IWR-1 withdrawal caused elevated ($P < 0.05$) expression of lineage markers including *CXCR4* (endoderm), *MEOX1* (mesoderm) and *NESTIN* (ectoderm), although other lineage markers *FOXA1*, *MIXL1*, *PAX6*, *SNAI1*, *SOX1*, *EOMES* and *SOX17* remained unaffected ($n = 4$). These data suggested that IWR-1 can prevent bESC from differentiating into certain lineages of the three germ layers. Next, we investigated inhibitory effects of IWR-1 on tankyrase activity. Abundance of TNKS1 and TNKS2 protein was assessed in bESC treated with or without IWR-1 for 1 day by western blotting. Results showed that TNKS2 but not TNKS1 level was reduced ($P < 0.05$) if IWR-1 was withdrawn from the medium. We confirmed that the decreased TNKS2 was not due to transcriptional regulation by qPCR. These data indicated that IWR-1 mitigated the poly-ADP-ribose polymerase activity of TNKS2, and therefore likely disrupted self-poly(ADP-ribosyl)ation and degradation of TNKS2. Subsequently, bulk RNA-seq analysis was performed on bESC treated plus/minus IWR-1 for 1 day ($n = 5$). We noticed that a WNT/Hippo targeted gene *CYR61* and a transcription factor of WNT pathway *TCF7* were both up-regulated ($P < 0.05$) in IWR-1 minus cells. Transcription of four genes coding the Hippo pathway components TEAD4, AMOTL2, WWC2, and AJUBA were stimulated ($P < 0.05$) by IWR-1 removal as well. Treatment effects on the transcription of these genes except AJUBA were validated by qPCR in another cell culture experiment ($n = 6$–8). Changes in TEAD4 protein expression were further confirmed by western blotting ($n = 3$). In all, our study suggests that IWR-1 negatively regulates Hippo signaling pathway besides disrupting the canonical WNT pathway to sustain cell potency in bovine, possibly through inhibition of TNKS2 activity. This highlights an important role of Hippo pathway activity in regulation of pluripotency, and perhaps also provides targets for improving the current culture system of ESC in livestock.

223 Wharton's jelly mesenchymal stromal/stem cell–derived conditioned medium effect on equine endometrial cell viability

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Endometritis is the major cause of reduced fertility in mares, with a consequent measurable economic loss. Mesenchymal stromal/stem cells (MSCs) and their derivates gained extremely high attention in recent years for their promising effects in the treatment of inflammation (Carrade *et al.* 2012 *Cell Med.* **4**, 1–12). Their application could help in the modulation of the immune inflammatory response, providing regeneration and remodeling of injured tissue. The aim of this preliminary study was to evaluate *in vitro* the effect of Wharton's jelly (WJ) MSC-derived conditioned medium (CM) on equine endometrial cells with lipopolysaccharide (LPS)-induced inflammation. Equine WJMSCs were isolated from 3 samples, pooled and then frozen at passage (P) 3. The WJMSCs were thawed and maintained in culture medium (Dulbecco's Modified Eagle Medium (DMEM) + 10% fetal bovine serum) until 80%–90% confluence, and then the culture medium was replaced with serum-free Ringer lactate solution. The CM was collected after 24 h of starvation and concentrated 10 times by centrifugation at 4000*g* for 30 minutes at 13°C using 3-kDa molecular weight cutoff filter units and then stored at −80°C until use. Endometrial cells were obtained from 3 diestrus mare uteri. Cells at P1 were plated in 24-well plates at a density of 20 000 cells/well. Four different conditions were tested: DMEM standard complete medium (CTRL), DMEM with the addition of (1) 10% of CM (CM), (2) 10 ng mL^{-1} LPS (LPS) and (3) LPS combined with CM (LPS + CM). Cell viability, apoptosis and necrosis were assessed after 6, 12 and 24 h of incubation, using acridine orange and propidium iodide fluorescent staining. Data were analysed by analysis of variance and Tukey's test. Results (mean ± s.d.) of 3 replicates are reported in Table 1. At each incubation time, CM gave similar results to the CTRL for all parameters. After 6 h of incubation, LPS increased the percentage of necrotic cells, while co-treatment with CM prevented the detrimental effect. After 12 h of incubation, LPS decreased cell viability and increased apoptosis and necrosis compared with the CTRL and CM groups, while the co-treatment LPS + CM gave intermediate results. In conclusion, these preliminary results suggest that equine WJMSCs with cytokines, chemokines, growth factors and microvesicles may in part mitigate the LPS-induced inflammation on endometrial cells, promoting further investigations on WJMSC-CM characterisation and its mechanisms of action.

Table 1. Results (mean ± s.d.) of 3 replicates.

% cells	Time	Conditions			
		CTRL	CM	LPS	LPS + CM
Live	6 h	87.9 ± 6.9	84.5 ± 5.8	60.5 ± 9.2	70.6 ± 10.3
	12 h	89.4 ± 6.4[a]	85 ± 13.9[a]	40.6 ± 21.9[b]	60 ± 8.9[ab]
	24 h	81.8 ± 14.5	77.8 ± 13.5	55.2 ± 14.0	72 ± 20.8
Apoptotic	6 h	12.1 ± 6.9	14.5 ± 4.8	25.7 ± 4.3	26.7 ± 12.6
	12 h	10.6 ± 6.4[a]	13 ± 10.4[a]	43.8 ± 19.4[b]	29.9 ± 16.3[ab]
	24 h	14.6 ± 9.4	18 ± 11.0	32.7 ± 12.7	20.5 ± 17
Necrotic	6 h	0[a]	1 ± 1.7[a]	13.8 ± 5.0[b]	2.6 ± 3.2[a]
	12 h	0[a]	2 ± 3.5[a]	15.7 ± 3.0[b]	10.4 ± 3.1[ab]
	24 h	3.6 ± 5.5	4.2 ± 2.5	12.3 ± 4.1	7.5 ± 4.0

[a,b]Values within rows with different superscripts are significantly different; $P < 0.05$.