GYNEMEDIA

Information and Suggestions from GYNEMED
March edition 2021

PREFACE

Dear Distributors and Friends,
in this recent edition we want to give you some information's about Assisted oocyte activation. Gynemed GM508 CultActive is designed to investigate if fertilization failure after previous ICSI-cycles is due to a deficient oocyte activation.

After that we introduce the new Gy-ML13 Filter (exclusively designed and produced by Camfil, Germany), enabling improved filter capacity for the Zandair 100C filter system.

Further we will introduce our our Microtech Pipettes which are a big success all around the world.

The single packed handmade pipettes are made out of borosilicate glass and sterilized by gamma-radiation in order to meet international standards as well as the requirements of the FDA.

Each Lot is will be mouse-embryo-tested before the final release of Gynemed.

Finally, we are happy to inform you that gynemed is on

LinkedIn

Assisted oocyte activation – a method to overcome fertilization failure in in ICSI

In the last two decades achievements in technology and science have led to the introduction of several new methods in the field of medical assisted reproduction which in turn caused a widespread change in practice of everyday lab work. The implementation of sequential culture media, prolonged embryo culture, preimplantation genetic testing, in-vitro maturation, vitrification and time-lapse imaging may illustrate such a scenario. One such new application that gained interest in the last couple of years is called assisted or artificial oocyte activation (AOA).

According to an international expert group (ESHRE and Alpha, 2017) fertilization rates are expected to be 60-75% in conventional IVF and 65-80% in ICSI. Reality, however, is somewhat different since not even the direct injection of a spermatozoon with presumed optimal quality does guarantee oocyte activation and fertilization. In fact, 2-3% of all ICSI cycles experience complete fertilization failure with another 10% having fertilization problems (less than 30% fertilized eggs).

Physiological oocyte activation is a well-orchestrated course of biochemical processes which start upon entrance of the sperm into the cytoplasm of the oocyte. In detail, the male gamete brings an enzyme named phospholipase C zeta (PLCζ) in the egg which then reacts with membrane-bound

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proteins to form inositol trisphosphate (IP_3). This key molecule in turn binds to its receptors which are located at the outer membrane of the smooth endoplasmic reticulum (sER). As a consequence, calcium is released from the sER in a time-dependent manner, thus forming characteristic intracellular Ca^{2+} oscillations. These Ca^{2+} peaks drive oocyte activation and they cease once the two pronuclei, representing the ultimate sign of fertilization, form.
Any deviation in the level or the potency of the involved crucial substances (e.g., PLCζ, IP_3) will automatically cause a dramatic change in intracellular calcium levels, which would be noticed as absence or reduction of Ca^{2+} oscillations. This obvious drawback can be compensated by artificially increasing calcium in the oocyte which then would trigger oocyte

activation. This so-called AOA can be done with different methods. The most obvious approach would be to perform a more invasive ICSI technique which would lead to a calcium increase via mechanical depletion of the sER. However, due to its invasiveness this modified ICSI would have a distinct effect on oocyte survival rate. Alternatively, Ca^{2+} ions can be requisitioned from the culture medium the oocytes are cultured in. In order to allow such an accumulation of extracellular Ca^{2+} inside the cell pores in the outer membrane of the egg have to be generated either using direct/alternate current (electrical oocyte activation) or a variety of chemical agents. Amongst the latter substances calcium ionophores, in particular ionomycin and calcimycin (also called A23187) lead the way (Heindryckx et al., 2008).

