Beneficial Microbes, September 2011; 2(3): 177-181

Wageningen Academic Publishers

OPINION PAPER

Quo vadis – EFSA?

G. Reid

Canadian Research & Development Centre for Probiotics, Lawson Health Research Institute, 268 Grosvenor St., London N6A 4V2 Ontario, Canada; gregor@uwo.ca

Received: 18 August 2011 / Accepted: 28 September 2011

Abstract

In times when human life and quality of life remain threatened by infectious diseases, increasing microbial resistance and a diminished armamentarium of treatments, you might think that the European Commission would urgently seek and encourage alternative approaches. On the contrary, the European Food Safety Authority (EFSA) has created a panel that has ignored good science, over-ridden peer-reviewed medical studies, and seems intent on breaking the back of a validated approach to infectious disease management, namely probiotics. The recent rejection of a simple and quite bland claim related to vaginal health emphasises the travesty of this bureaucracy. Herein, the science fights back and counters the critique of EFSA.

Keywords: European Food Safety Authority, probiotics, vagina

This opinion paper of Prof. dr. Gregor Reid entitled '*Quo vadis* – EFSA?' has been sent to the European Food Safety Authority (EFSA), prior to publication of this issue. The opinion paper criticizes the evaluation of probiotics dossiers in general and the one on the use of probiotics for vaginal health specifically. The EFSA was given the opportunity to react to the manuscript. However, the EFSA expressed to the journal that they had no wish as to respond prior to its publication.

It should be noted that any findings, opinions, conclusions or recommendations expressed in this publication are those of the author and do not necessarily reflect the views of this journal.

Introduction

The numbers speak for themselves. An estimated one billion females suffer from urogenital infection each year, and a similar number of adults and children from inflammatory bowel disease, diarrhea, irritable bowel syndrome, and antibiotic-associated diarrhea (Boschi-Pinto *et al.*, 2006; McFarland, 2009; Read, 2002). Are current pharmaceutical interventions solving the problems? No. Are there many suitable alternatives? Yes, probiotics. Could these help even 10% of the cases? At least.

Yet, official authorities in Europe are doing there level best to kill off the alternative. They seem to have no accountability. The net effect of some of their decisions is that research and development will suffer, companies will suffer, jobs will be lost and products will exit the shelves. The European Food Safety Authority (EFSA) has a lot to answer for.

When EFSA reject claims, their decisions represent a black mark against scientists, and their colleagues, students,

fellows and dedicated staff who worked on these organisms. It suggests 'your work can't be good, EFSA didn't approve it!'

The following is a rebuttal to an EFSA Scientific Opinion on the substantiation of health claims related to *Lactobacillus rhamnosus* GR-1 (ATCC 55826) in combination with *Lactobacillus reuteri* RC-14 (ATCC 55845) and defence against vaginal pathogens by increasing the proportion of lactobacilli and/or decreasing the proportion of potentially pathogenic bacteria and/or yeasts (ID 945) pursuant to Article 13(1) of Regulation (EC) No 1924/20061 (EFSA NDA, 2011). Of note, I had no role whatsoever in the submission of that claim application, and have not discussed my rebuttal with Chr. Hansen, who presumably did.

EFSA statement 1

The vagina is considered irrelevant to human nutrition. This means the vagina is not affected by the intestine or by the nutrients we consume.

Science

Bacteria present in the vagina have been shown to originate in the intestine (El Aila *et al.*, 2009, 2011; Meyn *et al.*, 2009). In addition, bacteria that infect the bladder have been shown to ascend from the intestine and reach the bladder via the vagina (Xie *et al.*, 2006). Lactobacilli consumed as food and dietary supplements have been shown by several independent laboratories to pass through the intestine and rectum and ascend to the vagina (Antonio *et al.*, 2005; Colodner *et al.*, 2003; Morelli *et al.*, 2004; Shalev *et al.*, 1996). Are bacteria regarded as food or do they contain components of nutrition? Of the six categories of nutrients that the body needs to acquire from food, namely: proteins, carbohydrates, fat, fibres, vitamins and minerals, and water, bacteria contain most. Thus, even if bacteria were only added as nutrients directly into the vagina, this would be valid. Nutrients are administered in various ways, including via the bloodstream, so why not directly into the vagina via the intestine? Is the vagina irrelevant because no nutrients are adsorbed from there into the bloodstream? This, of course, is not true and there is no evidence to suggest that the vaginal epithelium has some sort of adsorption barrier function.

