

## Enzymatic characterization of zona pellucida hardening in human eggs and embryos.

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**PURPOSE:** To characterize possible hardening of the human zona pellucida (ZP) and evaluate the effect of culture duration, patient age, and ZP thickness, ZP of unfertilized eggs (experiment 1, n = 367; experiment 2, n = 174) and abnormal embryos (experiment 1, n = 52) were randomly designated for alpha-chymotrypsin treatment after 0, 24, 48, 72, 96, 120 h (experiment 1) and 48 h, 72 h, and 1 week (experiment 2) of in vitro culture in HTF medium supplemented with 0.5% human serum albumin. Mean ZP thickness was predetermined in experiment 2.

**METHODS:** The dispersion of the ZP glycoproteins was assessed, and the duration of time for complete digestion was recorded as an index of ZP hardness.

**RESULTS:** In experiment 1, enzyme digestion duration increased ( $P < 0.05$ ) in the first 24 h in vitro from 18.0 +/- 2.0 to 34.6 +/- 2.5 min, and tended to decrease over the next 4 days in culture (25.2 +/- 1.3, 29.4 +/- 0.9, 27.3 +/- 0.6, 26.6 +/- 1.1, and 20.7 +/- 1.5 min on Day 2-6 ZP, respectively). Zona hardening of fertilized eggs was revealed by a longer ( $P < 0.01$ ) digestion time (32.2 +/- 1.8 vs 25.8 +/- 0.6 min).

**CONCLUSIONS:** There were significant patient-to-patient variation (16.4 +/- 0.7 to 39.6 +/- 2.2 min); however, age was not correlated to enzyme digestion duration. In experiment 2 we determined that ZP thickness (range 8.4-21.6 microns; mean 14.6 +/- 0.2 microns) was not correlated ( $r = 0.09$ ) to the digestion interval (mean 24.3 +/- 0.8 min). Based on our enzymatic ZP digestion measurements, it is apparent that spontaneous zona hardening does occur within 24 h of in vitro culture, similar to levels achieved postfertilization. The data do not support, however, the concept that additional, abnormal hardening of the ZP occurs during extended culturing.