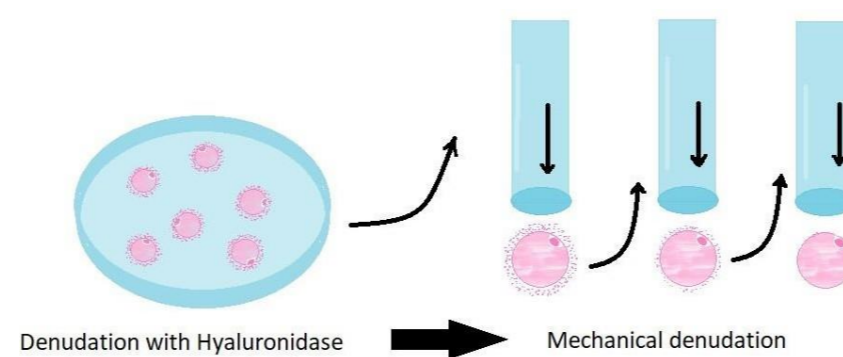


INTRODUCTION

Denudation of oocytes includes both enzymatic and mechanical removal of the cumulus cell surrounding the oocytes. Denudation is mandatory in order to make possible visualize the first polar body for precise alignment of oocyte prior to sperm injection. The follicular fluid is screened in order to collect the oocytes. Each oocyte is surrounded by cumulus-corona- oocyte complex. It is composed of cumulus granulosa cells surrounded in a bed of long hyaluronan oligosaccharide chains cross linked by a complex of hyaluronan binding cell surface and extracellular matrix proteins and proteoglycans. The COCC is removed prior to ICSI in order to gain visual of oocyte grade and first polar body. Conventionally, this has been attained using enzymatic breakdown of substance with hyaluronidase, followed by mechanical denudation of the oocyte with the pipette. Exposure of COCC in hyaluronidase affect the oocyte quality, comprising artificial interruption of COCC depending on exposure length of oocyte complex in hyaluronidase and the concentration of hyaluronidase. This hypothetical study based on the results of diluted concentration of hyaluronidase used for denudation of oocytes for women undergoing treatment for assisted reproduction by ICSI and their fertilization rate. There is increase in fertilization rate while diluting the hyaluronidase with fertilization media.



RESULTS

Surelife Hyaluronidase (SL- HYS- 1) is ready to use. This is HEPES based fertilization medium added with 80IU of hyaluronidase. The product contains Fertilization medium, hyaluronidase, Human serum albumin, HEPES of pharmaceutical grade. HSA has been tested negative for HbsAg, Anti- HCV, Anti- HIV 1/-HIV 2 and CJD. Surelife Hyaluronidase has made known to be safe and effective in removal of cumulus cells in the human oocyte prior to ICSI. Surelife Hyaluronidase is HEPES buffered and pH stable for use outside the incubator without former equilibration. The study was conducted with 80% (800 µl) of Fertilization media and 20% (200 µl) of Hyaluronidase in a center-well dish. Warm to 37°C about 30 minutes prior denudation. The cumulus oocyte complex were incubated in 06% CO2 incubator at 37°C for 2 hours. It is recommended to denude the cumulus oocyte complex in hyaluronidase solution for time less than 60 seconds or as less time possible, until the cumulus is separated from the oocytes by drawing them in and out of the glass capillary pipette avoiding air bubbles. Exposure of cumulus oocyte complex in hyaluronidase affect the oocyte quality, comprising artificial interruption of cumulus oocyte complex depending on exposure length of oocyte complex in hyaluronidase and the concentration of hyaluronidase. The cumulus oocyte complex is removed prior to Intra cytoplasmic sperm injection(ICSI) in order to gain visual of oocyte grade and first polar body. Conventionally, this has been attained using enzymatic breakdown of substance with hyaluronidase, followed by mechanical denudation of the oocyte with the pipette. The oocytes are then denuded mechanically using 170µm Flexipet stripper under the stereo microscope. It is observed that few cells are sticking to the oocytes. Remaining cells are removed using 140µm Flexipet stripper until nuclear maturation is seen. Total 380 oocytes denudation was performed with 20% dilution form. The denudation was done with the upper limit of 60 sec to the max. At the end of denudation, oocytes are placed in 30µL drops of Culture NX media(Irvine Scientific) overlaid with mineral oil(Surelife) and incubated until ICSI. After 2hrs of incubation period oocytes were injected with sperm by ICSI technique. After 14 to 16hrs the oocytes were evaluated for fertilization. In our study, out of 380 oocytes 311 were fertilized having fertilization rate of 82%.



CONCLUSION

The diluted hyaluronidase showed better results in fertilization rates. This study exhibits that the use of diluted hyalase enzyme, according to the following protocol is favorable and can be successfully used for oocyte denudation and its cost effective strategic to the laboratory. To conclude, using 20 % of diluted hyalase from ready to use hyalase gives better fertilization rate. This reduces stress to the oocytes during denudation process which favors better fertilization.

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