Video guide for Physiological ICSI: PICSI® and Sperm Slow™, two ready-to-use systems designed for sperm-hyaluronic acid (HA) binding selection

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Summary

Hyaluronic acid (HA) plays a fundamental role in sperm selection both in natural and in vitro fertilization. Only mature sperm which have completed their plasma membrane remodeling, the cytoplasmic extrusion and nuclear maturation have specific receptors to bind to HA in in vitro selection systems (1-3). It has been shown that the injection of spermatozoa bound to HA may improve the quality of the embryos and their development (4). This approach to ICSI with HA-bound sperm, when using HA-viscous medium (SpermSlow™ Origio, Denmark) or HA-culture dishes (PICSI® Sperm Selection Device-Origio), has been called “Physiologic ICSI” (4). It has been demonstrated that both these sperm selection systems are equally efficient (5, 6).

A recent systematic review and meta-analysis (performed on 7 studies/1437 cycles) concluded that at the moment there is no firm clinical advantage in terms of pregnancy rate by using HA sperm selection technique (7). However, during ICSI, without the physiological check-points of natural fertilization, suboptimal spermatozoa may generate embryos, and subsequently babies. Although we have as yet real knowledge about human ICSI adults in the long term, by Physiologic ICSI is possible at very least to mimic nature in order to restore physiological selection and prevent hypothetical fertilization by DNA damaged and chromosomal unbalanced spermatozoa (6).

KEY WORDS: physiological ICSI, hyaluronic acid, PICSI, sperm slow.

PICSI® procedure

PICSI® dishes are conventional plastic culture dishes pre-prepared with 3 microdots of powdered HA. The powdered HA is rehydrated by adding a 5 µL droplets of fresh culture medium to each of the three microdots. A 2 µL droplet with suspension of treated spermatozoa is placed near each 5 µL culture medium droplet and subsequently connected to the droplet using the tip of a Gilson pipette. The PICSI® dish is incubated at 37°C under oil; within 5 minutes the bound spermatozoa are attached by their head to the surface of the HA-microdots and are spinning around their head. An ICSI injecting pipette is used to pick HA-bound sperm up and inject them one by one into an oocyte. Spermatozoa spinning faster should be preferred; the speed of spinning is evaluated on screen by a different operator from the one performing ICSI. The ICSI injecting pipette is previously loaded with Sperm Slow™ to facilitate sperm micromanipulation.
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**Video 1: Motility patterns of HA-bound spermatozoa in PICSI®**

In PICSI®, HA-sperm are bound by the head to the bottom of the dish and have vigorous motility with the tail spinning around their head. This motility pattern allows a “fine tuning” of HA-bound spermatozoa selection based on observation of their degree of motility. HA-unbound spermatozoa, in contrast, swim free all around the droplet of culture medium with varied motility.

**Sperm Slow™ procedure**

On a plastic culture dish, a 2 µL droplet with suspension of treated spermatozoa is connected with a pipette tip to a 5 µL droplet of fresh culture medium. Simultaneously, a 5 µL droplet of Sperm Slow™ is connected with a pipette tip to the 5 µL droplet of fresh culture medium. The spermatozoa on this culture dish are incubated for 5 min at 37°C under oil. HA-spermatozoa are slowed in the junction zone of the 2 droplets; these spermatozoa can be selected and detached by injecting pipette and subsequently injected into oocytes.

**Video 2: Motility patterns of HA-bound spermatozoa in Sperm Slow™**

In Sperm Slow™, HA-sperm appear very “slowed” due to the HA-binding combined with the viscosity of the medium. HA-spermatozoa appear as if “trapped in a net” compared with HA-unbound spermatozoa which travel much further. A specific droplet preparation is suggested and specific training is needed to distinguish HA-bound spermatozoa from HA-unbound, which are also slowed by the viscosity of the medium.

**References**