

PICSI[®] Sperm Selection Device Instructions for Use

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Intended Use: In the treatment of infertile couples by ICSI, PICSI is indicated for the selection of mature sperm for injection.

Principle of the Device: Hyaluronan is a main component of the *cumulus oophorus* layer that surrounds the oocyte. The head of a mature sperm carries a hyaluronan-specific receptor that enables mature sperm to bind to hyaluronan (1). In contrast, immature sperm do not bind. Mature sperm exhibit a high DNA chain integrity, a normal frequency of chromosomal aneuploidies and provide a paternal contribution to the zygote comparable to that of sperm selected by the zona pellucida during natural fertilization (2).

In the usual practice of ICSI, sperm are visually selected for injection on the basis of their morphology and motility. However, this approach does not reflect the genomic integrity of the sperm and its ability to provide the best paternal contribution to the zygote. The PICSI dish provides a means to select mature sperm based on their ability to bind to hyaluronan hydrogel. PICSI mimics the natural binding of mature sperm to the cumulus oophorous, an important selective step in natural fertilization.

Device Description: The PICSI dish is a polystyrene culture dish with three microdots of hyaluronan attached to the interior bottom. Three locating lines embossed on the bottom exterior of the dish facilitate the location of the microdots. The microdot is found in an area approximately 2 mm wide and 3 mm long projecting from the end of the locating line. The device is sterile, essentially free of endotoxin and non-toxic to embryos (Each lot is assayed by the 1-cell Mouse Embryo Assay).

Preparation for use: Hydrate the hyaluronan microdots by placing single 10- μ L droplets of Human Tubal Fluid (HTF) or other suitable sperm diluent at the end of each locating line covering the area where the microdot is situated (Figure 1.) Alternatively, the sperm suspension can be added directly to the dry microdot. Drops of PVP or other fluids useful for manipulating sperm may also be placed elsewhere on the dish at this time. Carefully flood the dish with tissue culture oil to prepare it for use.

Hydrating the microdot before applying the sperm gives the hyaluronan time to swell. Swelling and sperm binding begin normally in 5 min. or less but there is some evidence that aged microdots may require 30 minutes or more to reach full binding capability. Therefore, whenever marginal sperm binding is observed, pre-hydrate for 30 min. or more, or allow sperm to incubate on the dot for 30 min. or more before selecting sperm. Hydrated microdots are stable for hours.

Using the PICSIS dish: Add the sperm to the pre-hydrated microdot in a volume equal to or greater than that used to pre-hydrate the dot (approximately 10 μ L). Touch the tip of the micropipette containing the sperm to the edge of the hydrating drop at the bottom of the dish under the oil and expel the sperm. By delivering the sperm in a volume equal to the hydrating fluid, immediate mixing and delivery of sperm to the vicinity of the microdot is assured. If the sperm are delivered in a smaller volume at the edge of the drop, a long time (> 30 min.) may be required for them to swim through the hydrating fluid to the vicinity of the microdot. Once bound, hyaluronan-bound sperm are easily identified: they exhibit no progressive migration despite a vigorous tail cross-beat frequency.

Factors governing sperm binding: To rapidly populate the microdot with bound sperm, approximately 100,000 hyaluronan-binding sperm per mL (approximately 1,000-2,000 total sperm in 10-20 μ L volume) are needed over the microdot, see Figure 2. As time passes, the number of bound sperm will increase as more swimming sperm make contact with the hyaluronan microdot.

Selection from the wall or the interior? The wall of the hyaluronan microdot is a physical barrier to which many sperm will bind since this is usually the first point of contact. It is sometimes difficult to distinguish whether the sperm are bound or they are simply swimming against the edge of the microdot. You may be sure of selecting bound sperm by selecting them from the interior of the microdot.

Obtaining a good density of bound sperm: If the density of bound sperm is too high or too low for good sperm selection, dilute or concentrate the prepared sperm sample and use the adjusted sperm sample to seed the next microdot. Three microdots are provided on each PICSIS dish to give a sufficient opportunity.

Sperm collection: To collect a bound sperm, position the tip of the ICSI micropipette next to the sperm and gently suck fluid into the pipette, drawing in the sperm. Continue collecting until 20-50 sperm are captured. Expel the captured sperm into a PVP drop to process them for ICSI (inactivating the tail, re-evaluating motility and morphology.) From the PVP droplet, select and load single, processed sperm for injection into the oocytes according to your standard injection protocol.