Conclusion

There is unequivocal evidence that bacteria present in food, ingested in food, and utilising the nutrients of the intestine, reach the vagina. There is also clear evidence that nutrients are adsorbed via the vagina, and that the vagina requires nutrients.

EFSA statement 2

The official view is that the intra-vaginal route of administration (of lactobacilli) is not relevant to human nutrition.

Science

This is a strange ruling since good nutrition can help prevent disease and promote health, and as the claim pertains to orally administered lactobacilli which pass through the intestinal tract, where nutritional adsorption affects all body sites including the vagina. If the claim was for direct administration of lactobacilli (themselves nutrients as per above), or if the EFSA Panel regard the main applicability of the claim to mean the orally administered lactobacilli's main function is via the vagina, the argument that nutrition is irrelevant for the vagina, and the body in general, still makes no sense.

Conclusion

The studies that show direct and indirect administration of lactobacilli into the vagina are meritorious and relevant to the use of lactobacilli as a human nutritional intervention for vaginal defence. Indeed, without nutrition, the reproductive tract would die along with the ability of humans to reproduce. Arguably also without lactobacilli, reproduction per se might even be at risk, as suggested by studies of women undergoing *in vitro* fertilisation (Eckert *et al.*, 2003; Verstraelen and Senok, 2010).

EFSA statement 3

L. rhamnosus GR-1 (ATCC 55826) in combination with *L. reuteri* RC-14 (ATCC 55845) do not defend against vaginal pathogens by increasing the proportion of lactobacilli and/or decreasing the proportion of potentially pathogenic bacteria and/or yeasts.

Science

If EFSA's position is correct, there can be no studies that disagree with their conclusions. Likewise, if ingestion of *L. rhamnosus* GR-1 in combination with *L. reuteri* RC-14 increase the proportion of lactobacilli in the vagina in any number of women, it proves EFSA's position to be untenable. Several studies have indeed shown that ingestion of *L. rhamnosus* GR-1 in combination with *L. reuteri* RC-14 lead to increased lactobacilli in the vagina. This is shown by a return to a healthy status that by definition means higher numbers of lactobacilli than pathogens (Eckert *et al.*, 2003; Reid *et al.*, 2001a,b, 2007; Verstraelen and Senok, 2010). One of these studies, a randomised, placebo-controlled study of 64 women, specifically analysed the proportion of potentially pathogenic bacteria and/or yeasts in the

vagina, and showed them to be significantly reduced with increased presence of lactobacilli (Reid *et al.*, 2003). This is further documented in four studies in which pathogens were treated with antibiotics or anti-fungal agents plus placebo or oral *L. rhamnosus* GR-1 in combination with *L. reuteri* RC-14 (Anukan *et al.*, 2006a, 2009; Martinez *et al.*, 2009a,b). The fact that the lactobacilli food significantly improved cure rate in the vagina demonstrated restoration of a *Lactobacillus*-dominated microbiota and reduced pathogens. Furthermore, when the two strains were administered directly into the vagina, bacterial vaginosis was cured (Anukan *et al.*, 2006b).

Conclusion

EFSA's position is untenable, otherwise in no human cases would *L. rhamnosus* GR-1 in combination with *L. reuteri* RC-14 increase the proportion of lactobacilli and decrease the proportion of pathogens in the vagina. By EFSA's own statement, 'The Panel considers that defense against vaginal pathogens by increasing the proportion of lactobacilli and/or decreasing the proportion of potentially pathogenic bacteria and/or yeasts is a beneficial physiological effect', this should be sufficient to approve the claim.

