Article

Human recombinant hyaluronidase (Cumulase®) improves intracytoplasmic sperm injection survival and fertilization rates

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Abstract

The cumulus–corona–oocyte complex, composed of cumulus granulosa cells embedded in a matrix of hyaluronan oligosaccharide chains cross-linked by hyaluronan binding proteins and proteoglycans, surrounds each oocyte and must be removed prior to intracytoplasmic sperm injection (ICSI). This is traditionally achieved using enzymatic digestion of the matrix with a bovine-derived hyaluronidase followed by mechanical denudation through pipetting. A human recombinant hyaluronidase (Cumulase®) has been developed with the intent of circumventing the problems and concerns associated with the animal origin and lack of purity of the bovine-derived form of the enzyme. In order to compare the effect of Cumulase with that of the bovine enzyme on the rates of normal fertilization and oocyte damage, a retrospective study using four experienced practitioners was performed. In 2006, using Cumulase, a significantly increased rate of normal fertilization (P = 0.0003) and a significantly decreased rate of oocyte damage (P < 0.0001) were observed compared with 2005, during which time bovine-derived hyaluronidase was predominantly used. This study indicates that Cumulase is safe and effective for use in the removal of the cumulus-corona-oocyte complex prior to ICSI, and may have several distinct advantages over the animal-derived form of the enzyme in terms of safety and efficacy.

Keywords: Cumulase®, human, hyaluronidase, ICSI, oocyte, recombinant

Introduction

Intracytoplasmic sperm injection (ICSI), involving the injection of a single sperm into the cytoplasm of an oocyte using a fine glass needle, was first successfully used in 1992 and has subsequently become the widely accepted treatment for couples with severe male-factor infertility (Van Steirteghem et al., 2002). Indeed, the outcome of several thousands of ICSI cycles in terms of fertilization, embryo cleavage, and implantation is similar to that for conventional IVF in couples with tubal or idiopathic infertility (Van Steirteghem et al., 2002).

When collected, each oocyte is surrounded by a matrix of cells called the cumulus–corona–oocyte complex (CCOC). The CCOC is composed of cumulus granulosa cells embedded in a matrix of long hyaluronan oligosaccharide chains cross-linked by a complex of hyaluronan binding cell surface and extracellular matrix proteins and proteoglycans (Richards, 2005; Russell and Salustri, 2006). Appropriate composition and assembly of the CCOC is essential for ovulation, oocyte maturation, and for fertilization (Wongsrikeao et al., 2005). However, prior to ICSI, the CCOC must be removed in order to visualize, grade, and manipulate the oocyte. Traditionally, this has been achieved using enzymatic digestion of the matrix with a bovine-derived hyaluronidase, followed by mechanical denudation of the CCOC with pipetting (Van Steirteghem et al., 1995; Taylor et al., 2006). However, as a result of the animal origin (bovine) and lack of purity (<10%) of the hyaluronidase agents, there are obvious safety issues to be considered. Moreover, bovine-derived hyaluronidase has been shown to affect oocyte quality
in several areas, including artificial disruption of the CCOC depending on exposure length of the oocyte to hyaluronidase and concentration of hyaluronidase (Van de Velde et al., 1997). A recombinant human hyaluronidase (rHuPH20, Cumulase®; Halozyme Therapeutics, Inc., San Diego, CA, USA) has been developed which may be considered in preference to the bovine-derived product because of its human origin, purity, safety, and effectiveness. Indeed, Cumulase may limit damage to the oocyte, thereby improving fertilization and embryo quality.

In November 2005, four experienced practitioners at the Woking Nuffield Hospital in Surrey, UK switched from traditional bovine-derived hyaluronidase to Cumulase for CCOC dissection prior to ICSI. A retrospective analysis of the yearly rates of normal fertilization and oocyte damage during ICSI procedures for these four ICSI practitioners was performed for the years 2005 and 2006, in order to compare the traditional bovine-derived hyaluronidase with the recently developed recombinant human-derived enzyme.

Materials and methods

Oocyte retrieval and sperm and oocyte handling for ICSI procedures were performed as previously described (Nagy et al., 1995).

