

Comparison of bovine- and recombinant human-derived hyaluronidase with regard to fertilization rates and embryo morphology in a sibling oocyte model: a prospective, blinded, randomized study

The objective of the present study was to compare a traditionally used bovine-derived hyaluronidase (Hyase) with the newly developed recombinant human-derived enzyme product (Cumulase) in intracytoplasmic sperm injection (ICSI) procedures using a sibling oocyte model in a prospective randomized design. The results of the study demonstrate that Cumulase is safe and effective in an ICSI treatment program and can provide comparable if not improved parameters, including fertilization and embryo developmental rates. (*Fertil Steril*® 2006;85:1544–6. ©2006 by American Society for Reproductive Medicine.)

Recombinant DNA technology has allowed for the development of highly purified and effective products for use in medicine and in vitro fertilization (IVF). For example, Serono Laboratories first introduced recombinant human FSH (rFSH), which became the first recombinant product used in an infertility setting.

Another recombinant product, recombinant human hyaluronidase (rHuPH20, Cumulase) (Halozyme Therapeutics, San Diego, California), has been introduced as a recombinant alternative to bovine-derived hyaluronidase. This product, owing to its purity and effectiveness, could be considered a preference to the bovine-derived hyaluronidase.

One of the major constituents of the cumulus-oocyte complex (COC) is hyaluronan, which is involved in maintaining the structure and shape of the COC (1). Hyaluronidase digests the hyaluronan interspaced between the cumulus cells, a process that removes the COC and consequently provides the possibility of clearer visualization of the oocyte and allows maturity grading together with other observation of the egg (2).

Current bovine-derived hyaluronidase can affect egg quality in several areas, including artificial disruption of the COC, exposure length of oocytes to hyaluronidase, and concentration of hyaluronidase (3). Recombinant human hyaluronidase may limit damage to the oocyte, improving fertilization and embryo quality.

It was the objective of this present study to compare the traditional bovine-derived hyaluronidase with the newly developed recombinant human-derived enzyme in ICSI procedures using sibling oocytes in a prospective randomized design.

The prospective study included 26 couples undergoing IVF treatment between January 2005 and May 2005 and was approved by the corresponding institutional review board committee. The inclusion criteria included patients with a maximum of three previous IVF cycles, minimum of eight eggs at egg retrieval (ER), and patient age of at least 20 and at most 38 years old at time of ER. Couples who had testicular sperm aspiration or previous fertilization failure with ICSI were excluded.

Controlled ovarian hyperstimulation is discussed elsewhere (4). Egg retrieval and sperm and oocyte handling for ICSI procedures were performed as described by Nagy et al. (5). After ER, the COCs designated for ICSI insemination were randomized into two groups (A and B) for denudation in either Cumulase (Halozyme Therapeutics, San Diego, CA) or Hyase (Vitrolife, Englewood, CO). Both groups were blinded with regard to solution A or B. Furthermore, the embryologist during the denudation was blinded to which solution correlated to which product.

Denudation consisted of the removal of the cumulus cells by short exposure to HEPES-buffered media (Cooper/Sage Biopharma, Trumbull, CT) containing 20 IU/mL of either Hyase or Cumulase. A lower concentration of enzyme was used to limit the influence of any toxins that may be present within the Hyase. To improve the removal, the COCs were pipetted up and down with a 20- μ L pipette (Eppendorf, Brinkmann Instruments, Inco, Westbury, NY) and disposable sterile pipette tips (VWR International, West Chester, PA). Pipetting was stopped before complete removal of the COC, and the time the oocytes were in the solution was recorded. After partial removal of the COC, the oocytes were rinsed in a 5.0% CO₂-equilibrated dish containing 5 mL fertilization media supplemented with 10% serum protein substitute (Cooper/Sage Biopharma, Trumbull, CT) and covered by Ovoil (Vitrolife, Gothenberg, Sweden). Rinsing was performed three consecutive times, disposing and replacing with a new sterile pipette

Received July 28, 2005; revised and accepted October 25, 2005.
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TABLE 1**Embryo statistics between oocytes denuded in Hyase or Cumulase.**

Groups	Hyase	Cumulase	Wilcoxon test
No. of patients	26	26	
No. of injected oocytes	203	199	
Egg maturity			
% oocyte in vivo mature	90.7 ± 15.0	87.3 ± 19.8	NS
% oocyte in vitro mature	9.3 ± 15.0	12.7 ± 19.8	NS
Pronuclear development			
% fertilization from in-vivo matured eggs only (% ± SD)	70.0 ± 19.6	85.3 ± 15.1	.015
% fertilization from all oocytes mature at ICSI (% ± SD)	70.7 ± 20.3	84.9 ± 21.8	.006
Embryo development (% ± SD)			
% A (excellent quality)	25.0 ± 27.6	43.4 ± 28.5	.051
% B (good quality)	42.1 ± 28.4	35.9 ± 30.7	NS
% C (fair quality)	16.4 ± 25.2	13.7 ± 15.4	NS
% D (poor quality)	16.5 ± 25.3	7.0 ± 12.6	NS
Cell Number			
Day 2 cell no. (±SD)	3.3 ± 0.7	3.4 ± 0.7	NS
Day 3 cell no. (±SD)	6.4 ± 1.4	6.9 ± 1.0	NS

Note: NS = not significant.