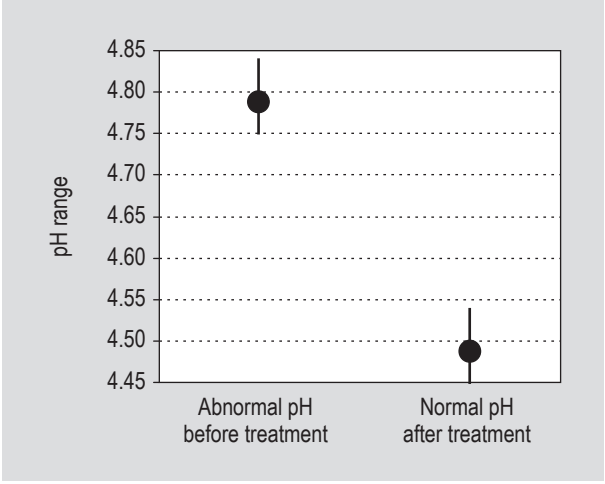

Figure 1. Pregnant women treated with *Lactobacillus rhamnosus* GR-1 in combination with *Lactobacillus reuteri* RC-14 at doses of one billion organisms per capsule daily for 30 days (22 patients). The mean changes (and standard error) of the vaginal pH levels were measured using Merck pH 4.0-7.0 indicator test strips. The statistic analysis of the mean changes of the vaginal pH levels was carried out based on t-Student test for independent groups (*P*=0.000011). Adapted from Oleszczuk *et al.* (2007).

EFSA statement 4

The 'beneficial physiological effect' is not defined by EFSA but presumably includes a return to a physiologically normal pH, a cessation of some or all symptoms and signs of illness including malodor (the primary reason for women to seek medical assistance for vaginal ailments), pain, discomfort and discharge associated with inflammation.

Science

The scientific evidence clearly shows that *L. rhamnosus* GR-1 in combination with *L. reuteri* RC-14 ingested daily for more than one week and within one month results in: (1) a return to a physiologically normal pH in pregnant women (Figure 1; Oleszczuk *et al.*, 2007), as well as in women whose BV was resolved by the treatment (Anukam *et al.*, 2006a,b, 2009; Hummelen *et al.*, 2010; Martinez *et al.*, 2009a,b; Oleszczuk *et al.*, 2007; Reid *et al.*, 2001a,b); (2) cessation of some or all symptoms and signs of illness including malodor (Figure 2; Anukam *et al.*, 2006a,b, 2009; Kira *et al.*, 2007; Martinez *et al.*, 2009a,b; Reid *et al.*, 2001a,b); (3) cessation in pain, discomfort and discharge associated with inflammation (Anukam *et al.*, 2006a,b, 2009; Martinez *et al.*, 2009a,b; Reid *et al.*, 2001a,b).

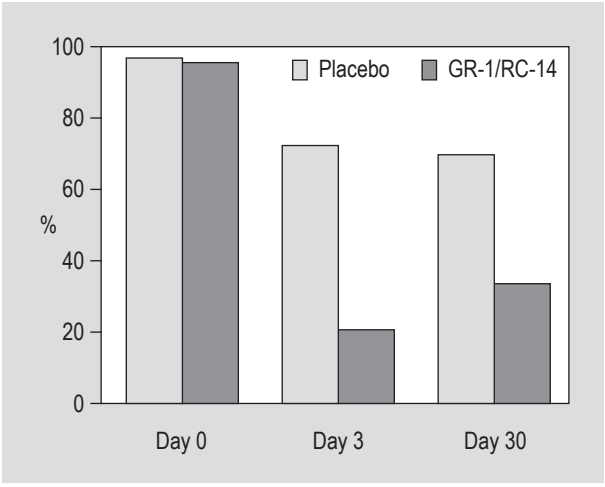

Figure 2. The changes in amine test as a measure of vaginal malodor following the treatment of 30 women with bacterial vaginosis with *Lactobacillus rhamnosus* GR-1 in combination with *Lactobacillus reuteri* RC-14 capsules (10^9 cfu each) or placebo capsules once per day for 15 days. Adapted from Kira *et al.* (2007).

Conclusion

The claim that was proposed was not indicating efficacy or superiority over standard medical practice. Thus, there is no requirement for weight of evidence. Rather, if an effect is noted in one woman definitively, that should be proof that it is possible for lactobacilli to increase and pathogens to decrease following oral use of *L. rhamnosus* GR-1 in combination with *L. reuteri* RC-14.

EFSA statement 5

There is no requirement by EFSA for providing studies that show mechanistically that all or any of the points in a claim are actually feasible with respect to the probiotic organisms. Indeed, the only requirement is that lactobacilli increase in proportion over pathogens in the vagina. For this to occur, however, and for the patient to return to heath, the treatment (in this case *L. rhamnosus* GR-1 in combination with *L. reuteri* RC-14) should be able to have an effect on pathogen growth, colonisation and survival, as well as ameliorate to some degree the host's adverse response to infection indicated by inflammation, pain and discharge.

