Sperm Collection and Processing Methods

A Practical Guide

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Introduction

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Recent advances in sperm procurement and processing have made fertility conditions once deemed hopeless a thing of the past. Even cases of sterility are now either treatable or entirely circumvented through various clinical techniques, including surgical correction, hormone treatment, and various sperm enhancement technologies.

This anthology presents these latest sperm procurement and processing methods in chapters written by acknowledged experts in their respective fields. The editor hopes that this collection will provide clinical and laboratory professionals from all areas of fertility science with both a comprehensive overview and a handy reference resource.

The primary biological function of the male reproductive system is procreation facilitation. The system accomplishes this through two fundamental processes: generation of fertility potential within the male, and subsequent male genome delivery directly into the female reproductive tract.

Spermatozoa (or "sperm"), as the exclusive male genome vehicle, is the vital carrier and component of male fertility potential. "Sperm procurement methods" are clinical procedures designed to acquire viable sperm for analysis, analysis interpretation and, if deemed necessary, sperm utilization for various assisted reproductive techniques and artificial insemination procedures.

Any difficulty with sperm procurement, analogous to problems with male fertility as a whole, can involve biological deficiencies with sperm creation, delivery, or both. The various procurement

methods described in this text are all designed with one purpose in mind: acquisition of as many viable spermatozoa as possible, given initial patient conditions. Once sperm are procured, a myriad of sperm processing methods, described in the latter portion of this text, are available to optimize fertilization potential.

The fundamental goal: viable sperm

Physically, sperm are comprised of a head and tail (Figure 1): the head, which contains the essential male fertility components, is oval and flat, tapering progressively toward an apex, while the tail, which provides propulsion for the entire cell, is narrow and long.

Average sperm volume is 16 μm^3. Overall cell length is approximately 50–60 μm. The sperm head measures 4.0–5.5 μm in length, 2.5–3.5 μm in width, with a typical length-to-width ratio of 1.50–1.75. The head contains the spermatozoon nucleus, appropriating approximately 65% of overall volume. The nucleus itself is comprised of tightly packed chromosomal material (mostly deoxyribonucleic acid, or DNA) and numerous proteins, accomplishing diverse cellular functions. Sperm also feature a sac-like structure called the acrosome, externally covering one-half to two-thirds of the anterior portion of the head. This acrosome contains enzymes essential for sperm penetration into the female egg, or oocyte.

The tail, approximately 10 times head length, is enclosed within a thin sheath and is comprised of the principal piece and end piece, known as the terminal filament. Two central fibers and nine outer pairs of fibers provide underlying tail support and structure. Another sheath, located within the tail's anterior portion (flush with the area where tail and head connect) encloses tightly packed mitochondria. These mitochondria provide the necessary energy for tail movement, sustaining and facilitating the mechanism by which sperm motility is achieved.

Sperm are produced within the testicles, a pair of reproductive glands located, in turn, within the scrotum. The testicles are

Figure 1 Scanning electron photograph of human spermatozoa.

each oval-shaped and approximately 25 ml in volume. Spermatozoa production (spermatogenesis; see below) is an elaborate cell differentiation process taking place within the seminiferous tubules of the testes. Spermatogenesis begins with a germ cell (called spermatogonia) and terminates with a fully differentiated and highly specialized male fertility cell, the spermatozoon, or sperm cell.

Awareness of the spermatogenesis process can, particularly for instances of azoospermia and other male genome deficiencies, facilitate overall male fertility diagnosis and treatment.

Spermatogenesis

Spermatogenesis involves, briefly, the following process: primary male germ cells, created in the seminiferous tubules of the testes, originate the overall process, and are named spermatogonia. These cells are comprised of two types:

- Type A spermatogonia cells, which in turn consist of:
  - Type A dark: these dark cells divide mitotically into both the ongoing type A pale cells (see below) and their own original type A dark form, capable of perpetuating the process.
    Note: Consequently, type A dark cells continually replenish themselves, enabling spermatogenesis (and therefore, male fertility) to continue throughout the male adult life span.
  - Type A pale: these pale cells mitotically divide to produce only type B spermatogonia, continuing the overall spermatogenesis process, as such.
- Type B spermatogonia cells: these type Bs, in turn, divide mitotically to produce the primary spermatocyte.
  - Primary spermatocyte: these cells then undergo meiotic division, giving rise to:
    - Secondary spermatocyte: these haploid spermatids then undergo various morphological changes, eventually developing into mature spermatozoa.
      Note: Spermatogenesis in humans, from originating spermatogonium to the finished spermatozoon sperm cell, requires approximately 70 days.

Following the aforementioned process, sperm production is constant and continues throughout human male adult life. Average sperm production per testicle is estimated to be approximately 85 million sperm per day.

Once sperm are created within the testes, they follow a circuitous route through the male reproductive system. During this journey, the sperm cells are augmented with various nutritional and other biochemical supplements, creating a viscous fluid ideal for transfer into the female reproductive tract.