At the beginning there was considerable concern about the safety of the AOA procedure due to the non-physiological nature of ionophores. With respect to this it should be clarified that ionophores do not cause physiological Ca^{2+} oscillations. Much rather ionophore treatment results in an intracellular increase of calcium and once a critical Ca^{2+} level is passed oocyte activation gets started. Another problem was their inconsistent application throughout literature (Vanden Meerschaut et al., 2014). Differences in ionophore type, concentration, exposure time and/or the number of stimuli impeded the standardization of AOA techniques. The launching of a ready-to-use calcimycin compound (GM508 CultActive, Gynemed) was the first step towards standardization of the AOA procedure (Ebner et al., 2012; 2015). To date, CultActive is the only commercially available AOA compound and the number of indications to use it is steadily growing. In fact it proved useful in cases of complete fertilization failure, fertilization problems, cryopreserved sperms, severe male factor infertility and developmental problems of the embryos in a previous treatment cycle. Meanwhile, over a hundred healthy live births by the use of CultActive are reported and it is very likely that the ready-to-use calcimycin (as well as the the other AOA techniques) can be considered safe. This statement is based on gene expression studies, chromosomal segregation error analyses, morphokinetic annotation of embryos and careful follow-up of children born so far.

GM508 CultActive

Although success rates and safety aspects with respect to AOA are reassuring it should be kept in mind that ionophores will not help in all cases of fertilization deficiency (e.g., if limited Ca^{2+} is not the causative factor). As such, AOA should still be considered experimental and only be used with proper indication. Informed consent from the patient should be obligatory.

References

- *Ebner T, Montag M, Oocyte Activation Group. Live birth after artificial oocyte activation using a ready-to-use ionophore: a prospective multicentre study. Reprod Biomed Online 2015; 30: 359-65.*
- *Ebner T, Köster M, Shebl O, Moser M, Van der Ven H, Tews G, Montag M. Application of a ready-to-use calcium ionophore increases rates of fertilization and pregnancy in severe male factor infertility. Fertil Steril 2012; 98: 1432-1437*
- *ESHRE Special Interest Group of Embryology and Alpha Scientists in Reproductive Medicine. The Vienna consensus: report of an expert meeting on the development of ART laboratory performance indicators. Reprod Biomed Online. 2017 Nov;35(5):494-510.*
- *Heindryckx B, De Gheselle S, Gerris J, Dhont M, De Sutter P. Efficiency of assisted oocyte activation as a solution for failed intracytoplasmic sperm injection. Reprod Biomed Online 2008; 17: 662–668.*
- *Vanden Meerschaut F, Nikiforaki D, Heindryckx B, De Sutter P. Assisted oocyte activation following ICSI fertilization failure. Reprod Biomed Online 2014; 28,: 560-571.*

Introducing GY-ML13 (HEPA) Filter for Zandair 100C

Gynemed is happy to introduce the new Gy-ML13 Filter (exclusively designed and produced by Camfil, Germany), enabling improved filter capacity for the Zandair 100C filter system.

The Gy-ML13 perfectly fits in the Zandair 100c housing and is classified H13 (according to EN 1822:2009) which attests a filtration efficiency of at least 99.95% (most penetrating particle size).
The filter material consisted of grid protected glass fibers built into an anodized aluminum profile.

The new GY-ML13 filter shows

GY-ML13 Filter

increased efficiency particularly with smaller particles (Fig. 1).
Each filter is individually tested and a test certificate declaring the H13 classification (EN1822:2009) is included.

For further information please contact us directly!

