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Use of MCWF (Settle®) in the Breeding Mare

Objectives:

1) Achieve successful pregnancy
2) Reduce/eliminate the use of medically important antibiotics in the breeding mare

Settle®

- Mycobacterium cell wall fraction (MCWF) derived from a non-pathogenic soil-borne bacterium
- Only immunomodulator approved for use as a treatment of equine endometritis caused by *Streptococcus zooepidemicus*
- Approved for intravenous (IV) and intrauterine (IU) administration
- Settle induces the expression of cytokines: pro-inflammatory (IFN, IL-8, TNFα), immunomodulating (IL-6, GM-CSF) and anti-inflammatory (IL-1RN)

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Endometritis

- Endometritis is inflammation of the uterus
- This invisible and often undiagnosed disease is a major cause of mare infertility, affecting up to 15% of broodmares
- It affects the delicate lining of a mare's uterus–the endometrium–which can become inflamed and create a hostile environment for sperm, as well as any resulting embryo, to live
- Because it frequently lacks clear clinical signs, it often goes undiagnosed
- Endometritis can occur at/around the time of breeding and post-partum

Presentation Overview

- Post-breeding Endometritis
  - Causes
  - Problem mares
  - Settle results in these mares

- Post-partum Endometritis
  - Involution
  - Foal heat breeding
  - Settle results in these mares

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Post-Breeding Endometritis

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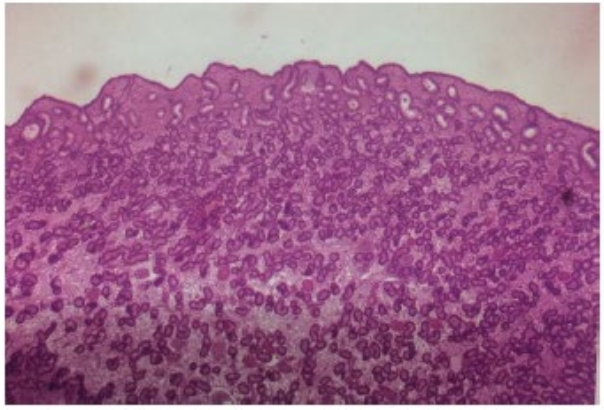
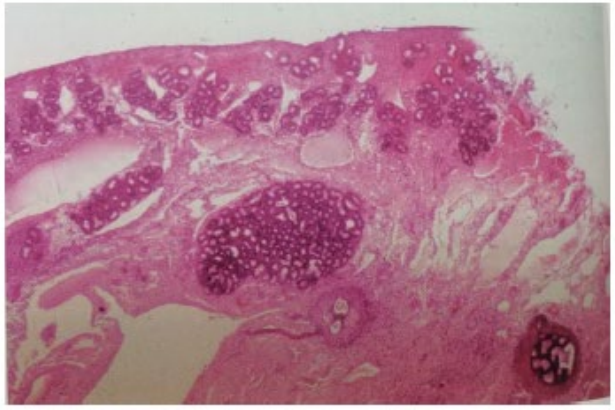
Persistent Breeding-Induced Endometritis (PBIE)

- The majority of mares are able to resolve inflammation unassisted
- **PBIE Resistant ("Normal Mare")**
  - Grade I-IIa biopsy
  - No fluid/PMN at 24-36 h
  - Acute inflammation



- 10–15% of the population are unable to resolve inflammation (Zent *et al*., 2005)
- **PBIE Susceptible ("Problem Mare")**
  - Grade IIb-III biopsy
  - Fluid/PMN more than 96 h
  - Chronic inflammation



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Uterine Defense: Immune Response

- A transient innate immune response to breeding in the mare can be caused by a variety of things:

✓ Bacteria ✓ Fungus ✓ Yeast	**Pathogenic endometritis**
✓ Sperm ✓ Seminal Plasma ✓ Detritus	**Breeding-induced endometritis**

- The innate immune response can be enhanced by the administration of Settle at breeding

