avoid heavy exertion or sitting submerged in hot water for 48 h postprocedure. Despite pre- and post-procedure instructions being discussed during the informed consent process, we believe that a written reminder of instructions reviewed at the time of the procedure might reduce the risk of side effects and complications from the procedure.

This study intends to convey the safety and effectiveness of using a testicular FNA procedure for studying male testicular hormone physiology; however, this study has some limitations. As this study summarizes data accrued over several years during six separate clinical trials, the reporting of adverse events differed somewhat between the studies. Nevertheless, the rate of bruising was very similar between studies. In addition, adverse event rates and volume of fluid aspirated can vary between investigators performing the procedures; however, a small number of physicians performed all of the procedures described in this report. Finally, given the rarity of the serious adverse events associated with testicular FNA, it is difficult to determine the true rate of these events other than to state that they are very uncommon, occurring in 0.5% of procedures performed.

Testicular FNA is a minimally invasive, highly effective procedure for studying the unique intratesticular hormonal environment in men. While immediate postprocedural pain is common, it is usually only mild or moderate in severity and only occasionally requires the use of an analgesic (usually acetaminophen monotherapy). Sustained postprocedure pain is uncommon, and there was no evidence of risk of infection with appropriate skin preparation. Adequate testicular fluid for hormone analysis was obtained from 85% of procedures. Testicular FNA is a safe and effective method for obtaining intratesticular fluid for research on testicular hormonal physiology and testicular pathology in men.

AUTHOR CONTRIBUTIONS

Fine-needle aspiration procedures completed by MYR, JJNN, KL, TJW, AMM, JKA, and BDA. Data acquisition and analysis completed by APL, MYR, JKA, STP, BDA. Drafting of the manuscript completed by APL, MYR, JKA, STP, and BDA. Study design and securing of grant funding for the studies completed by WJB, AMM, JKA, STP, and BDA. All authors read and approved the final manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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REFERENCES

1 Morse HC, Horike N, Rowley MJ, Heller CG. Testosterone concentrations in testes of normal men: effects of testosterone propionate administration. *J Clin Endocrinol Metab* 1973; 37:882–6.
2 Winters SJ, Troen P. Testosterone and estradiol are co-secreted episodically by the human testis. *J Clin Invest* 1986; 78: 870–3.
3 Maddocks S, Hargreave TB, Reddie K, Fraser HM, Kerr JB, *et al*. Intratesticular hormone levels and the route of secretion of hormones from the testis of the rat, guinea pig, monkey and human. *Int J Androl* 1993; 16: 272–8.
4 McLachlan RI, O'Donnell L, Stanton PG, Balourdos G, Frydenberg M, *et al*. Effects of testosterone plus medroxyprogesterone acetate on semen quality, reproductive hormones, and germ cell populations in normal young men. *J Clin Endocrinol Metab* 2002; 87: 546–56.
5 Obrant KO, Persson PS. Cytological study of the testis by aspiration biopsy in the evaluation of fertility. *Urol Int* 1965; 20: 176–89.
6 Turek PJ, Cha I, Ljung BM. Systematic fine-needle aspiration of the testis: correlation to biopsy and results of organ "mapping" for mature sperm in azoospermic men. *Urology* 1997; 49: 743–8.
7 Jarow JP, Chen H, Rosner TW, Trentacoste S, Zirkin BR. Assessment of the androgen environment within the human testis: minimally invasive method to obtain intratesticular fluid. *J Androl* 2001; 22: 640–5.
8 Coviello AD, Matsumoto AM, Bremner WJ, Herbst KL, Amory JK, *et al*. Low-dose human chorionic gonadotropin maintains intratesticular testosterone in normal men with testosterone-induced gonadotropin suppression. *J Clin Endocrinol Metab* 2005; 90: 2595–602.
9 Page ST, Kalhorn TF, Bremner WJ, Anawalt BD, Matsumoto AM, *et al*. Intratesticular androgens and spermatogenesis during severe gonadotropin suppression induced by male hormonal contraceptive treatment. *J Androl* 2007; 28: 734–41.
10 Coviello AD, Bremner WJ, Matsumoto AM, Herbst KL, Amory JK, *et al*. Intratesticular testosterone concentrations comparable with serum levels are not sufficient to maintain normal sperm production in men receiving a hormonal contraceptive regimen. *J Androl* 2004; 25: 931–8.
11 Roth MY, Lin K, Amory JK, Matsumoto AM, Anawalt BD, *et al*. Serum LH correlates highly with intratesticular steroid levels in normal men. *J Androl* 2010; 31: 138–45.
12 Roth MY, Page ST, Lin K, Anawalt BD, Matsumoto AM, *et al*. Dose-dependent increase in intratesticular testosterone by very low-dose human chorionic gonadotropin in normal men with experimental gonadotropin deficiency. *J Clin Endocrinol Metab* 2010; 95: 3806–13.
13 Roth MY, Nya-Ngatchou JJ, Lin K, Page ST, Anawalt BD, *et al*. Androgen synthesis in the gonadotropin-suppressed human testes can be markedly suppressed by ketoconazole. *J Clin Endocrinol Metab* 2013; 98: 1198–206.
14 Gorgy A, Meniru GI, Naumann N, Beski S, Bates S, *et al*. The efficacy of local anaesthesia for percutaneous epididymal sperm aspiration and testicular sperm aspiration. *Hum Reprod* 1998; 13: 646–50.
15 Harrington TG, Schauer D, Gilbert BR. Percutaneous testis biopsy: an alternative to open testicular biopsy in the evaluation of the subfertile man. *J Urol* 1996; 156: 1647–51.
16 Carpi A, Menchini Fabris FG, Palego P, Di Coscio G, Romani R, *et al*. Fine-needle and large-needle percutaneous aspiration biopsy of testicles in men with nonobstructive azoospermia: safety and diagnostic performance. *Fertil Steril* 2005; 83: 1029–33.
17 Turek PJ, Ljung BM, Cha I, Conaghan J. Diagnostic findings from testis fine needle aspiration mapping in obstructed and nonobstructed azoospermic men. *J Urol* 2000; 163: 1709–16.
18 Vinik R, Wanner N, Pendleton RC. Periprocedural antithrombotic management: a review of the literature and practical approach for the hospitalist physician. *J Hosp Med* 2009; 4: 551–9.
19 Friedler S, Raziel A, Strassburger D, Soffer Y, Komarovsky D, *et al*. Testicular sperm retrieval by percutaneous fine needle sperm aspiration compared with testicular sperm extraction by open biopsy in men with non-obstructive azoospermia. *Hum Reprod* 1997; 12: 1488–93.