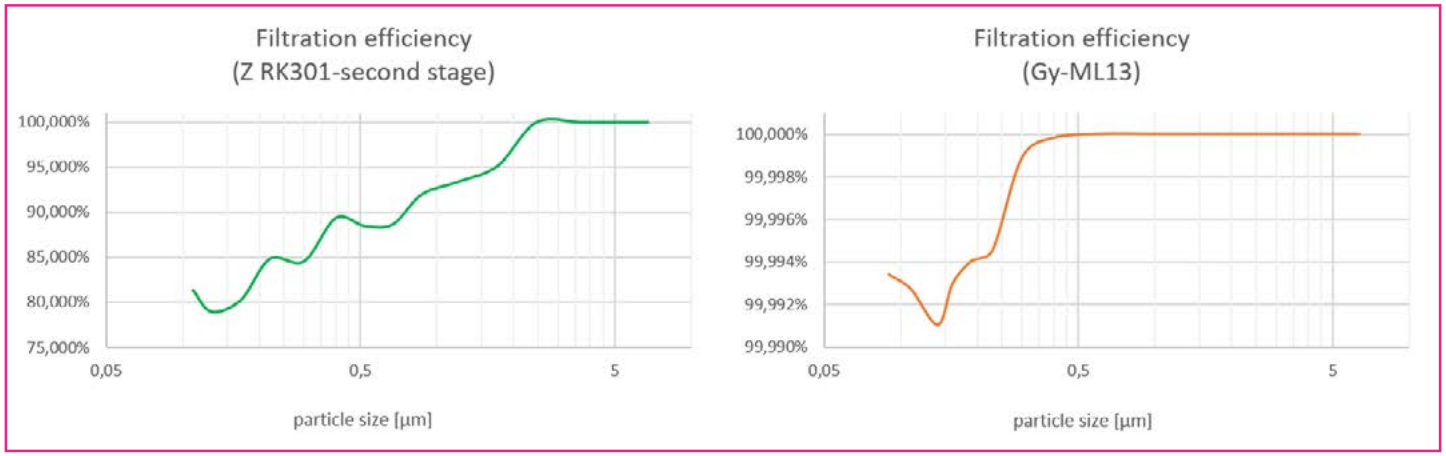

Figure 1:
Direct comparison of Filtration efficiency between the Z RK301-second stage and the Gy-ML13 HEPA filter

The Gynemed - Pipette - Team

Holding, ICSI, Biopsy and Hatching pipettes from Gynemed.

In addition to our own brand micropipettes we can also offer you a great alternative from Microtech – CE certified and MEA tested.

Holding Pipettes

Holding micropipettes are used for the fixation of oocytes, embryos or blastycysts and are therefore essential for all micromanipulation procedures in ART like ICSI, assisted hatching and polar body or blastomere biopsy.
The Holding micropipettes are manufactured from borosilicate glass tubing (outer diameter/ O.D. 1.00 mm, inner diameter/ I.D. 0.75 mm) total length 5.50 cm with length of arm 0.9mm and polished opening. Pipettes are available with 3 different outer diameters 80 µm (small), 100 µm (medium) and 120 µm (large). The holding pipettes are available straight (0°) or with bending angle ranging from 20° to 40°.

ICSI (intracytoplasmic sperm injection / spermatid) Pipettes

ICSI (intracytoplamic Sperm Injection) micropipettes are used to immobilze and aspirate spermatozoa and inject the sperm directly into the oocyte. The ICSI micropipettes are manufactured from borosilicate glass tubing (outer diameter/ O.D. 1.00 mm, inner diameter/ I.D. 0.78 mm) total length 5.50 cm with length of arm 0.5 mm, beveled 35° at the opening and I.D. of the 4.5-5 µm and with and without spike. The ICSI pipettes are available straight (0°) or with bending angle ranging from 20° to 40°.

Larger ICSI-Spermatid micropipettes are used for aspiration and injecting immature sperm directly

into the oocyte. ICSI-Spermatid micropipettes have I.D. 7.00 - 8.00 µm, O.D. 9.00 -10.00 µm at the the tip.

Hatching Pipettes

Hatching micropipettes are used for the mechanical opening of the Zona pellucida of embryos or blastocysts by partial zona dissection (mechanical assisted hatching / PZD). The Hatching micropipettes are prepared from borosilicate glass rods outer diameter 1.20 mm, total length 5.50 cm and length of the arm 2.00 mm with short thin taper (S) or with long thin taper (L), sharp point.
Hatching micropipettes may be ordered straight or with bending angle (20°, 30 and 35°).

Biopsy Pipettes

Biopsy micropipettes are used to perform biopsies on the embryo (blastocyst) or the oocyte (polar body) for Preimplantation Genetic Diagnosis - PGD.
The Biopsy micropipettes are prepared from borosilicate glass tubing (O.D. 1.00 mm, I.D. 0.78 mm) with total length 5.50 cm and 1.0 mm length of the arm. Available bending angles are 20° to 45°.
Depending on the intended use biopsy micropipettes are available with blunt opening (A) or beveled 40° and polished (B) opening and with inner diameter 10, 15, 20, 30 and 35 µm.

LEGAL NOTE

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