Supplementary material and methods accompanying:

Immune activation suppresses plasma testosterone level; A meta-analysis

Jelle J. Boonekamp[1], Albert H.F. Ros[2] & Simon Verhulst[1,*]

1. Behavioural Biology, University of Groningen,
P.O.Box 14, 9750 AA Haren, the Netherlands
2. Eco-Ethology Research Unit, ISPA, Lisbon, Portugal

* correspondence: s.verhulst@rug.nl

1. Search and selection of studies

We searched using the ISI Web of Knowledge and Medline (NCBI), using combinations of multiple keywords: e.g. testosterone, androgens, parasites, immunocompetence, srbc, lps, vaccination and sickness behavior, and by checking references of the relevant papers.

We selected studies that used adult male subjects and included an in vivo immune challenge followed by T plasma measurements. More specifically, studies were included in the meta-analysis when they satisfied the following criteria: (1) the immune system was challenged experimentally using parasites, viruses, or non-living antigens, and testosterone was subsequently measured. (2) The effect of the immune challenge could be compared with a proper control group, thus studies that used each individual as a 'self' control, without controlling for possible time-period effects, were excluded. (3) It had to be possible to extract the data in a way suitable for the meta-analysis.

2. Effect size calculations

For each experiment included in the meta-analysis we here describe how the effect size (r) was calculated. Numbers correspond to study numbers in table 1 in the main paper. References to tables and figures refer to data sources in that particular study. We calculated test statistics ourselves when these were not provided, using n, mean and S.D. when available, or measuring these from graphs when necessary. We computed exact P-values when possible when only threshold values were reported (e.g. $P<0.05$).

1 Hales et al (2000): *P*-value from 2-sample *t*-test ($t = 3.589$) calculated by us using *n*, means and standard deviations from paper (measured in figure 1a).

2 Barthelemy et al (2004): Average *P*-value from two successive sampling points (*t*-test statistics from paper: $t_{14} = -3.7$, $t_{14} = -3.86$).

3 Isseroff et al (1986): *P*-value from *t*-test ($t = 9.293$) calculated by us using *n*, means and standard deviations in text from paper.

4 He et al (2000): *P*-value directly from author.

5 Weil et al (2006): *P*-value from test statistic from paper ($F_{1, 60} = 38.815$).

6 Kasilima et al (2004): *P*-value from test statistic ($t = 0.998$) calculated by us, using *n*, weighed means and standard deviations. Multiple sample points were used (table 2b), and we averaged over sampling points (weighted by sample size).

7 O'Bryan et al (2000): *P*-value from *t*-test ($t = 5.673$) calculated by us, using *n*, weighed means and standard deviations. Multiple sample points were used (figure 1c), and we averaged over sampling points (weighted by sample size).

8 O'Bryan et al (2000): See study 7 ($t = 4.844$, data from figure 2c).

9 Mutayoba et al (1997): *P*-value from *t*-test ($t = 1.593$) calculated by us using *n*, means and

standard deviations from paper (table 1).

10 Garamszegi et al (2004): *P*-value directly from paper.

11 Boltz et al (2004): *P*-value directly from paper. (*P*-value from *t*-test (t = 2.067) calculated by us using *n*, means and standard deviations from table 1, resulted in P = 0.058. However, the authors report $P< 0.05$, a difference that could perhaps be a rounding effect. We assumed P=0.05 in our analyses).

12 Boltz et al (2007): *P*-value from *t*-test (t = 1.436) calculated by us using *n*, means and standard deviations in table 4, comparing experimental groups Nonvac with Lvac.

13 DeVaney et al (1977): *P*-value directly from paper.

14 Verhulst et al (1999): *P*-value from ordered heterogeneity test-statistic from paper ($F_{2, 77}$ = 4.93, $r_s P_c$ = 0.99).

References

Barthelemy, M., Gabrion, C. & Petit, G. 2004 Reduction in testosterone concentration and its effect on the reproductive output of chronic malaria-infected male mice. *Parasitol Res* **93**, 475-481.

Boltz, C. R., Boltz, D. A., Bunick, D., Scherba, G. & Bahr, J. M. 2007 Vaccination against the avian infectious bronchitis virus affects sperm concentration, sperm quality and blood testosterone concentrations in cockerels. *Brit Poultry Sci* **48**, 617-624.

Boltz, D. A., Nakai, M. & Bahr, J. M. 2004 Avian infectious bronchitis virus: A possible cause of reduced fertility in the rooster. *Avian Dis* **48**, 909-915.

Devaney, J. A., Elissalde, M. H., Steel, E. G., Hogan, B. F. & Delvarpetersen, H. 1977 Effect of Northern Fowl Mite, Ornithonyssus-Sylviarum (Canestrini and Fanzago) (Acarina Dermanyssidae) on White Leghorn Roosters. *Poultry Sci* **56**, 1585-1590.

Garamszegi, L. Z., Møller, A. P., Torok, J., Michl, G., Peczely, P. & Richard, M. 2004 Immune challenge mediates vocal communication in a passerine bird: an experiment. *Behav Ecol* **15**, 148-157.

Hales, K. H., Diemer, T., Ginde, S., Shankar, B. K., Roberts, M., Bosmann, H. B. & Hales, D. B. 2000 Diametric effects of bacterial endotoxin lipopolysaccharide on adrenal and leydig cell steroidogenic acute regulatory protein. *Endocrinology* **141**, 4000-4012.

He, H. B., Ohta, N. & Kawaguchi, H. 2000 Effect of *Schistosoma japonicum* infection on serum testosterone levels in mice. *Zhongguo Ji Sheng Chong Xue Yu Ji Chong Bing Za Zhi.* **18**, 173-175.

Isseroff, H., Sylvester, P. W. & Held, W. A. 1986 Effects of Schistosoma mansoni on androgen regulated gene-expression in the mouse. *Mol Biochem Parasitol* **18**, 401-412.

Kasilima, Y. S., Wango, E. O., Kigondu, C. S., Mutayoba, B. M. & Nyindo, A. 2004 Plasma bioactive LH and testosterone profiles in male New Zealand rabbits experimentally infected with Schistosoma mansoni. *Acta Trop* **92**, 165-172.

Mutayoba, B. M., OShaughnessy, P. J., Jeffcoate, I. A., Eckersall, P. D., Cestnik, V. & Holmes, P. H. 1997 Effect of experimental infection with Trypanosoma congolense and scrotal insulation on Leydig cell steroidogenesis in the ram. *Theriogenology* **48**, 411-422.

O'Bryan, M. K., Schlatt, S., Phillips, D. J., de Kretser, D. M. & Hedger, M. P. 2000 Bacterial lipopolysaccharide-induced inflammation compromises testicular function at multiple levels in vivo. *Endocrinology* **141**, 238-246.

Verhulst, S., Parmentier, H. K. & Dieleman, S. J. 1999 A trade-off between immunocompetence and sexual ornamentation in domestic fowl. *Proc. Natl. Acad. Sci. USA* **96**, 4478-4481.

Weil, Z. M., Bowers, S. L., Pyter, L. M. & Nelson, R. J. 2006 Social interactions alter proinflammatory cytokine gene expression and behavior following endotoxin administration. *Brain Behav Immun* **20**, 72-79.