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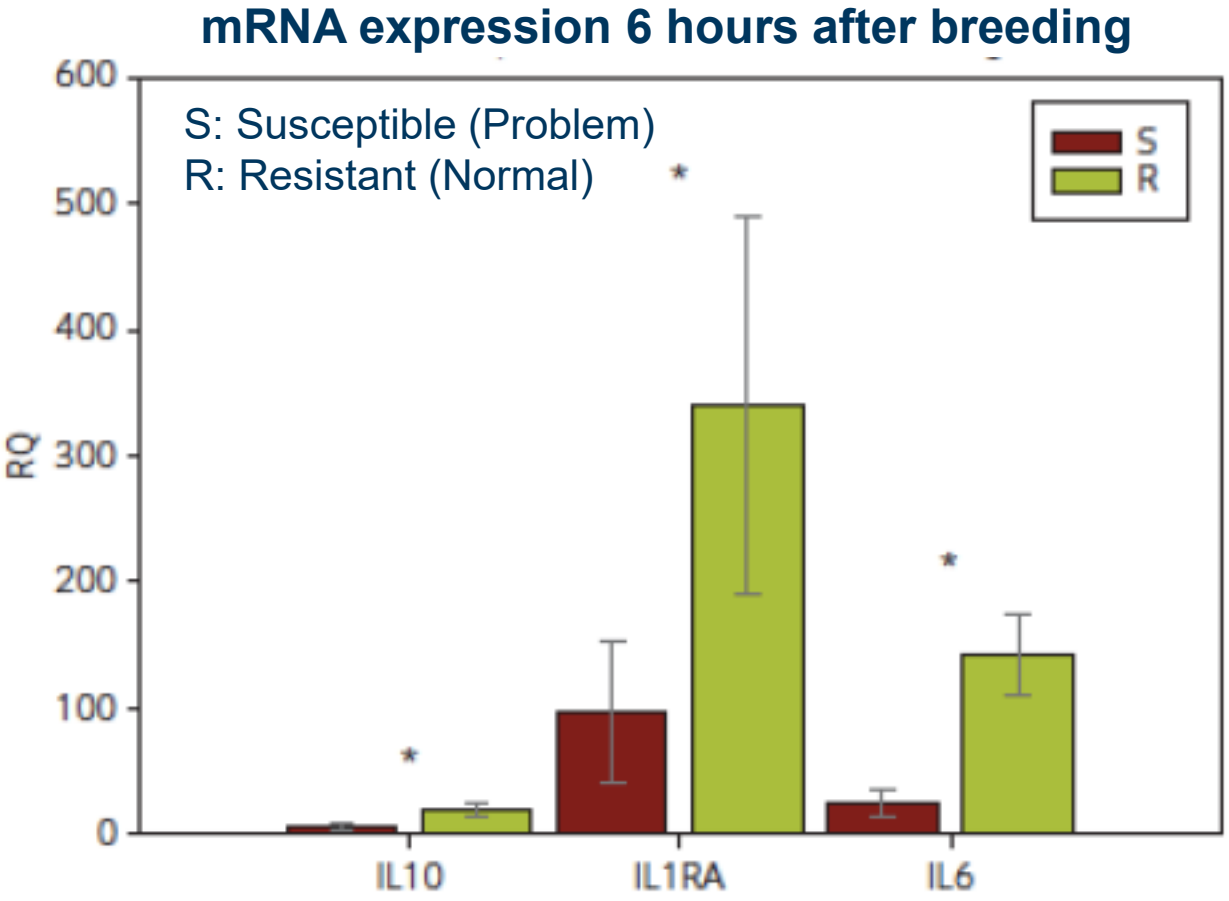
Natural Immune Response to Breeding



- 6 h post-breeding
- Susceptible mares lack anti-inflammatory response

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Use of Settle® in Problem Mares to Clear Experimentally Induced Endometritis with *S. zooepidemicus*

