Materials and methods: Using image analysis techniques to monitor intracellular calcium concentrations in human spermatozoa loaded with Fura 2/AM (4 μM), the responsiveness of these cells to 5 μM progesterone was investigated under conditions that promoted or suppressed the tyrosine phosphorylation status of the spermatozoa, as determined by a Western blot analysis.

Results: In the untreated controls, exposure to progesterone after 3 h capacitation in Biggers–Whitten–Whittingham medium was followed by a single calcium transient in 75% of cells (n = 70), while in a further 20% of spermatozoa this primary transient was followed by a series of secondary oscillations. If tyrosine phosphorylation was promoted with pentoxifylline (3 mM), the response to progesterone was enhanced in parallel with an increased proportion of cells exhibiting secondary calcium oscillations (38%; n = 90). Conversely, when spontaneous tyrosine phosphorylation was inhibited using the reducing agent 2-mercaptoethanol (0.05%), the biological response to progesterone was suppressed and only 12% of cells (n = 147) exhibited such secondary oscillations.

Conclusions: Although studies have shown that the primary influx of calcium does not operate through a voltage-sensitive channel (Aitken *et al.*, 1996a), it is known that this influx is accompanied by a chloride efflux (Meizel and Turner, 1995) which causes the membrane to depolarize. This depolarization may then cause voltage-sensitive calcium channels to open, enabling oscillations to occur. Therefore the ability of voltage-sensitive channel antagonists to inhibit these secondary calcium transients is currently being investigated.

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P-165. Purification and characterization of NADPH oxidase in human spermatozoa

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Introduction: The biochemical events involved in the capacitation of human spermatozoa are thought to be redox regulated (De Lamirande and Gangon, 1993; Aitken *et al.*, 1995). It is therefore postulated that human spermatozoa contain an enzyme system capable of producing reactive oxygen species (ROS), one component of which is thought to be an NADPH oxidase. In this study we present definitive evidence for an NADPH oxidase complex in human spermatozoa that is localized on the sperm plasma membrane and is sensitive to flavoprotein inhibitors such as diphenylene iodonium and quinacrine. We also succeeded in developing strategies for isolation by using a series of purification procedures and a biochemical assay to detect ROS production.

Results: The addition of NADPH to viable populations of motile spermatozoa induced a sudden dose-dependent increase in the rate of superoxide generation via mechanisms that could not be disrupted by inhibitors of the mitochondrial electron transport chain (antimycin A, rotenone, carbonyl cyanide *m*-chlorophenylhydrazone and sodium azide), diaphorase (dicoumarol), xanthine oxidase (allopurinol) or lactic acid dehydrogenase (sodium oxamate). However, NADPH-induced ROS generation could be stimulated by permeabilization and was negatively correlated with sperm function. Both NADH and NADPH were active electron donors in this system, while NAD^+ and $NADP^+$ exhibited little activity. Stereospecificity was evident in the response in that only the b isomer of NADPH supported superoxide production. The involvement of a flavoprotein in the electron transfer process was indicated by the high sensitivity of the oxidase to inhibition by diphenylene iodonium and quinacrine. The oxidase activity from human spermatozoa was purified by a combination of Mono Q anion exchange and size-exclusion chromatography. The purified protein was subjected to 10% PAGE. Bands at 30 and ~40 kDa were identified on silver staining. Isoelectric focusing was performed on the purified protein using a narrow pI range of 6.1–7.9 and two bands of activity were identified.

Conclusion: An NADPH-dependent oxidase was successfully isolated from human spermatozoa and was shown to produce ROS. Further studies will be needed to characterize this complex and determine its relationship with the classic NADPH oxidase of phagocytic leukocytes. Western blots on antibodies raised in rabbits and immunocytochemistry to localize its position within the spermatozoa will be performed.

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P-166. Duration of surgery increases adhesion formation in a rabbit model

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The aim of this study was to assess the influence of training, intra-operative blood, drying of peritoneal surfaces and duration of CO_2 pneumoperitoneum on adhesion formation in a rabbit model. The study was based at the Centre for Surgical Technologies, Katholieke Universiteit Leuven, Belgium.

Materials and methods: In mature female white New Zealand rabbits a standardized surgical trauma was induced to the peritoneal surface and uterine horn (opposing lesion), and to the pouch of Douglas (non-opposing lesion) during a standard three-puncture laparoscopy with conventional endoscopic equipment. Adhesions (type and extension) were scored at the injury and extragenital sites by visual assessment performed during laparoscopy after 7 days.