Science

There are numerous studies on the ability of strains GR-1 and RC-14 to confer these effects, as published in the peer-reviewed literature and explained in various reviews (Li *et al.*, 2011; MacPhee *et al.*, 2010; Reid *et al.*, 2011). These mechanisms include an ability to counter uropathogen virulence (Cadieux *et al.*, 2009; Li *et al.*, 2011a,b), penetrate urogenital pathogen biofilms and displace them from surfaces (McMillan *et al.*, 2010), and modulate mucosal immunity (Kim *et al.*, 2006; Yeganegi *et al.*, 2010).

Conclusion

These two probiotic organisms have been shown to have the mechanistic ability to counter urogenital pathogens, further supporting the claim.

In summary

The legislative process established by the European Commission to adjudicate claims for probiotics is severely flawed, as has been stated by many outstanding scientists, companies and organisations. In this article, the gravity of the situation is highlighted by the fact that an extremely simple claim, without reference to disease treatment or efficacy, for a two-strain probiotic can fail to be approved by EFSA. The volume of science countering the panel's decision is presented for others to review, but it seems clear that it far outweighs the reasons given for the claim refusal. It is ironic that the treatment options for women with acute and recurrent urogenital infections remain unchanged for decades, and the suffering of millions of women continues. In addition, this same European government sits idly, as does the US Food and Drug Administration in allowing so-called 'probiotic' products with zero scientific or clinical documentation to flood the markets using the term 'probiotic' without adhering to the FAO/WHO Guidelines in any way (Reid *et al.*, 2005). Allowing simple claims for documented products and enforcing bans on undocumented copy-cats, would serve the population much more than the current hopeless efforts of the EFSA panel. For now, we need a roadmap from the European Union (Guarner *et al.*, 2011), but we're running out of road. *Quo vadis* – EFSA?

Acknowledgements and conflicts of interest

The patents and technology my colleague and I developed on *Lactobacillus* strains GR-1 and RC-14 were transferred to Chr. Hansen in 2009 and I now have no conflict of interest as an independent scientist.

References

Antonio, M.A., Rabe, L.K. and Hillier, S.L., 2005. Colonization of the rectum by *Lactobacillus* species and decreased risk of bacterial vaginosis. Journal of Infectious Diseases 192: 394-398.

Anukam, K.C., Osazuwa, E., Osemene, G.I., Ehigiagbe, F., Bruce, A.W. and Reid, G., 2006. Clinical study comparing probiotic *Lactobacillus* GR-1 and RC-14 with metronidazole vaginal gel to treat symptomatic bacterial vaginosis. Microbes and Infection 8: 2772-2776.

Anukam, K., Osazuwa, E., Ahonkhai, I., Ngwu, M., Osemene, G., Bruce, A.W. and Reid, G., 2006. Augmentation of antimicrobial metronidazole therapy of bacterial vaginosis with oral probiotic *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14: randomized, double-blind, placebo controlled trial. Microbes and Infection 8: 1450-1454.

Anukam, K.C., Duru, M.U., Eze, C.C., Egharevba, J., Aiyebelehin, A., Bruce, A.W. and Reid, G., 2009. Oral use of probiotics as an adjunctive therapy to fluconazole in the treatment of yeast vaginitis: a study of Nigerian women in an outdoor clinic. Microbial Ecology in Health and Disease 21: 72-77.

Boschi-Pinto, C., Lanata, C.F., Mendoza, W. and Habte, D., 2006. Diarrheal diseases. In: Jamison, D.T., Feachem, R.G., Makgoba, M.W., Bos, E.R., Baingana, F.K., Hofman, K.J. and Rogo, K.O. (eds.) Disease and mortality in Sub-Saharan Africa. 2nd ed., World Bank, Washington, DC, USA, Chapter 9.

Cadieux, P.A., Burton, J., Devillard, E. and Reid, G., 2009. *Lactobacillus* by-products inhibit the growth and virulence of uropathogenic *Escherichia coli*. Journal of Physiology and Pharmacology 60 Suppl. 6: 13-18.