Removal of the CCOC using bovine-derived hyaluronidase

Oocytes were exposed to prewarmed bovine-derived hyaluronidase (Sydney IVF, Cook UK) 1–3 h post-oocyte collection for approximately 1 min (and no more than 5 min), during which time they were gently aspirated using a flame-bevelled glass pipette to ensure adequate exposure to the enzyme. Oocytes were then completely denuded using a 140 μm flexipet (Cook Medical), in prewarmed HEPES-buffered oocyte wash solution for approximately 2 min (and no more than 8 min), and then were transferred to a fresh equilibrated pre-ICSI culture dish (1 ml of fertilization media with 1 ml of oil). Oocytes were graded for maturity, and were then returned to the incubator. The ICSI procedure was performed approximately 1–3 h later.

Removal of the CCOC using Cumulase

The removal of CCOC was performed 1–3 h post-oocyte collection and involved exposure to a HEPES-buffered solution containing Cumulase (Rochford Medical, UK). Prewarmed HEPES-buffered oocyte wash solution (0.9 ml, Rochford Medical) was added to the Cumulase-10× vial (containing 0.1 ml of a ×10 solution), and the resultant Cumulase solution was placed in the centre of a well-dish, with 3 ml of oocyte wash solution added to the outer well. Between five and eight oocytes were then transferred into the Cumulase solution and gently aspirated using a flame-bevelled glass pipette to ensure adequate exposure of the oocytes to the Cumulase solution. After 40 s of exposure, the oocytes were washed in the wash solution in the outer well. Unlike with the bovine method, the oocytes were then transferred into a pre-equilibrated culture dish containing 1 ml of SAGE fertilization medium overlaid with 1 ml of SAGE oil, and cultured in an incubator for 20–30 min. The CCOC was then mechanically removed by pipetting with a 140 μm flexipet for approximately 1 min (but no more than 5 min), and the denuded oocytes were placed into a fresh equilibrated pre-ICSI culture dish containing 1 ml of fertilization medium with 1 ml of oil. Oocyte maturity was graded, and then oocytes were returned to the incubator. The ICSI procedure was performed 1–3 h later.

Assessment of fertilization

Oocytes were assessed for fertilization 14–18 h post-ICSI to assess the presence of pronuclei (PN). Fertilization was only considered normal when two distinct PN containing nucleoli were present (2PN). Damaged eggs were identified by diffuse or non-translucent cytoplasm due to the breakdown of the vitelline membrane.

Methods of analysis

A retrospective analysis of the four ICSI practitioners for the years 2005 and 2006 was performed, with particular focus on normal 2PN fertilization rates and the percentage of damaged oocytes. Statistical significance for the four individual practitioners was determined using both a chi-squared test and Fisher’s exact test. Significance for the total number of fertilizations was determined using the Cochran–Mantel–Haenszel test, stratified by practitioner. P-values ≤ 0.05 were considered statistically significant, although there was no adjustment for multiplicity of statistical testing. Significance for the baseline patient characteristics, including the number of oocytes collected during retrieval procedures and patient age, was determined using a two-way analysis of variance with effects for practitioner and year. P-values ≤0.05 were considered statistically significant.

Results

The baseline patient characteristics are presented in Table 1. In 2005, during the majority of which time bovine-derived hyaluronidase was utilised for CCOC removal, there were a total of 227 oocyte retrieval procedures, with an average of 11.5 oocytes collected per procedure. In 2006, during which time Cumulase was exclusively utilized for CCOC removal, there were a total of 313 oocyte retrieval procedures, with an average of 10.8 oocytes collected per procedure. The average ages of the patient at the time of retrieval in 2005 and 2006 were 35.57 and 35.38 years of age respectively. There were no significant differences between 2005 and 2006 in terms of the number of oocytes collected per procedure or the average age of the patient.

