Relationships Between Oxidative Status and Pregnancy Outcome in Dairy Cows

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In ruminants, oxidative stress may be involved in several pathological conditions, including conditions that are relevant for ruminant reproduction, production and the general welfare (Miller *et al* 1993). Oxidative stress resulting from increased production of pro-oxidants, and/or a decrease in antioxidant defence, leads to damage of biological macromolecules and disruption of normal metabolism and physiology. Recent evidence has identified a potential role for oxidative stress as a trigger for cell death during luteolysis. Corpus luteum is essential for the maintenance of pregnancy and an excessive free radical generation damages luteal cell membrane and affects progesterone (P4) production (Kato *et al* 1997). This condition may lead to a failure in embryo development, increasing days open and calving intervals. It is possible that the metabolic stress induced by lactation makes the corpus luteum more susceptible to stress and free radicals may be involved in the decrease in fertility.

This study was designed to evaluate if the establishment of pregnancy may be affected by metabolic and oxidative status of dairy cows. The study was conducted at the Faculty of Veterinary Science, the University of Sydney (Australia) and used 40 pasture fed Holstein-Friesian cows which were monitored daily for oestrous activity starting from day 50 postpartum. On the day of the artificial insemination (AI; Day 0), and on Days 30 and 42 after AI, blood samples were collected and reactive oxygen metabolites (ROMs) and biological antioxidant potential (BAP) concentrations were determined by a commercial kit (Diacron, Grosseto, Italy) on fresh blood by Free Radical Analytical System (FRAS4; H&D limited, Parma, Italy). Blood was centrifuged immediately and plasma was stored at -20˚C until assayed for its concentrations of P4, pregnancy associated glycoprotein (PAG), advanced oxidation protein products (AOPP), glucose, total proteins, urea, non-esterified fatty acid (NEFA), and glutathione (GSH). Plasma PAG concentrations at days 30 and 42 were used to perform a pregnancy diagnosis according to Zoli *et al* (1992). On the days of blood sampling, cows were monitored for body condition score (BCS; scale 1-5, arbitrary units). Data was analysed by linear regression (GenStat); analysis included: between-subjects main effect of AI outcome (AI resulted in pregnancy, AI+; AI not resulted in pregnancy, AI- and embryo mortality, EM), within-subjects main effect of postpartum period (Period 1: < 80 day; Period 2: 81-120 days; Period 3: >120 days) and their interaction. Pearson's correlation coefficients were calculated between the parameters measured in this study.

Overall, we observed 26 AI+ (33%), 49 AI- (62%), while EM was observed in 4 cases (5%). Cows that experienced EM had lower BCS values (2.5) compared to the AI+ (2.8) and AI- (2.9) cows ($P<0.05$). A reduction in BCS during early lactation doubles the rate of pregnancy losses and embryo mortality in dairy cows (Lopez-Gatius *et al* 2003). During the postpartum BCS increased and NEFA decreased ($P<0.05$) suggesting a recovery from the negative energy balance (NEB). The observation that on Day 0 plasma NEFA concentrations were higher in the AI+ (0.62 mmol/L; $P<0.05$) cows compared to the AI- (0.58 mmol/L) and EM (0.58 mmol/L) cows was unexpected as high serum concentrations of NEFA are known to have detrimental effects on fertility. Plasma glucose and ROMs concentrations increased during the postpartum ($P<0.03$ and $P<0.01$, respectively) with their highest values observed during Period 3. The gradual increase in glucose concentration suggests a recovery from the NEB. It is likely that the increase in ROMs concentration was due to the parallel increase in glucose, indeed the two were significantly correlated ($r=0.2$; $P<0.05$). Concentrations of BAP decreased during the postpartum ($P<0.05$) possibly as a consequence of parallel increase in ROMs levels; antioxidant depletion indeed, is considered the consequence and not the cause of oxidative stress. GSH and AOPP showed a similar trend during Period 1 their concentrations were higher in EM than in AI+ and AI- ($P<0.05$). However, because of the low number of EM cases, this finding needs to be confirmed in further studies. It seems that plasma ROMs and BAP concentrations are not related to AI outcome. Considering that the maintenance of redox homeostasis is quite complex, further studies are required to clarify the role of oxidative status on cows' fertility.

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