Temperature: Sperm bind best to hyaluronan hydrogel at temperatures below 30°C. At temperatures above 30°C, sperm swimming vigor increases and the swimming force may overcome the binding force. The result is that about one-third of sperm bound at room temperature will show some progressive migration at 37°C and may be deemed not bound, immature. In practice, most ICSI microscope stages are heated to 37°C. PICSIS dishes placed on a 37°C heated stage will come to about 33°C and then remain at that temperature. At 33°C or even at 37°C, many bound sperm will still be available for selection.

Potential Problems: Microdot shape: The PICSIS hyaluronan microdot is crater-shaped. The edge of the microdot is a raised wall of hydrogel surrounding a low, flat interior

layer. The wall is flexible and may be irregular in shape due to uneven hydration of the hydrogel.

The hydrogel wall can be pierced and torn by an ICSI micropipette driven directly into it. It is best to position the elevated micropipette tip over the microdot interior and lower it to the microdot surface for recovery of sperm.

Microdot caves: During manufacture, uneven hydration can cause segments of the microdot perimeter edge to buckle and lift off the dish creating small “caves”. The cave’s floor is uncoated polystyrene and the walls are the buckled hyaluronan gel. The cave mouth opens from the inside edge of the wall. Sperm that swim into a cave are trapped, not bound. The trapped sperm are actually underneath the hyaluronan hydrogel, not bound to its surface. Trapped sperm often occur in clusters, when several sperm swim into the same cave. Trapped sperm usually all face away from the center of the microdot and show vigorously beating tails. The heads of trapped sperm can move laterally and sometimes back and forth within the walls of the cave. Trapped sperm should not be selected since their binding status is unclear.

Microdot stability: If a part of the wall separates from the polystyrene, the same forces that create caves can cause the microdot wall to progressively detach from the dish and coil up like a spring. When this occurs, some or the entire wall will separate from the microdot. However, the microdot interior hyaluronan layer will remain intact. The interior hyaluronan layer is stable for hours, it collects and houses bound sperm that may be used for ICSI. The curled up wall remnant can often be found, covered with sperm, but not useful for sperm selection and isolation.

Troubleshooting: If sperm do not bind to the microdot:

1. Determine that the sperm sample contains mature, hyaluronan-binding sperm by assaying with HBA[®] (Hyaluronan Binding Assay). If the HBA score is essentially zero (<5%), no sperm binding is expected (In some laboratories, as much as 10% of the sperm donor population shows no sperm binding. This is a significant factor contributing to infertility).

2. How long have the sperm been incubated on the microdot? Binding is directly related to the time allowed for binding. Sperm bind when they make contact with the hyaluronan microdot, swimming randomly. The number of sperm bound to the dot will grow with time as more sperm encounter the hyaluronan microdot. Check the microdot periodically over two hours to see if sufficient bound sperm have accumulated for an ICSI procedure.

3. The density of hyaluronan-binding sperm is critical. Rapid population of the microdot requires a density of at least 100,000 hyaluronan-binding sperm per mL. If the HBA score is low, the total sperm density must be increased to deliver an effective number of hyaluronan-binding sperm. For example: if the HBA score is 10%, 1,000,000 total sperm per mL will be required to deliver 100,000 hyaluronan-binding sperm per mL.

4. Pre-hydration of the microdot assures that the hyaluronan is sufficiently hydrated to allow immediate sperm binding. Very dry, aged microdots may require 30 minutes or more hydration before rapid binding will occur.

Warning: U.S. Federal law restricts this device to the sale by, or on the order of, a physician. A Certificate of Analysis, if not already provided with the shipment, is available on request from the distributor. **This device is covered under US patent 5,897,988 and the corresponding foreign patent applications.**

Questions or Comments: Please contact the Distributor at the telephone or e-mail address given above.

References: 1. Huszar, G., *et al.* 2003. Hyaluronic acid binding by human sperm indicates cellular maturity, viability, and unreacted acrosomal status. *Fertil. Steril.* **79**(3):1616-24.
2. Jakab, A. *et al.* 2005. Intracytoplasmic sperm injection: a novel selection method for sperm with normal frequency of chromosomal aneuploidies. *Fertil. Steril.* **84**(6):1665-73.

Figure 1, the PICSU dish.

Figure 2, Sperm binding versus time and sperm density.



