Collagenase treatment

1. Transfer 1.5 ml GM501 Collagenase into a 5 ml round-bottom centrifugation tube.
2. Warm the GM501 Collagenase at 37 °C. GM501 Collagenase is HEPES-buffered. Incubation in a CO_2-Incubator will lower the pH.
3. For digestion of testicular tissue carefully pick up the chosen tissue pieces with a fine syringe cannula. For easier handling, if necessary, fill the tissue suspension into a 60 mm petri dish. Let adhesive transport or cryo medium drop off occur as much as possible and transfer into Collagenase tubes.
4. Close the tube completely and place in the incubator (or ideally in a heat cabinet for digestion of the tissue) for 60 minutes. Slight agitation every 20-30 min will facilitate the formation of a single cell suspension.
5. Suspend the digested tissue by carefully pipetting up and down. Under ideal conditions a suspension of single testicular tissue cells and free semen cells has been formed. If coarse tissue pieces are still visible, repeat step 4 for a further 20 to 30 minutes.
6. Now centrifuge the tissue cell suspension and wash twice with 1–2 ml HEPES-buffered sperm processing medium (e. g. GM501 SpermAir). Discard the obtained supernatant. Alternatevily, the cell suspension can be processed using a density gradient system (e. g. GM501 Gradient).
7. Resuspend the pellet in a small volume of 30–80 µl HEPES-buffered sperm processing medium. Add a few µl of this suspersion into a dish.
8. Continue IVF treatment according to internal standard procedures.
9. If no motile sperms can be found Gynemed recommends the application of GM501 Sperm-Mobil.

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