

<p>2nd Symposium of the Croatian Society of Clinical Embryologists and Andrology Workshop</p>	<p>Workstation 3</p>
<p>HBA</p>	

HBA - Hyaluronan Binding Assay

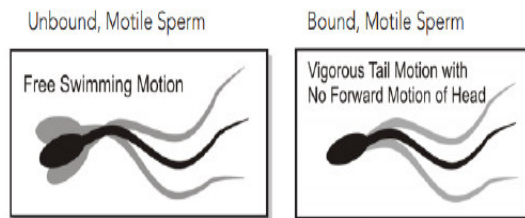
HBA provides a rapid assessment of sperm quality, maturity, and structural integrity. In natural fertilization mature sperm bind to **hyaluronan**, the main component of the cumulus oophorus matrix; immature sperm do not bind.

A sperm's ability to bind to HA correlates to: cellular maturity, less chromosomal aneuploidy, less DNA fragmentation, increased chromatin integrity, normal head morphology → *better fertilizing potential*.

HBA slide

Mature sperm also bind to a hyaluronan chemically attached to a support such as the hyaluronan-coated glass slides that make up the HBA slide.

Viewed in the microscope, bound sperm are differentiated from unbound sperm by their beating tails with heads that make no progressive movement.



Materials

HBA® slide with two identical hyaluronan-coated assay chamber. Cover slip provides a grid of 100 squares, 0.1 x 0.1 mm, within a viewing circle.



Procedure

The assay is performed at room temperature (20-30° C)

1. Mix the semen sample and pipette a drop (7-10 µL) onto the center of the assay chamber.
2. Place the cover slip over the drop Carefully to avoid air bubbles formation.
3. Incubate the chamber for 10 to 20 minutes.
4. Count only the motile sperm, bound and unbound, in the same number of grid squares. Preferably count at least 100 total motile sperm.
5. Calculate the percent of sperm binding to the hyaluronan:

$$\% \text{ Bound} = 100 \times \text{Bound Motile Sperm} / \text{Total Motile Sperm}$$

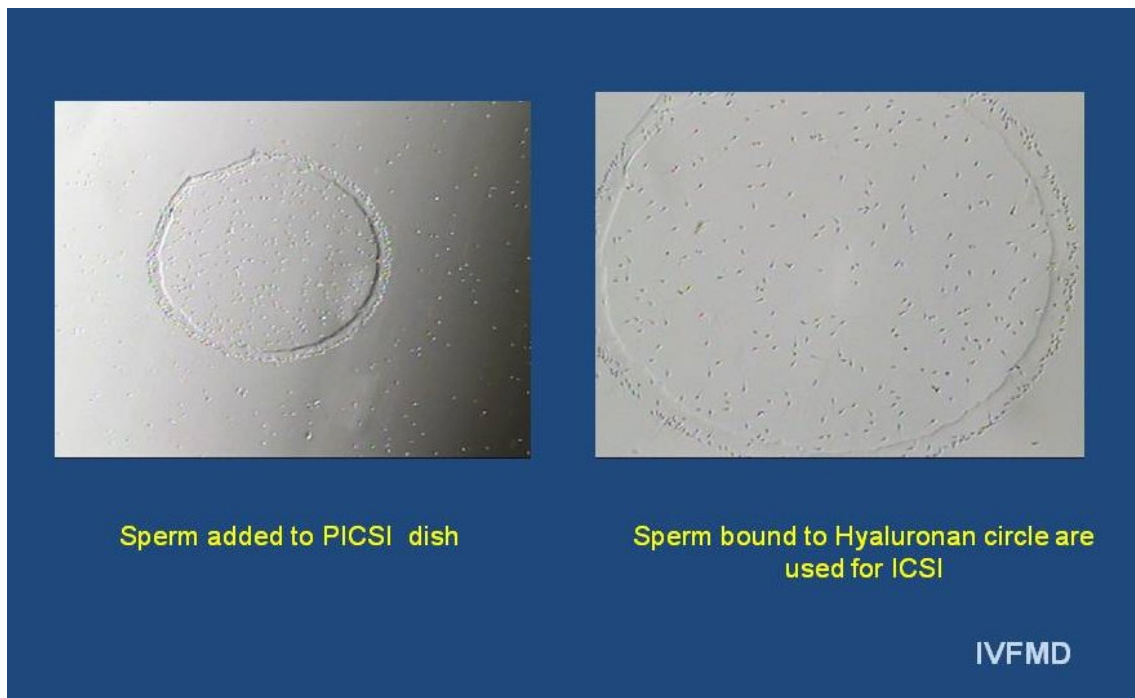
Results Interpretation

HBA® Score (% binding)	Interpretation
>80 % binding	Normal maturity and physiological function
<80 % binding	Diminished maturity and physiological function

PICSI dish

Sperm selection for ICSI based on Hyaluronan binding

The PICSI dish can be used for standard ICSI procedure and is a sterile plastic dish containing 3 microdots of Hyaluronic acid, where only mature DNA intact spermatozoa will bind for easy picking.



A large multi-center ICSI trial (Worrilow et al., 2012) found that combining the diagnostic abilities of the Hyaluronic Binding Assay (HBA), and the HA - sperm selection in the PICSI dish, lead to improved clinical Pregnancy Rates (CPL) and a significantly reduced Pregnancy Loss Rate in ICSI patients diagnosed to have low HA-binding ability (HBA) score ($\leq 65\%$).

Indications for modified ICSI - PICSI

- Very low numbers of motile sperm with normal appearance.
- Problems with sperm binding to and penetrating the egg.
- Prior or repeated fertilization failure with standard IVF culture and fertilization methods.
- Frozen sperm collected prior to cancer treatment that may be limited in number and quality.
- Absence of sperm secondary to blockage or abnormality of the ejaculatory ducts that allow sperm to move from the testes. In this situation, sperm are obtained from the epididymis by a procedure called microsurgical epididymal sperm aspiration (MESA).
- Absence of sperm in the ejaculate, but presence of sperm in the testes. Sperm can be obtained by testicular biopsy.
- Patients with high sperm DNA fragmentation.
- Previous history of poor embryo development day- 3 to day-5.

- Previous history of low or poor fertilization.
- Repeated implantation failure.
- High miscarriage rate.
- Reduced sperm morphology
- Reduced sperm motility
- Low fertilization with ICSI has occurred
- The woman is over 38 years old

For more information and other questions please contact moderators:

Dejan Ljiljak – dejan.ljiljak@kbcsm.hr

Marijana Baričević - marijana.baricevic@idb.hr

Sanja Zovkić - zovkic.sanja@gmail.com