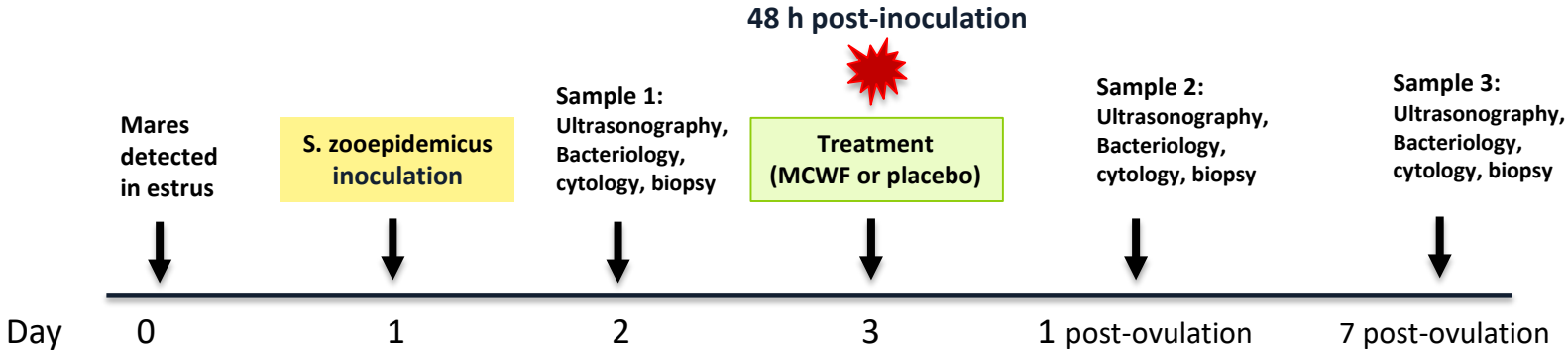
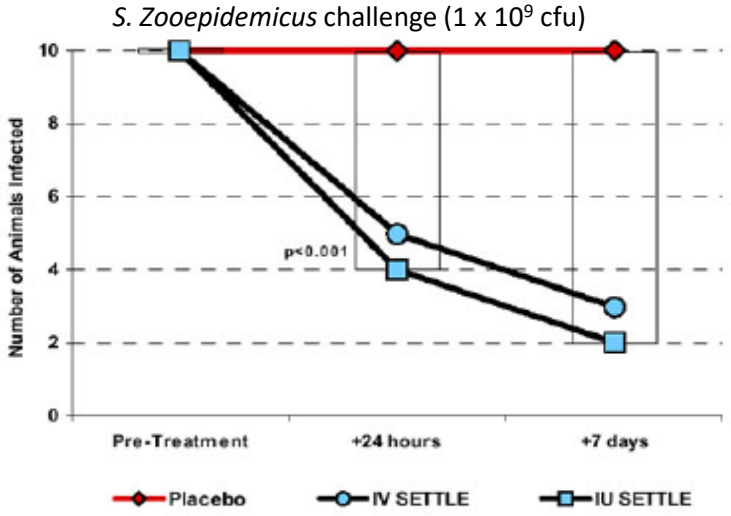


Figure 1. Experimental schedule, showing mare inoculation and sampling points



Rogan *et al*. 2007

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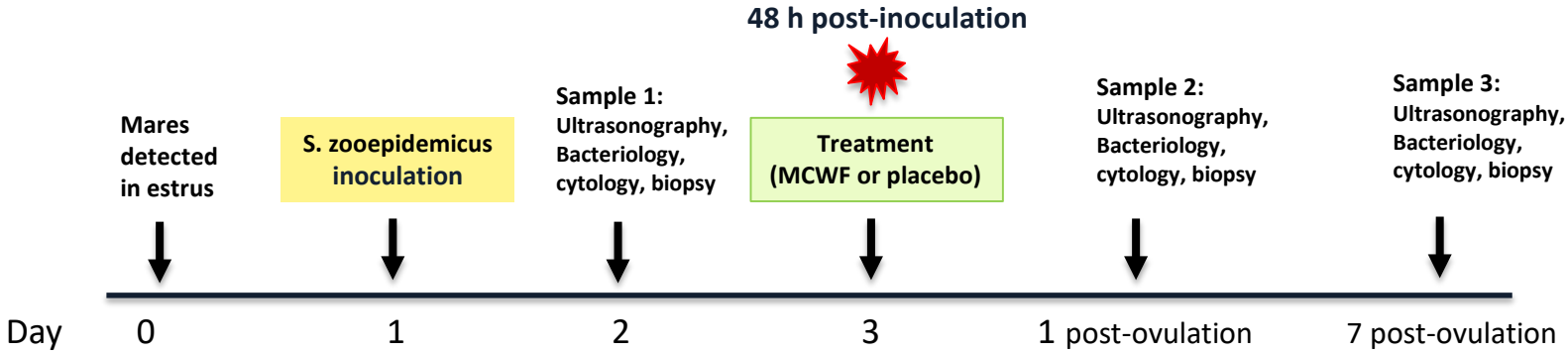

