prepared for each patient. Microdrops for each concentration of HSF in medium were placed under oil and equilibrated in an atmosphere of 5% CO_2 in air at 37°C for 6 h before culture. The osmolarity and pH of the HSF were measured before and after equilibration. To evaluate embryo development, nine to 15 embryos were cultured in each microdrop and the percentage of blastocysts after 72 h was examined. To assess the mitotic activity of the embryos, all embryos were fixed by 1% glutaraldehyde in PBS and stained with Hoechst 33342. Then the number of cells in each embryo was counted.

Results: The average pH and osmolarity of HSF were 8.2 and 283.8 mOsm respectively. After equilibration in a 5% CO_2 incubator, the pH value changed to 7.47, which was within the physiological range. The ratios of blastocyst development were not significantly different between each concentration of the patients' HSF ($P = 0.34$). The mean ratios of blastocyst development in media containing 0 (T6, T6 + BSA), 10, 50 and 100% HSF were not significantly different, i.e. 88.9, 85.4, 89.9, 89.6 and 75.6% respectively ($P > 0.05$). The numbers of blastocyst were 57, 52, 80, 79 and 70 respectively. Mean ± SEM cell numbers in the blastocysts were significantly decreased in 50 and 100% HSF [90.0 ± 2.9, 93.1 ± 4.1, 92.9 ± 2.7, 82.6 ± 3.5[a] and 62.5 ± 2.6[a] for 0 (T6, T6 + BSA), 10, 50 and 100% HSF respectively; [a]$P < 0.01$, ANOVA and Duncan's multiple range test].

Conclusion: The ratio of blastocyst development *in vitro* was not significantly affected. However, the mitotic activity of the blastocyst decreases in high concentrations of HSF. Therefore HSF does not have an embryotoxic effect but does have a mild adverse effect on embryo development. Further studies on the capacity of the embryo to implant are required to evaluate the negative effect of hydrosalpinx.

Andrology 02

Monday 23 June 1997
Hall D: Tinto Suite

17.15–17.30

O-075. Induction of the acrosome reaction by angiotensin II

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Introduction: The physiological functions of the renin–angiotensin system on male fertility have not yet been clarified. Angiotensin-converting-enzyme (ACE), which converts angiotensin I into angiotensin II, has been shown to be released from human spermatozoa during capacitation. Therefore the purpose of this study was to assess the influence of angiotensins I and II on the acrosome reaction.

Materials and methods: After glass wool filtration and two washing steps in HTF medium (1% HSA), spermatozoa were treated with 0.1–10.0 µM angiotensin I or 10 nM–100 µM angiotensin II for 6 h at 37°C. In addition, spermatozoa pretreated with an angiotensin II antagonist specific for AT-2 receptors (1–100 µM) were incubated with 100 nM angiotensin II for 4 h at 37°C. The percentages of living acrosome-reacted spermatozoa were determined by triple staining and compared with the corresponding values obtained after incubation with HTF medium–HSA (negative control) and 10 µM ionophore A 23187 (30 min after 3.5 h capacitation; positive control).

Results: Compared with the negative control, the percentage of acrosome-reacted spermatozoa was increased significantly after treatment with 100 nM angiotensin II (15.1 ± 4.3 versus 9.9 ± 3.4% respectively; $n = 11$; $P < 0.05$), whereas angiotensin I failed to induce the acrosome reaction. The specificity of acrosome reaction induction by angiotensin II was proved by pretreatment of the spermatozoa with an angiotensin II inhibitor. In these experiments the percentages of acrosome-reacted spermatozoa were comparable with those of the negative controls (3.9 ± 2.5 versus 4.4 ± 1.5% respectively; $n = 5$). The effect of angiotensin II on the acrosome reaction was not observed in calcium-free medium.

Conclusion: The renin–angiotensin system may be involved in induction of the human sperm acrosome reaction. The effect of angiotensin II on this exocytic process seems to be mediated by a calcium-dependent process.

17.30–17.45

O-076. Double fluorescence labelling of acrosin and tubulin in human spermatozoa: a rapid diagnostic procedure to identify sperm samples with poor fertilizing ability

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Introduction: Acrosin and tubulin are structural components of the spermatozoon involved in zona pellucida digestion and tail movement respectively. Their cellular localization can be achieved easily by indirect immunofluorescence. In a previous study using polyclonal antibodies they were shown to be involved in the fertilization process. The commercial availability of monoclonal antibodies against pro-acrosin and tubulin has renewed the interest for a rapid diagnostic test to identify those sperm samples capable of fertilizing *in vitro*. The purpose of this study was (i) to correlate the indirect immunofluorescence and morphology (strict criteria) results with the fertilization rates obtained after regular IVF or ICSI and (ii) to provide biologists with a quick and efficient tool for the selection of the most appropriate fertilization technique (IVF or ICSI).

