

## Long term amniotic fluid ureaplasma colonization with low dose *Ureaplasma parvum* serovar 3 induces severe, chronic chorioamnionitis

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**Background:** Ureaplasmas are the most prevalent microorganisms in amniotic fluid from preterm births. We compared severity of chorioamnionitis in sheep, after long-term colonization of amniotic fluid with *Ureaplasma parvum* serovar 3 or serovar 6.

**Methods:** At 50 days of gestation (d, term=150d) ewes bearing single fetuses received an ultrasound-guided intra-amniotic injection of either:  $2 \times 10^7$  colony forming units (cfu: high dose) of *U. parvum* (serovar 3, n=7; serovar 6, n=8);  $2 \times 10^4$  cfu (low dose) of *U. parvum* (serovar 3, n=6; serovar 6, n=8); or media control, (n=6). At 125d, fetuses were delivered by caesarean section; amniotic fluid and chorioamnion samples were collected for quantitation of ureaplasmas by culture. A chorioamnion sample was fixed and prepared for quantitation of inflammatory cells (H&E), and immunohistochemistry (anti-ureaplasma rabbit antisera, secondary HRP- or Alex Fluor 488-labeled antibodies). Sections were viewed by light or confocal microscopy.

**Results:** Amniotic fluid ureaplasma titers at delivery ranged from  $2.77 \times 10^5$  to  $3.25 \times 10^6$  cfu/mL. The highest numbers of ureaplasmas were from low dose serovar 3 pregnancies. All ureaplasma infected/colonized fetal membranes had higher inflammatory cell counts ( $p < 0.05$ ) than controls. Patterns of histologic inflammation differed between inoculate dose and serovar; monocyte and lymphocyte (not neutrophil) numbers were higher ( $p < 0.05$ ) in chorioamnion after low dose *U. parvum* serovar 3 than other groups. Inflammatory changes and tissue repair were evident in chorioamnion of these same animals. Immunohistochemistry demonstrated localization of ureaplasmas within chorioamnion, and typical ureaplasma morphology.

**Conclusion:** The severity of chorioamnionitis induced by ureaplasmas was dose-and serovar-dependent. The most severe changes were induced by low dose *U. parvum* serovar 3.