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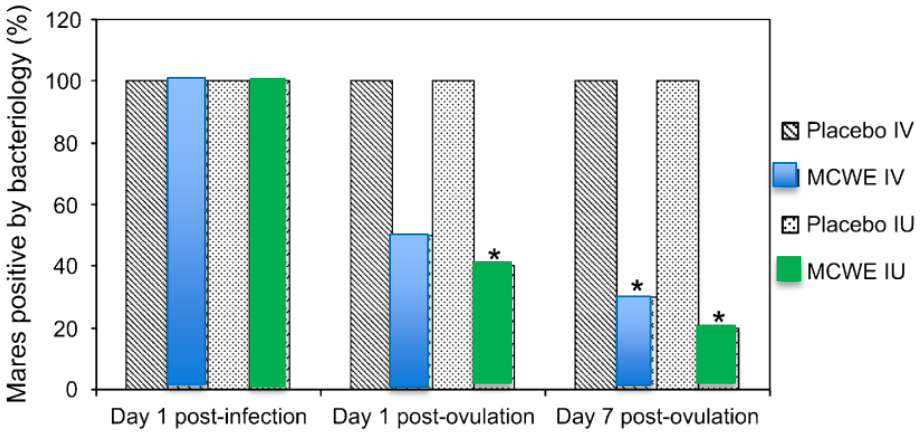

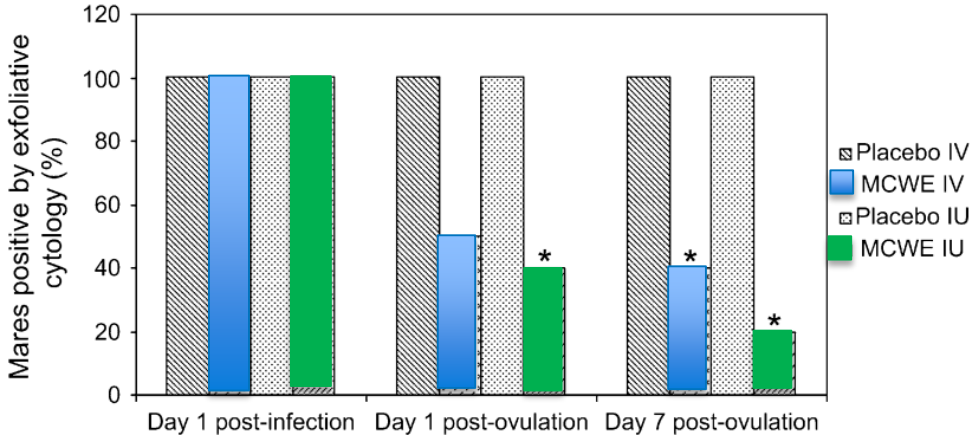

Rogan *et al*. 2007

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Summary of Results – Post-Breeding

Settle®:

- Decreased pro-inflammatory cytokine production (IL-1β, IL-8) (Fumuso *et al*. 2006; 2008; Woodward *et al*. 2014)
- Increased anti-inflammatory cytokine production (IL-10) (Fumuso *et al*. 2007)
- Decreased PMN infiltration – decreased fluid (Fumuso *et al*. 2007; Christoffersen *et al*. 2014)
- Decreased bacterial infiltration (Fumuso *et al*. 2010; Christoffersen *et al*. 2014)