Materials and methods: A 5 µl aliquot from a washed sperm suspension used for regular IVF or ICSI was smeared onto a microscopic slide, fixed and kept at –20°C until use. Monoclonal antibodies against pro-acrosin (Biomérieux, France) or

tubulin (Sigma, USA) were used for indirect immunofluorescence assessment of the sperm acrosome and tail in a double labelling assay. The percentages of spermatozoa exhibiting normal acrosomes or normal tails were recorded separately by a single observer unaware of the fertilization outcome using an epifluorescence microscope (×400, MTII; Olympus). The whole indirect immunofluorescence procedure was performed in <1 h. A Papanicolaou-stained smear was prepared in parallel for morphological assessment using Kruger's strict criteria.

Results: Double immunofluorescent labelling of pro-acrosin and tubulin allowed the easy identification of both acrosomal and tail defects in a single reading. In regular IVF, fertilization rates (zygotes/oocytes, %) were strongly correlated ($P < 0.001$) with the percentages of normal fluorescence patterns, allowing the identification of threshold values (60% normal acrosomes; 80% normal tails) below which the fertilization rates were 0–30%. In contrast, a much weaker correlation was found with the morphology determinations, for which 30% fertilization rates were still obtained even with only 0–10% normal forms. In ICSI cycles, the fertilization rates were >50% and correlated with neither the indirect immunofluorescence results nor the percentage of morphologically normal forms.

Conclusions: The indirect immunofluorescence procedure described can be easily implemented in a busy IVF unit. It provides clinicians and patients with more detailed descriptions of sperm abnormalities. In cases of abnormal spermatozoa, it helps biologists to choose rapidly which fertilization technique should be employed (IVF or ICSI).

17.45–18.00

O-077. Immunocytochemical analysis of oscillin in spermatozoa from a globozoospermic patient

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Recently we identified a globozoospermic patient with spermatozoa that were totally incapable of activating either mouse or human oocytes following ICSI. Because the 33 kDa protein oscillin has been shown to be the sperm-derived protein that is a probable factor responsible for oocyte activation following ICSI, as well as during natural fertilization, an immunocytochemical analysis was performed to determine the level of expression of this protein in the spermatozoa of the globozoospermic patient.

Materials and methods: Indirect immunocytochemistry was carried out with a mouse monoclonal antibody (J4A3) raised against hamster sperm oscillin, that cross-reacts with a related epitope in boar and human spermatozoa. A fresh semen sample was obtained from the patient and washed twice in M2 medium. A sperm fraction with an increased proportion of living spermatozoa was prepared by Percoll centrifugation (25, 50, 75 and 90% isotonic Percoll) and washed twice in PBS. At this stage, sperm motility was evaluated to be 50%, and half of the spermatozoa were treated by two cycles of unprotected freeze–thawing (2×UFT). Both portions were processed for immunocytochemistry as described earlier by Parrington *et al.* (1996). The swim-up fraction with 100% motility obtained from a healthy donor was used as a positive control. The same fraction treated by 2×UFT was used as a negative control.

Results: A bright equatorial staining in almost 100% of intact donor spermatozoa was observed with J4A3 under the fluorescence microscope. This staining was clearly observed in only 5% of 2×UFT-treated donor spermatozoa. In another 2% a weak staining at the equator was observed. This demonstrated the disappearance of the epitope in most of the sperm cells after membrane permeabilization by freezing. A weak, homogeneous, unspecific staining of the sperm cytoplasm was observed with J4A3 in all round-headed spermatozoa from the globozoospermic patient. Furthermore, no changes in staining intensity were found by two independent observers between intact and 2×UFT-treated spermatozoa from the patient.

Conclusions: The oscillin epitope, recognizable by the J4A3 antibody, is abnormal in spermatozoa from the patient with globozoospermia associated with oocyte activation deficiency. This may reflect an altered level of expression or mutation of the gene for this protein. Further molecular analysis of such cases may help to provide an insight into the regulation of oscillin gene expression during spermatogenesis and reveal the mechanisms of pathology in globozoospermic patients.

Reference:

Parrington *et al.* (1996) *Nature*, **379**, 364–368.

18.00–18.15

O-078. A peptide related to fertilization-promoting peptide (FPP) can inhibit FPP's stimulation of capacitation: a possible cause of male subfertility?

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Introduction: Fertilization-promoting peptide (FPP; pGlu–Glu–ProNH_2) is structurally similar to thyrotrophin-releasing hormone (TRH) and is present in the prostate gland and seminal plasma of several mammalian species. It is found in human seminal plasma at a mean concentration of ~50 nM. FPP has been shown not only to stimulate capacitation but to inhibit spontaneous acrosome loss in mouse spermatozoa, suggestive of a role *in vivo* for maximizing the fertilizing potential of the few spermatozoa to reach the ampulla. Here we report that FPP-related peptides vary in their ability to modulate sperm function, with some having no effect but one being able to competitively inhibit the stimulatory effects of FPP.

Materials and methods: Epididymal sperm suspensions were prepared, treated as detailed below and assessed for functional state using either chlortetracycline (CTC) fluorescence (for capacitation/acrosome reaction) or IVF (for fertilizing ability).