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Effects of relaxin in a co-culture of Sertoli and germ cells

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Summary

Spermatogenesis is controlled by FSH, testosterone and paracrine factors produced by Sertoli cells. The knockout of relaxin decreases sperm maturation in mice. Studies from our laboratory have shown that relaxin and its receptor RXFP1 are expressed in rat Sertoli cells, and exogenous relaxin stimulates Sertoli cell proliferation. Relaxin receptors are also detected in the rat germ cells at specific stages of development. Relaxin could therefore affect spermatogenesis either indirectly, by stimulating Sertoli cell proliferation, or directly, by affecting germ cells. The aim of the present study was to explore a role of relaxin at specific stages of spermatogenesis using a co-culture of rat Sertoli and germ cells. Relaxin seems to increase the number of pre-meiotic and meiotic cells.

The role of relaxin in male reproduction is still unclear (Ivell *et al*., 2011). In the reproductive tract of human males, relaxin is mainly produced by the prostate and secreted into the seminal fluid, where it seems to play a role in sperm function. It has been found recently that RXFP1 is expressed in human spermatozoa and that relaxin stimulates sperm motility, mitochondrial function, apoptosis, capacitation and acrosome reaction (Ferlin et al., 2011), providing additional evidence that relaxin is important for fertilizing ability and preservation of sperm functionality.

The knockout of relaxin decreases sperm maturation (Samuel et al., 2003), and studies from our laboratory and others have shown that the testes are also a source of relaxin (Cardoso et al., 2010; Kato et al., 2010). Relaxin and RXFP1 are expressed in rat Sertoli cells (Filonzi et al., 2007), and relaxin stimulates Sertoli cell proliferation (Cardoso et al, 2010; Nascimento et al., 2012), which could indirectly affect spermatogenesis. We also detected RXFP1 in elongating spermatids, which suggests that relaxin may directly affect spermatogenesis. To investigate a role of relaxin in spermatogenesis, we developed a co-culture of Sertoli and germ cells. Cells were extracted from 7-day old rats, and plated on Matrigel that was depleted of diffusible components by incubation with growth medium to avoid interference of diffusable growth factors in germ cell differentiation. Cells were then cultured for 48 h in the absence or presence of 0.5% fetal bovine serum (FBS) or 100 ng/mL H2 relaxin. Cells were then kept for 24 h in supplement-free medium, and cultured for an additional 48 or 120 h in the absence or presence of FBS or relaxin. Cell number and differentiation were analyzed by immunohistochemistry, confocal microscopy and flow cytometry. The ratio between germ and somatic cells (G/S) fell drastically from culture days 2 to

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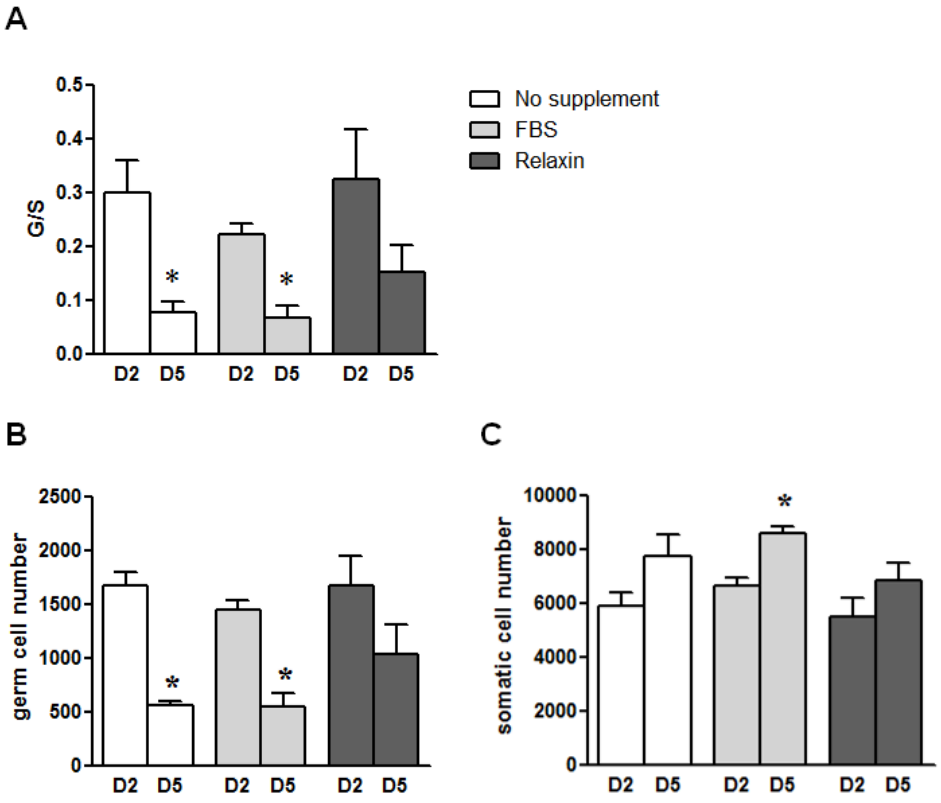

Figure 1. Somatic and germ cell number after 2 and 5 days in culture, in the absence or presence of FBS or relaxin. Data are expressed as means ± SEM of 3 independent experiments (different co-cultures). A. G/S: ratio between total germ cell number and total somatic cell number. B. Total number of germ cells. C. Total number of somatic cells. FBS: 0.5% fetal bovine serum; Relaxin: 100 ng/mL H2-relaxin. *Indicates statistically significant difference ($P<0.05$, t test). No statistical difference was detected between different supplementations at the same period of culture.

5, and this fall was not affected by FBS but it was less pronounced when cells were treated with relaxin (Figure 1A). Relaxin did not affect the proportion of somatic cells (Figure 1C), but seemed to preserve the germ cells (Figure 1C), especially diploid and tetraploid germ cells (spermatogonia and spermatocyte). Furthermore relaxin seemed to favor organization of cells in tubular-like structures. These findings suggest that relaxin may play a role in spermatogenesis by increasing the number of pre-meiotic and meiotic germ cells and affecting testicular architecture.

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