A new simple and quick method for sperm preparation and selection prior to ICSI procedure

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Summary

Introduction. **Semen preparation procedures before performing ICSI (Intra Citoplasmatic Semen Injection) help either to select the best spermatozoa in terms of motility and morphology, or to separate ROS (Reactive Oxygen Species) through washing and centrifugation. Actually in IVF laboratories the most used semen preparation techniques are swim-up and gradient centrifugation. During these procedures, the sample is centrifuged and passed from a tube to another many times in a period of time that ranges from 45 to 60 min for swim-up to 60 to 90 min for gradient centrifugation making the process time-consuming. In the last two years, we have set-up a simpler procedure for semen preparation, based on horizontal sperm migration directly in the injection dish.**
Objective. **The aim of this study is to validate our new sperm preparation technique in a selected population of patients using as reference the standard ones. The primary outcome measure is clinical pregnancy rate. Secondary outcome measure included fertilization, cleavage and blastocyst formation rates.**
Materials and methods. **This is retrospective cohort study including 460 couples treated in our Centre between January 2013 and December 2104. Inclusion criteria were: male partners with at least 2 millions motile spermatozoa in the ejaculate, female age <38 years, no previous pregnancy, no PCOS/PCOD, no history of endometriosis, normal AMH, and at least 4 MII oocytes retrieved. The patients were divided in 2 groups: in Group A the new sperm preparation technique was used (N=230) and in Group B the standards preparation techniques were used (swim-up or gradient centrifugation) (N=230). The new preparation technique consists in the preparation of the ICSI plate with 3 additional 50 microliters drops of culture medium. These drops were then linked to each other through a small culture medium bridge, using a stripper pipette. Depending on the concentration of the sperm sample, 1 to 3 microliters of ejacuate were directly injected in the proximal drop about 10 minutes before adding the oocytes in the dedicated separated drops (Figure 1). We were able to select motile spermatozoa also in case of hyper viscous sperm samples, simply using a 10 microliters pipette to take a very small amount of sample (video 1). No additional procedures were necessary.**
Results. **At the moment of ICSI, an adequate number of spermatozoa (>20) reached the superior edge of the distal drop in 100% of cases. *De facto* we performed an "horizontal layering swim-up", removing spermatozoa from the oxidant environment of the seminal plasma. The final dilution of the semen in the clean buffered culture medium was from 1:60 to 1:20.**
The clinical pregnancy rates obtained in the 2 groups were similar: 87/230 (37,7%) in Group A and 84/230 (36,5%) in Group B. Laboratory

outcomes were all similar in terms fertilization rate, cleavage rate and blastocysts formation. Moreover, none of the cases developed bacterial infection during handling.
Conclusions. **The results show that our sperm preparation method for ICSI is not inferior compared to traditional techniques, involving several benefits. First of all, about 1 hour of work is avoided and less culture media/plastic tubes are necessary. Less steps also mean a lower risk of samples mix-up. Unnecessary semen centrifugation may also be beneficial for the spermatozoa avoiding mechanical stress. The great majority of patients undergoing ICSI (about 70% for our centre) can benefit from this simple procedure.**
It remains to evaluate if our method can also be beneficial from a biological point of view reducing spermatozoa stress during *in vitro* manipulation.

KEY WORDS: sperm selection, ICSI, swim-up.

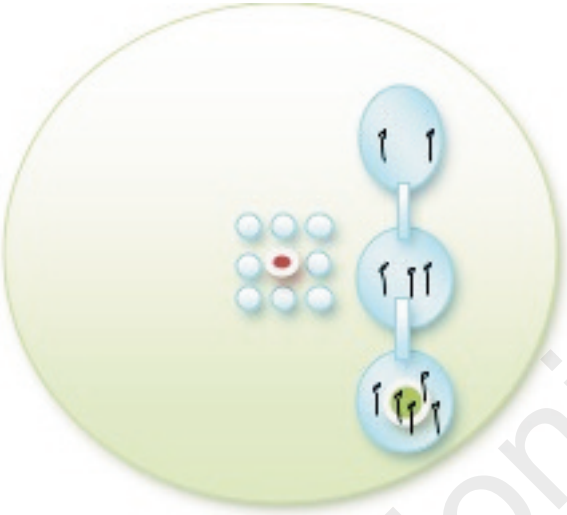

Figure 1