Taylor. Comparison of two types of hyaluronidase. *Fertil Steril* 2005.

with each fresh media drop. After rinsing, the oocytes were placed back into the incubator for 30 minutes.

The COC was then removed mechanically by pipetting with a 150- μ m-diameter plastic pipette followed by a 135- μ m pipette (Stripper, MidAtlantic Diagnostic, Marlton, NJ). After stripping, the oocytes were rinsed several times and then transferred into new drops of fertilization media and assessed under an inverted microscope for nuclear maturation. Finally, they were incubated at 37°C in 5.0% CO₂, 5% O₂, 90% N₂, and 98% humidity for 2–4 hours before ICSI.

The methods for the ICSI procedure have been described in detail elsewhere (5, 6).

At 16 to 20 hours after ICSI, the oocytes were evaluated for fertilization. Embryo assessment took place 42 to 46 hours and 66 to 70 hours after ICSI.

Statistical analyses were performed using Wilcoxon signed ranks test when appropriate. $P < .05$ was considered to be statistically significant.

Twenty-six patients met the requirements and signed consent to participate in the study. The mean female age was 32.0 (± 3.8) years, and an average of 19.0 (± 9.7) eggs per patient were recovered. A mean of 7.8 (± 5.2) eggs per patient were injected after Hyase treatment, and 7.7 (± 4.7) after Cumulase. An average of 76.2 (± 26.8) seconds was required for complete denudation of the eggs after Hyase, and 76.6 (± 31.3) seconds after Cumulase (not significant). No significant difference was observed in maturation rates

from the metaphase I to metaphase II oocyte between the two groups (Hyase 9.3% \pm 15.0%, Cumulase 12.7% \pm 19.8%; $P = .4887$).

Fertilization rates between the groups were significantly different. The average per-patient fertilization rate was 70.0% \pm 19.6% for oocytes denuded in Hyase and 85.3% \pm 15.1% for those denuded in Cumulase ($P = .015$) (Table 1). Fertilization rates were also significantly different when all mature oocytes at time of ICSI were examined individually (Hyase 70.7% \pm 20.3, Cumulase 84.9% \pm 21.8; $P = .006$) (Table 1).

Cell number was assessed on day 2 and day 3. Average embryonic blastomere number on day 2 and day 3 was not significantly different between the two groups (Table 1).

Embryo morphology on day 3, where “A” represents the highest quality and “D” the lowest quality, took into account blastomere number and symmetry, embryo fragmentation, cytoplasm quality, and multinucleated blastomeres, along with several other parameters. The percentage of “A” quality embryos trended strongly in favor of oocytes denuded in Cumulase but did not reach statistical significance (Hyase “A” embryos 25.0% \pm 27.6, Cumulase “A” embryos 43.4% \pm 28.5; $P = .051$) (Table 1).

Our objective was to test a new enzyme and its effectiveness at denuding the COC. The results of the present study demonstrate that the Cumulase enzyme is effective for denuding oocytes prior to ICSI treatment. Furthermore, the data suggest that Cumulase significantly improves fer-

tilization rates and may positively affect embryo development compared to the bovine testes-derived product Hyase.

The times exposed to each solution were not significantly different between the two groups, indicating that Cumulase is as effective as traditionally used bovine-derived hyaluronidase for disrupting the COC. However, the time oocytes are in each solution is subjective in that each embryologist has different criteria before oocytes are removed.

Many clinics use a hyaluronidase concentration of 80 U/mL, which would lead to shorter exposure times in hyaluronidase. Van de Velde et al. (3) found that a lower concentration of hyaluronidase and a pipette of larger diameter could effectively limit the exposure time of oocytes to hyaluronidase. Furthermore, those authors discuss the fact that the effect on the fetus to long exposure to hyaluronidase is unknown, so finding a protocol that allows for short exposure and effective removal of COC is critical (3).

To assess fertilization, two different aspects were examined: oocytes that were mature after stripping (in vivo mature) and all oocytes that were mature at ICSI (includes in vitro mature). It was important to separate the in vitro matured oocytes from the in vivo matured oocytes owing to the decrease in fertilization rates between the two groups (7). Both of these categories showed statistical significance in favor of Cumulase, illustrating the effectiveness of Cumulase in achieving higher fertilization rates.

The results of the present study show that the Cumulase enzyme is effective for use in an ICSI treatment program for obtaining high fertilization and embryo developmental

rates. It was also demonstrated that the newly developed Cumulase enzyme is similarly effective as the traditional bovine-derived Hyase for disrupting the COC and achieves similar if not improved fertilization and embryo quality.

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