Colodner, R., Edelstein, H., Chazan, B. and Raz, R., 2003. Vaginal colonization by orally administered *Lactobacillus rhamnosus* GG. Israel Medical Association Journal 5: 767-769.

Eckert, L.O., Moore, D.E., Patton, D.L., Agnew, K.J. and Eschenbach, D.A., 2003. Relationship of vaginal bacteria and inflammation with conception and early pregnancy loss following *in-vitro* fertilization. Infectious Diseases in Obstetrics and Gynecology 11: 11-17.

El Aila, N.A., Tency, I., Claeys, G., Verstraelen, H., Saerens, B., Santiago, G.L., De Backer, E., Cools, P., Temmerman, M., Verhelst, R. and Vaneechoutte, M., 2009. Identification and genotyping of bacteria from paired vaginal and rectal samples from pregnant women indicates similarity between vaginal and rectal microflora. BMC Infectious Diseases 9: 167.

El Aila, N.A., Tency, I., Saerens, B., De Backer, E., Cools, P., Dos Santos Santiago, G.L., Verstraelen, H., Verhelst, R., Temmerman, M. and Vaneechoutte, M., 2011. Strong correspondence in bacterial loads between the vagina and rectum of pregnant women. Research in Microbiology 162: 506-513.

European Food Safety Authority Panel on Dietetic Products, Nutrition and Allergies (EFSA NDA), 2010. Scientific Opinion on the substantiation of health claims related to *Lactobacillus rhamnosus* GR-1 (ATCC 55826) in combination with *Lactobacillus reuteri* RC-14 (ATCC 55845) and defence against vaginal pathogens by increasing the proportion of lactobacilli and/or decreasing the proportion of potentially pathogenic bacteria and/or yeasts (ID 945) pursuant to Article 13(1) of Regulation (EC) No 1924/20061. EFSA Journal 9: 2232. Available at: http://www.efsa.europa.eu/en/efsajournal/doc/2232.pdf.

Guarner, F., Sanders, M.E., Gibson, G., Klaenhammer, T., Cabana, M., Scott, K., Reid, G., Delzenne, N.M., Fahey, G.C. and Hill, C., 2011. Probiotic and prebiotic claims in Europe: seeking a clear roadmap. British Journal of Nutrition, in press. DOI: http://dx.doi.org/10.1017/S0007114511002248.

Hummelen, R., Changalucha, J., Butamanya, N.L., Cook, A., Habbema, J.D.F. and Reid, G., 2010. *Lactobacillus rhamnosus* GR-1 and *L. reuteri* RC-14 to prevent or cure bacterial vaginosis among women with HIV. International Journal of Gynecology and Obstetrics 111: 245-248.

Kim, S.O., Sheik, H.I., Ha, S.-D., Martins, A. and Reid, G., 2006. G-CSF mediated inhibition of JNK is a key mechanism for *Lactobacillus rhamnosus*-induced anti-inflammatory effects in macrophages. Cellular Microbiology 8: 1958-1971.

Kira, E.F., Artymuk, N.V., Bezhenar, V.F., Savicheva, A.M., Gaitukieva, R.A., Gamirova, E.V., Beliakina, I.V. and Melko, A.I., 2007. Multicenter, randomized, placebo-controlled study of the efficacy and safety of Lactogyn in the treatment of bacterial vaginosis. In: Russian Gynecological Congress, 2 October 2007, Moscow, Russia, abstract.

Li, J., McCormick, J., Bocking, A. and Reid, G., 2011. Probiotics for reproductive medicine. Reproductive Sciences, in press.

Li, J., Wang, W., Xu, S.X., Magarvey, N.A. and McCormick, J.K., 2011. *Lactobacillus reuteri*-produced cyclic dipeptides quench *agr*-mediated expression of toxic shock syndrome toxin-1 in staphylococci. Proceedings of the National Academy of Sciences of the USA 108: 3360-3365.

MacPhee, R., Hummelen, R., Bisanz, J., Miller, W.L. and Reid, G., 2010. Probiotic strategies for the treatment and prevention of bacterial vaginosis. Expert Opinion on Pharmacotherapy 11: 2985-2995.

Martinez, R.C., Franceschini, S.A., Patta, M.C., Quintana, S.M., Candido, R.C., Ferreira, J.C., Pereira De Martinis, E.C. and Reid, G., 2009. Improved cure of bacterial vaginosis with single dose of tinidazole (2 g) and *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14: a randomized, double-blind, placebo-controlled trial. Canadian Journal of Microbiology 55: 133-138.

