## 32. THE EXPRESSION OF OVULATORY MEDIATORS BY MACROPHAGES ISOLATED FROM THE GONADOTROPHIN-STIMULATED MOUSE OVARY

*K.H. Van Der Hoek, N.K. Ryan, S.A. Robertson and R.J. Norman*
Reproductive Medicine Unit, Department of Obstetrics and Gynaecology, University of Adelaide, The Queen Elizabeth Hospital, SA.

IL-1beta (IL-1β), TNF alpha (TNFα), nitric oxide (NO) and macrophages have been shown to be important in stimulating the ovulatory event [1-4]. Since macrophages produce these mediators elsewhere, it was our aim to determine if ovarian macrophages are a source of these known ovulatory mediators. Ovaries from gonadotrophin-primed immature mice were removed at various times and enzymatically digested. Macrophages were tagged with the specific antibodies F480 or anti-MHC II (anti- Ia) and isolated using antibody panning. mRNA was isolated from some macrophages and analysed by quantitative RT-PCR; other macrophages were cultured and secreted protein levels measured. mRNA levels, expressed as fold changes from the first isolation time point and normalised to the housekeeper gene HPRT, show that ovarian macrophages exhibit regulated cytokine mRNA profiles in response to hCG. Both IL-1β and TNFα mRNA increased significantly (*) post hCG injection (IL-1β:Ia. 7.3 ± 2*, F480 5.1 ± 0.8*, TNFα:Ia 2.39 ± 0.5, F480 2.47 ± 0.21*). Secreted TNFα protein increased 6 h post hCG and increased further 24 h post ovulation and then returned to pre-hCG levels 48 h after ovulation. Prior to hCG administration IL-1β was not detectable in conditioned media, but could be detected in media from Ia cells following hCG administration. No changes in total NO activity were detected across the stimulated cycle. Therefore, ovarian macrophages exhibit regulated cytokine expression, particularly of TNFα, in response to hCG. Since TNFα is known to modulate follicular rupture, its production by ovarian macrophages may contribute to the ovulatory process.

(1) Brannstrom M *et al*. (1993) *Endocrinology* **132**: 399-404. (2) Bonello N *et al*. (1996) *Biol. Reprod*. **54**: 436 445. (3) Brannstrom M *et al*. (1995) *Reprod. Fertil. Dev.* **7**: 67-73. (4) Van der Hoek KH *et al*. (2000) *Biol. Reprod.* **62**: 1059-1066.