P2

How many sperm to inseminate with? Results from a pilot study

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INTRODUCTION:
Standard *in vitro* fertilization procedures employ the combined effect of multiple spermatozoa to dissociate cumulus cells surrounding the oocyte during insemination, attachment to the zona pellucida and ultimate fertilization of the oocyte. Based on sperm parameters, the insemination concentration is a balance between sperm concentration and optimal fertilization.
The hemizona bioassay was selected to portray the binding of spermatozoa to oocytes, since supra or sub-minimum sperm concentrations per insemination can compromise fertilization outcome *in vitro*.

AIM:
The aim of this study was to determine the minimum spermatozoa concentration that would still provide adequate sperm-zona binding as indicated by the hemizona assay.

MATERIALS AND METHODS:
Unfertilized non-viable oocytes (n=107) obtained through a standard IVF cycle, were bisected using a micromanipulator. Zona halves were placed in 50µL fertilization media drops and incubated with six different test concentrations (0.05, 0.1, 0.2, 0.5, 1.0 & 2.0 x 10^4 motile sperm, with a mean of 6% normal sperm morphology), with 15 experimental repeats. Zona halves were randomly placed in either the test or control droplet containing 5.0 x 10^4 sperm. Dishes were incubated at 6% CO_2 for 18 hours, where after zona halves were washed in fresh media and the number of sperm bound to each zona half was counted independently by two qualified embryologists.

RESULTS:
Zona-halves from the control group with <50 spermatozoa bound were identified and the data from the control and the corresponding test hemi-zona, was excluded. The remaining zonae were analyzed and the number of sperm bound evaluated. The average number of sperm bound was 12 ± 6, 30 ± 26, 31 ± 18, 46 ± 27, 77 ± 42, 106 ± 77, 116 ± 33 (0.05, 0.1, 0.2, 0.5, 1.0 & 2.0 x 10^4 sperm inseminated). Percentage of zonae with >30 and >50 sperm bound was 0 and 0%, 22 and 11%, 47 and 11%, 68 and 37%, 94 and 63%, 100 and 88%, 100 and 100% (0.05, 0.1, 0.2, 0.5, 1.0 & 2.0 x 10^4 sperm inseminated) .

CONCLUSION/DISCUSSION:
A direct association between insemination concentration and sperm bound to the zona pellucida was observed. Comparing lower sperm doses to a standard insemination number of 5 x 10^4 spermatozoa, insemination with 0.5 x 10^4 spermatozoa has a 37% possibility to present with >50 spermatozoa bound to the hemizonae, and similarly a 68% likelihood >30 bound spermatozoa. The average number of sperm bound to a hemizona after insemination with a dose of 0.5 x 10^4 spermatozoa was 46 ± 27. It is possible that insemination with <0.05 x 10^4 spermatozoa may negatively influence fertilizing potential *in vitro*. Using a minimum of 20 sperm-zona binding as a cut-off indicator, no less than 0.1 x 10^4 spermatozoa should be used for insemination.