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## 1 Introduction

The hypoosmotic swelling (HOS) test evaluates the functional integrity of the sperm plasma membrane and also serves as a useful indicator of its fertility potential. The functional integrity can be demonstrated by allowing sperm to react in a hypoosmotic medium. The hypoosmotic swelling test presumes that only cells with intact membranes (live cells) will swell in hypotonic solutions. The results of the HOS test correlate closely with the hamster egg penetration test. Spermatozoa with intact membranes swell within 5 min in hypoosmotic medium and all flagellar shapes are stabilized by 30 min [1–3].

### 1.1 Specimen Collection

The physician instructs the patient on proper collection technique. The patient collects the specimen into a sterile container and brings it to the laboratory within 1 h, keeping the sample at body temperature. Sperm vitality should be assessed as soon as possible after liquefaction of the semen sample, preferably at 30 min, but within 1 h of ejaculation to prevent the deleterious effects of dehydration, or large changes in temperature, on vitality.

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### 1.2 Equipment and Materials

A. Hypoosmotic solution—prepare monthly (or as needed) as follows:

**Combine the following in 100 mL of distilled water:**

1. 0.735 g sodium citrate dihydrate (Fig. 12.1)
2. 1.351 g fructose (Fig. 12.2)

- B. Microcentrifuge tubes
- C. 3" × 1" glass slides (plain)
- D. 22 × 22 mm coverslips
- E. Pipette tips (100 µL, 1000 µL)

### 1.3 Quality Control

A positive control using a donor or patient specimen should be run monthly or when fresh reagents are prepared with a result of 58 % or greater vitality to verify the integrity of the media. Record initial results on testing control sheet the day when the control is run.

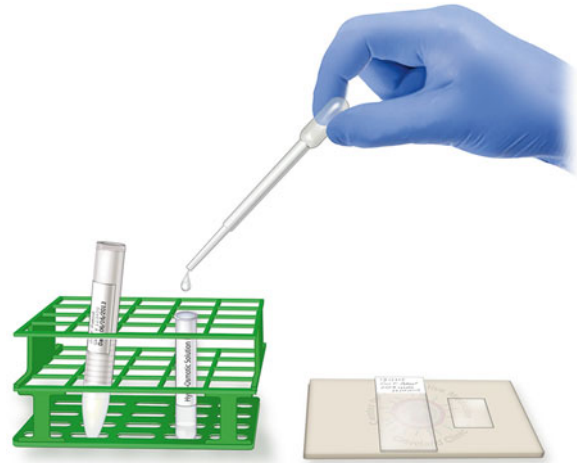
### 1.4 Procedure

**Note:** Specimens to be tested should be liquefied and no more than 1 h old.

- A. Combine 0.1 mL well-mixed semen with 1 mL hypoosmotic solution (HOS) (Fig. 12.3).
- B. Mix gently by drawing sample in and out of the pipette.
- C. Incubate at 37 °C for 30–60 min.
- D. After incubation, place one drop of the semen mixture on a glass slide and top with a coverslip (Fig. 12.4).



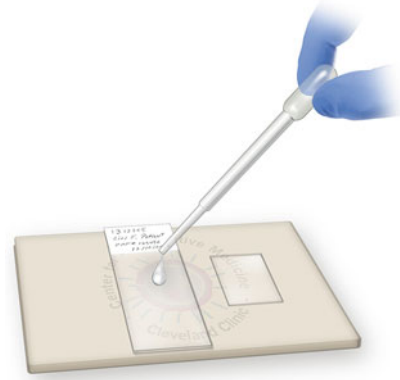
**Fig. 12.1** Sodium citrate dihydrate [Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2015. All Rights Reserved]



**Fig. 12.3** Adding well-mixed semen sample to HOS solution [Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2015. All Rights Reserved]



**Fig. 12.2** D-(-)-Fructose [Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2015. All Rights Reserved]



**Fig. 12.4** Droplet of semen sample and HOS mixture placed on slide [Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2015. All Rights Reserved]

- E. Observe for tail swelling (Fig. 12.5) under 40× phase contrast lens.
  - F. Using a two-channel cell counter, differentiate 100 sperm by swelling vs. non-swelling tails.
- Example:

$$\frac{55(\text{swollen tails})}{100 \text{ total sperm counted}} \times 100 = 55\%$$



**Fig. 12.5** Degrees of hypoosmotic swelling of sperm tails [Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2015. All Rights Reserved]

## 1.5 Results

Reference range:  $\geq 58\%$  (CI: 55–63 %) Tail swelling  
HOS test values approximate those of the eosin test [4]. The lower reference limit for vitality (membrane-intact spermatozoa) is 58 % (fifth centile, 95 % CI 55–63).

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## References

1. Hossain AM, Selukar R, Barik S. Differential effect of common laboratory treatments on hypoosmotic swelling responses of human spermatozoa. *J Assist Reprod Genet.* 1999;16(1):30–4.
2. Jeyendran RS, Van der Ven HH, Perez-Pelaez M, Crabo BG, Zaneveld LJ. Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. *J Reprod Fertil.* 1984;70: 219–28.
3. WHO Manual. Laboratory manual for the examination of human semen and semen-cervical mucus interaction. 5th edition, Geneva. World Health Organization Manual. Switzerland, 2010.
4. Ramirez JP, Carreras A, Mendoza C. Sperm plasma membrane integrity in fertile and infertile men. *Andrologia.* 1992;24(3): 141–4.

# HOS Test (Hypoosmotic Swelling Test)

## Procedure

The membrane integrity of the spermatozoa can be tested by the hypoosmotic swelling test.

### I. Equipment and Materials

- A. Hypo-osmotic solution – prepare monthly (or as needed) as follows:  
Combine the following in 100mL of distilled water:
  - 1) 0.735g Sodium citrate dihydrate (Figure 1)
  - 2) 1.351g fructose (Figure 2)



Figure 1. Sodium citrate dihydrate



Figure 2. D-(-)-Fructose

### Preparing for HOS test

- A. Combine 0.1 mL well-mixed semen with 1 mL hypo osmotic solution (HOS) (Figure 3).



Figure 3. Adding well-mixed semen sample to HOS solution.

- B. Mix the sperm suspension gently by drawing sample in and out of the pipette.
- C. Incubate at 37°C for 30-60 minutes.
- D. After incubation, place 1 drop of the semen mixture on a glass slide and place a coverslip (Figure 4).

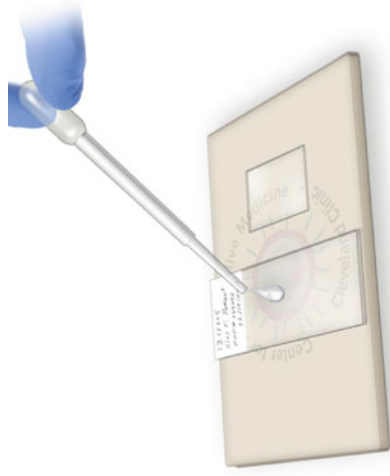


Figure 4. Add well-mixed semen sample to HOS solution.

- E. Observe tail swelling (Figure 5) under 40X phase contrast lens



Figure 5. Type of hypo-osmotic swelling of sperm tails.

Using a 2-channel cell counter, differentiate 100 spermatozoa by counting spermatozoa with tails swollen vs. non-swelling tails.

Example:  $\frac{55 \text{ (swollen tails)}}{100 \text{ total sperm counted}} \times 100$   
 = 55% spermatozoa with swollen tails

### Results:

Normal (fertile):  $\geq 58\%$  (CI: 55-63%) Tail swelling