➢ A single administration of Settle to problem mares at/within 24 h of breeding resulted in normalizing the immune response and enhancing bacterial clearance without the use of medically important antibiotics

Post-partum Endometritis

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Settle® and Foal Heat Breeding

The day after foaling: mares were treated with 1.5 mL of Settle IV or with placebo

	Settle		Placebo	
Mares treated	n=45		n=44	
	1 day after foaling	7 days after foaling	1 day after foaling	7 days after foaling
BACTERIOLOGY:				
S. zooepidemicus	4	1	8	6
S. equisimilis	2	0	1	1
S. equi	0	0	1	0
E. coli	7	1	5	2
Klebsiella spp.	1	0	0	0
Proteus spp.	0	0	1	0
All bacterial isolates	14	2	16	9
Bacterial cfu reduction		**86 %**		**44 %**
CYTOLOGY:		n=13		n=12
PMN reduction		**92 %**		**59 %**
Mares bred at foal heat	n = 43		n = 38	
Pregancy rate	**28/43 (65 %)[a]**		**9/38 (24 %)[b]**	

ab: p< 0.05

Fumuso *et al*. 2003

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Summary of Results – Post-Partum

Settle®:

- Reduces inflammation
- Enhances bacterial clearance
  (University of Kentucky – Gluck Equine Research Centre)
- Hastens uterine involution
- Shortens days to ovulation (11 days)*
  (University of Kentucky – Gluck Equine Research Centre)
- Enables normal pregnancy at foal heat**
  (65% (Settle) vs. 24% (placebo) (Fumuso *et al*. 2003)

**two doses one week apart (day after foaling and seven days later)*

***one dose (day after foaling)*

Settle® - Summary

- ✓ Settle is the only approved immunomodulator for the treatment of uterine infections due to *Streptococcus zooepidemicus* in the mare (regulator approved in the U.S.A., Australia and New Zealand)
- ✓ Settle has been shown to reduce infections of both Gram-positive and Gram-negative bacterial colonies in the mare
- ✓ Settle is safe and effective when given by either intravenous or intrauterine routes of administration
- ✓ Settle has been shown to induce efficient involution of the uterus resulting in the ability to prepare the mare for early breeding (foal heat)
- ✓ Settle is compatible with, and non-toxic to, spermatozoa (can be mixed with semen extender)
- ✓ In all studies conducted with Settle, it was demonstrated that **Settle can replace medically important antibiotics** in the broodmare