In 2005, the four practitioners in this study injected a total of 2010 oocytes (Table 2). During the majority of this period (January to October), bovine-derived hyaluronidase was utilized for CCOC removal. The mean damage and normal (2PN) fertilization rates for the four practitioners were 8.46% (range 6.62–9.74%) and 68.86% (range 61.97–73.54%) respectively. In 2006, using Cumulase exclusively for CCOC removal, the four practitioners injected a total of 2470 oocytes. During this period, the mean total damage rate [3.80% (range 2.33–3.80%)] was significantly [P < 0.0001 (CMH test)] decreased compared with the mean total rate in 2005, as were the damage rates for the individual practitioners A [P < 0.0001 (chi-squared and Fisher’s
The aim of this study was to compare the yearly rates of normal (2PN) fertilization and oocyte damage using Cumulase, a recombinant human form of hyaluronidase, and the traditional bovine-derived form of the enzyme, for CCOC removal prior to ICSI. The results indicate that Cumulase is safe and effective as a method of CCOC removal and suggest that the rate of normal fertilization is significantly increased and the rate of oocyte damage significantly decreased compared with the rates historically achieved using the traditional bovine form of the hyaluronidase enzyme. This study reflects the improvements in fertilization and oocyte damage rates using Cumulase compared with the bovine-derived enzyme previously reported in prospective blinded sibling oocyte studies (Nagy et al., 2005; Taylor et al., 2006).

The animal origin (bovine) and lack of purity (<10%) of the traditional hyaluronidase agents result in a number of concerns in the clinical setting. These include the risk of transmission of animal-derived pathogens or contaminants, batch-to-batch consistency, and effects on oocyte quality. In order to combat possible adverse effects on the oocyte, studies have been performed to decrease possible toxic effects of bovine-derived hyaluronidase on the oocyte by decreasing the concentration and the length of exposure time (Van de Velde et al., 1997; Joris et al., 1998). It is anticipated that a human recombinant form of the enzyme should represent a safer and more effective agent, with no risk of viral and other pathogen transmission and a consistent activity (Yocum et al., 2007). Further work will be required to elucidate the mechanisms involved in damage caused to oocytes with the use of bovine hyaluronidase.

While this study does indicate a significant advantage to using Cumulase for CCOC removal compared with traditional bovine-derived enzyme, there are some potential limitations to the current study that should be considered. This was a
retrospective study with a historical control and a number of important factors could potentially have changed during the 2-year period. The equipment used for ICSI is known to be critical for the success of the procedure, and improvements in such equipment have been shown to markedly improve the outcome of ICSI (Joris, 1998). A new microscope with new microinjectors was introduced during the 2-year period of the current study and may have contributed to the improved observations observed. It should be noted that the ICSI equipment was updated to a newer model from the same manufacturer and thus was a similar and familiar set-up. With the exception of the denudation protocol (i.e. Cumulase versus traditional bovine-derived enzyme exposure after which mechanical denudation was delayed by 20–30 min in the Cumulase group only), no other laboratory technique or product, including tissue culture media, was changed. No other drug regimes and stimulatory protocols altered, and the clinical management of cycles was conducted by the same experienced consultants. Another issue that could have influenced the results is patient age. However, it should be noted that the average patient age at retrieval was consistent between 2005 and 2006. These observations help support the premise that the improvements demonstrated in the rate of normal fertilization and oocyte damage were due to the use of Cumulase and not the result of differences in other laboratory procedures/products or in the patient populations.

In addition, the practitioners in this study were all experienced (with a minimum of 3 years of ICSI clinical experience prior to the start of 2005), and so it is unlikely that increased technical competence would lead to the improvement observed (Dumoulin et al., 2001; Rosen et al., 2006). It should also be noted that the data presented here involve oocyte survival and fertilization rate, immediate endpoints of the ICSI procedure. While outcomes such as embryo quality or pregnancy rate may represent more clinically relevant outcomes of the ICSI procedure, they were considered too complex and potentially affected by too many external factors to be considered reliable for comparison in a retrospective study such as this. Finally, it should also be acknowledged that the improvements observed in this study between 2005 and 2006 may have actually been greater had bovine-derived enzyme been used for the entire year of 2005, rather than only from January to October.

In conclusion, this study suggests that for use in CCOC removal prior to the ICSI procedure, Cumulase is safe and effective and has several significant advantages over the traditional bovine-derived hyaluronidase, as demonstrated by increased normal fertilization rates and decreased oocyte damage rates. Moreover, this retrospective study confirms the findings of several prospective blinded sibling oocyte studies (Nagy et al., 2005; Taylor et al., 2006) in a real-life clinical setting (i.e. no patient inclusion/exclusion criteria) over an extended period of time. These clinical advantages justify the continued use of Cumulase in the ICSI setting.

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References


Richards JS 2005 Ovulation: new factors that prepare the oocyte for fertilization. Molecular and Cellular Endocrinology 29, 75–79.


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