Spermatozoa route through male reproductive tract

From spermatogenesis to ejaculation, sperm undertake the following path:

- Seminiferous tubules to rete testis and ducts: spermatozoa leave the testis by way of the rete testis, and a number of vasa (ductuli) efferentia.
- Ducts to epididymis: these ductuli join to form a single and very long, highly convoluted duct that comprises the tubular portion of the epididymis, a structure only a few centimeters long.
- Epididymis to vas deferens:
  - The head (caput) of the epididymis is attached to the testicle.
  - Full spermatozoon maturation and fertilizing capacity cannot be achieved until passage through the epididymis is realized.
    - During this passage, sperm undergo physical and biochemical changes.
    - Sperm also attain motility capacity.
  - Epididymal passage of spermatozoa requires 4–10 days, depending on daily sperm production rate.
    - As a general rule, a higher rate of daily sperm production implies faster epididymal transit time.
    - The primary sperm storage site is the epididymal tail (cauda).
- Tail (cauda) leading into vas deferens: vas deferens is a muscular tube of approximately 37 cm, extending from epididymis and entering the body through the inguinal canal to reach the prostate and urethra.
- Vas deferens to prostate and urethra: at the urethral end, the vas enlarges into an ampullary portion, forming the ejaculatory ducts with the seminal vesicles' excretory canals, joining the urethra.

  Note: Few sperm actually flow into the seminal vesicles: these sperm are generally found in the terminal portion of the ejaculate.

  Note: Sperm not ejaculated gradually die and dissolve (through cytolysis) like any other cell in the body.

Spermatozoa route through female reproductive tract

The second necessary male reproductive step, male genome delivery, culminates in semen deposition within the female reproductive tract.

The capacity to sustain an erection is vital. Natural fertilization involves penile insertion within the vagina, followed by ejaculation, resulting in the release of sperm inside the female.

Subsequent sperm transport to and through the female genital tract, despite independent cilial movement within, and overall contractions of the tract throughout, requires sperm motility. Sufficient sperm motility is also vital to avoid phagocytization by polymorphous white blood cells contained in body fluids, such as those present in the uterine and fallopian tube lumen.

The sperm, while physically fully formed when ejaculated, is not fertile until undergoing certain biochemically induced membrane changes within the female reproductive tract – a process known as capacitation. Essentially, only direct physical interaction with the female reproductive tract can activate male fertilization potential. In other words, only direct biochemical contact within the female reproductive system will induce necessary sperm capacitation, thereby sufficiently preparing the spermatozoon for potential fertilization of the oocyte. (Such sperm capacitation can also be achieved artificially in vitro, using actual or analogous biochemical factors.)

Statement of purpose

Statistically, of the millions of spermatozoa normally ejaculated into the vagina, only 1000–5000 sperm actually reach the fallopian tube ampulla and the potential fertilization site.

Therefore, procurement of functionally intact and potentially fertile sperm in statistically sufficient quantity is absolutely fundamental to male infertility assessment, therapy, and therapeutic procedures.

Outline

The text is divided into two primary sections: Part 1 – sperm procurement methods, and Part 2 – sperm processing methods.

Semen analysis: references and supplementary texts

The equally vital intermediary step, fertility assessment through semen analysis (and subsequent semen analysis result interpretation), is discussed in detail elsewhere, and will not be considered in this particular text.

For further details, the following sources may prove relevant:

- World Health Organization (1999). *The WHO Laboratory Manual for the Examination of Human Semen and Sperm–Cervical Mucus Interaction*. World Health Organization Cambridge, UK: Cambridge University Press.
- Keel, B.A. and Webster, B.W. (eds) (1990). *The Handbook of Laboratory Diagnosis and Treatment of Infertility*. Boca Raton, Florida: CRC Press.
- Mortimer, D. (ed.) (1994). *Practical Laboratory Andrology*. New York: Oxford University Press.

A text directly addressing semen analysis interpretation:

- Jeyendran, R.S. (ed.) (2000). *The Interpretation of Semen Analysis Results: A Practical Guide*. Cambridge: Cambridge University Press.

Sperm procurement methods

These methods primarily include the following:

- Nonclinical methods of semen collection, including masturbation and retrieval from urine
- Clinical semen retrieval, via:
  - Mechanical and electrical procedure

  - Medical (hormonal) management
  - Surgical interventions
- Clinical sperm retrieval, from male reproductive tract

Following sperm procurement and subsequent semen analysis, the sperm retrieved from semen must be sufficiently manipulated and processed to be effectively utilized for intrauterine insemination and other assisted reproductive technology procedures. The second part of this text summarily addresses these underlying processing principles, concepts, and methodological details.

Sperm processing methods

These methods, elaborated in the second half, primarily include:

- General procedures
  - Sperm washing
  - Sperm treatment
  - Cryopreservation
- Sperm processing procedures for intrauterine insemination
  - Sperm migration method
  - Density gradient centrifugation method
  - Column adherence method
- Sperm processing for assisted reproductive techniques
  - In vitro fertilization
  - Gamete intrafallopian transfer
  - Intracytoplasmic sperm injection
    - Epididymal sperm aspiration
    - Testicular biopsy sperm extraction
    - Testicular and epididymal sperm thawing and processing