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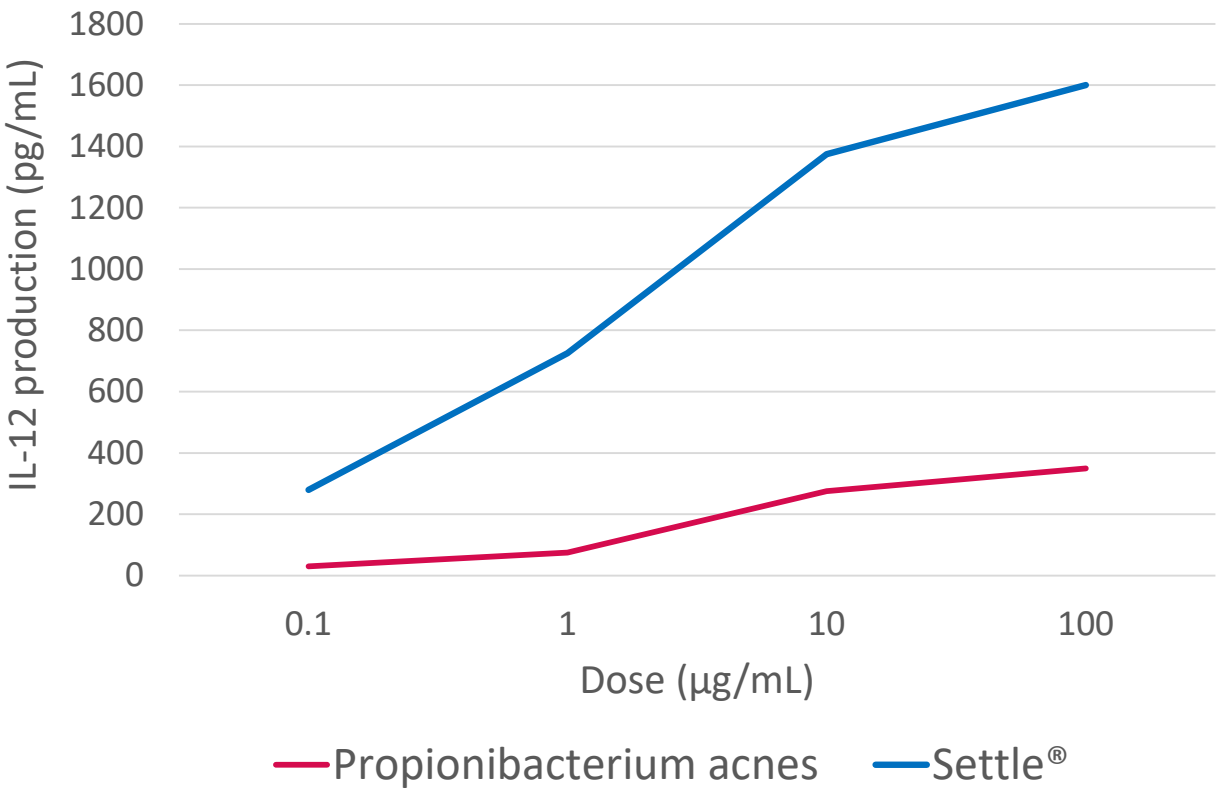
Comparison of Immunomodulators

- Settle® and *P. acnes* cultured with 1 x 10^6/mL human monocytes (THP-1 cells)
- IL-12 measured at 48 h after culture



- IL-12 is a T cell-stimulating factor (Hsieh et al. 1993) and is produced by activated antigen-presenting cells (dendritic cells, macrophages, neutrophils) (Kaliński et al.1997)
- IL-12 promotes the development of Th1 responses and is a powerful inducer of IFNγ production by T and NK cells
- IL-12 represents a bridge between the innate resistance and adaptative immune response (Trinchieri 1993)

Neogen Corporation, Lansing, MI, USA

Filion 2004. Proc. Modern Vaccine/Adjuvant Formulation, Czech Rep, Sep 15-17. (Bioniche Therapeutics Inc., Canada)

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- Use of a Mycobacterial Cell Wall Extract (MCWE) in susceptible mares to clear experimentally induced endometritis with *Streptococcus zooepidemicus*. *D. Rogan, DVM, MSc, PhD, E. Fumuso, DVM, PhD, E. Rodriguez, DVM, MSc, J. Wade, DVM, PhD, and S.F. Sánchez Bruni, DVM, PhD* (Journal of Equine Veterinary Science, March 2007)
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- Non-specific immunomodulation (NSI) can rectify an imbalanced uterine/ovarian milieu in mares susceptible to endometritis. *E. Fumuso, J. Aguilar, S. Giguère, I. Videla Dorna, J. Wade and D. Rogan* (6th International Symposium on Equine Embryo Transfer, August 4-6, 2004, Rio de Janeiro, Brazil)
- Endometrial IL-1β and TNF-α, mRNA expression in mares resistant or susceptible to post-breeding endometritis; Effects of estrous cycle, artificial insemination and immunomodulation. *Elida Fumuso, Steeve Giguère, José Wade, Dragan Rogan, Ignacio Videla-Dorna, Raúl A. Bowden* (Veterinary Immunology and Immunopathology 96 (2003) 31-41)
- Non-specific immunomodulation at post-partum improves uterine condition and fertility in mares. *Fumuso, E., Alvarez, G., Bruno, S., Videla-Dorna, I., Wade, J., Rogan, D., and Bowden, R.A.* (8th World Equine Veterinary Association, October 15-17, 2003, Buenos Aires, Argentina)

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Settle®

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