

The Effect of Equine Viral Arteritis on Follicular Fluid Nitric Oxide in Experimentally Infected Mares

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Equine arteritis virus (EAV) is a horizontally and venereally transmitted arterivirus known to cause respiratory disease, vasculitis, and abortion. Persistently infected stallions serve as the reservoir for the virus within the equine population. Sero-naïve mares bred to stallions persistently infected with EAV become infected and fail to conceive during that cycle. In addition to virus associated vasculitis detectable within the ovary on histopathologic examination, we have also anecdotally noted that mares have reduced ovarian follicular activity during the viremic phase of infection as observed via ultrasonography.

An emerging mediator in immunological and reproductive physiology is the secretory product nitric oxide (NO). NO is inducibly and constitutively expressed, and decomposes rapidly to form free radicals, nitrites and nitrates. Inducible NO pathways generate free radicals during the immune response. In laboratory animals, constitutive NO mediates ovarian steroidogenesis, follicular development, and ovulation. It was hypothesized that increased ovarian NO levels induced by EAV could be a factor in EAV-related infertility.

Within the physiologic breeding season nine seronegative, reproductively normal mares were observed daily for follicular development by ultrasonographic examination. From each mare baseline NO content of ovarian follicular fluid (ff) was examined in small, medium, and large equine follicles. Follicular fluid samples were obtained via transvaginal, ultrasound-guided needle aspiration and assayed for nitrites and nitrates by chemiluminescence and colorimetric methods. Normal ranges (1.0 - 5.6 μM nitrite, and 11.7 – 173.0 μM nitrate) were established and baseline ff NO content was shown to increase as follicle size increased. A significant difference was observed between mean NO concentration in large vs. small follicles (0.57 μM , $p = 0.006$) and medium vs. small follicles (0.40 μM , $p = 0.006$). These baseline results provide evidence for the presence of NO activity in equine ovarian physiology, and suggest that the level of NO activity increases with follicular development. After the previremic sampling phase of the study, ff NO content was examined in the same mares following exposure to a field isolate of EAV. The NO levels within ff during viremia were then compared to a standard curve of expected values based on follicle size. We found that during the viremic phase of infection with EAV, nitrite and nitrate concentrations were significantly decreased from normal values (-0.53 μM nitrite, $p = 0.0175$, and -33.7 μM nitrate, $p = 0.00001$). While our working hypothesis was that NO content would be increased due to inflammatory and / or immunological responses to EAV infection, it is now evident that depressed rather than elevated ovarian NO levels may be involved in the pathogenesis of the short term EAV-related mare infertility.