Martinez, R.C., Franceschini, S.A., Patta, M.C., Quintana, S.M., Candido, R.C., Ferreira, J.C., Pereira De Martinis, E.C. and Reid, G., 2009. Improved treatment of vulvovaginal candidiasis with fluconazole plus probiotic *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14. Letters in Applied Microbiology 48: 269-274.

McFarland, L.V., 2009. Evidence-based review of probiotics for antibiotic-associated diarrhea and *Clostridium difficile* infections. Anaerobe 15: 274-280.

McMillan, A., Dell, M., Zellar, M.P., Cribby, S., Martz, S., Hong, E., Fu, J., Abbas, A., Dang, T., Miller, W. and Reid, G., 2010. Disruption of urogenital biofilms by lactobacilli. Colloids and Surfaces B: Biointerfaces 86: 58-64.

Meyn, L.A., Krohn, M.A. and Hillier, S.L., 2009. Rectal colonization by group B *Streptococcus* as a predictor of vaginal colonization. American Journal of Obstetrics and Gynecology. 201: 76.e1-76.e7.

Morelli, L., Zonenenschain, D., Del Piano, M. and Cognein, P., 2004. Utilization of the intestinal tract as a delivery system for urogenital probiotics. Journal of Clinical Gastroenterology 38 Suppl. 6: S107-S110.

Oleszczuk, J., Leszczyńska-Gorzelak, B., Haberek, M. and Dziduch, P., 2007. Clinical evaluation of LaciBios femina in treatment of disturbances of the vaginal bacterial flora in pregnant women. In: Congress of the Polish Gynecological Society, 28 September 2006. Poznan, Poland, abstract.

Reid, G., 2002. Probiotics for urogenital health. Nutrition in Clinical Care 5: 3-8.

Reid, G., 2005. The importance of guidelines in the development and application of probiotics. Current Pharmaceutical Design 11: 11-16.

Reid, G., Beuerman, D., Heinemann, C. and Bruce, A.W., 2001. Probiotic *Lactobacillus* dose required to restore and maintain a normal vaginal flora. FEMS Immunology and Medical Microbiology 32: 37-41.

Reid, G., Bruce, A.W., Fraser, N., Heinemann, C., Owen, J. and Henning, B., 2001. Oral probiotics can resolve urogenital infections. FEMS Immunology and Medical Microbiology 30: 49-52.

Reid, G., Charbonneau, D., Erb, J., Kochanowski, B., Beuerman, D., Poehner, R. and Bruce, A.W., 2003. Oral use of *Lactobacillus rhamnosus* GR-1 and *L. fermentum* RC-14 significantly alters vaginal flora: randomized, placebo-controlled trial in 64 healthy women. FEMS Immunology and Medical Microbiology 35: 131-134.

Reid, G., Younes, J., Van der Mei, H.C., Gloor, G.B., Knight, R. and Busscher, H.J., 2011. Microbiota restoration: natural and supplemented recovery of human microbial communities. Nature Reviews Microbiology 9: 27-38.

Shalev, E., Battino, S., Weiner, E., Colodner, R. and Keness, Y., 1996. Ingestion of yogurt containing *Lactobacillus acidophilus* compared with pasteurized yogurt as prophylaxis for recurrent candidal vaginitis and bacterial vaginosis. Archives of Family Medicine 5: 593-596.

Verstraelen, H. and Senok, A.C., 2005. Vaginal lactobacilli, probiotics, and IVF. Reproductive Biomedicine Online 11: 674-675.

Xie, J., Foxman, B., Zhang, L. and Marrs, C.F., 2006. Molecular epidemiologic identification of *Escherichia coli* genes that are potentially involved in movement of the organism from the intestinal tract to the vagina and bladder. Journal of Clinical Microbiology 44: 2434-2441.

Yeganegi, M., Leung, C.G., Martins, A., Kim, S.O., Reid, G., Challis, J.R.G. and Bocking, A.D., 2010. *Lactobacillus rhamnosus* GR-1-induced IL-10 production in human placental trophoblast cells involves activation of JAK/STAT and MAPK pathways. Reproductive Sciences 17: 1043-1051.