Title: Scaled Production of Human Placental Extract (HPE), through Pooling of Donor Tissues, Improves Wound Healing and Reduces Overall Disease Severity in Necrotizing Enterocolitis

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Background: Necrotizing Enterocolitis (NEC) is an acute disorder of premature infants characterized by intestinal necrosis. NEC is the most serious GI disease of premature babies. These neonates weigh <1.5kg and often suffer from a multifaceted array of medical issues. NEC occurs in 2.6k-3k premature babies annually (30% mortality rate). Mechanistically, NEC is an inflammatory condition of the GI tract with a combination of risk factors exaggerating the inflammatory response, leading to hemorrhagic necrosis.

Hypothesis: An anti-inflammatory therapeutic mimicking the natural mid-pregnancy amniotic fluid, HPE, will reduce NEC severity by promoting homeostasis and normal maturation of the GI tract.

Methods: HPE is produced from pooled, donated term placental discs from n=10 donors. Plakous has received an exemption to pool product from the FDA to reduce donor variability, improve product consistency and increase scalability. Comparisons of individual versus pooled batches of HPE de-identified lot release factors are presented in **Table 1**. To determine a direct effect of HPE on injured neonatal gut epithelial cells, scratch wound assays were performed on confluent monolayers derived from ~1-day old piglet ileal crypts over 24 hours. Next, NEC was induced in piglets through premature (90%) cesarean delivery followed by 48 hours of fasting then hyperosmolar formula feeding. Prior to formula feeding, piglets were *nil per os* or enterally supplemented with HPE. GI tissues were assessed grossly and histologically, with increasing NEC score reflecting severe disease. Statistical evaluation was by 2-Way ANOVA and Tukey post-test, $p<0.05$.

Results: The average values and corresponding %CV results from individual versus pooled batches for each of the lot release factors indicate reduced or equal variation for the three pooled lots when compared to the thirty individual tissues and supports pooling of tissues in the production of HPE. In vivo administration of HPE reduced overall disease severity by 28% in treated compared to untreated piglets (**Fig.1**) and increased weight gained (**Fig.2**). In vitro, HPE increased the closure of scratch wounds compared to untreated wounds in neonatal porcine ileal epithelial monolayers. HPE also expanded the proportion of proliferative cells in scratch wound leading edges compared to untreated wounds.

Conclusions: Plakous has successfully scaled HPE production by pooling multiple placental tissues per lot, decreasing donor variability and increasing yield, minimizing the concentration variability of the constituent proteins and producing a more consistent HPE product lot to lot. Plakous has demonstrated preclinical bioactivity of pooled lots of product and its effectiveness to decrease NEC severity and improve gut healing, and in the future believes patients will benefit from a more consistent HPE product with lower lot to lot variability of the constituent protein concentrations.

Funding: Plakous Investment, NIH R44HD100243, K01 OD019911-01A1
Ethical animal research: Inotiv IACUC Approved, NCSU IACUC Approved

Supporting Documentation

	Individual			Pooled		
	Avg	**SD**	**%CV**	**Avg**	**SD**	**%CV**
Factor 1	0.8595	0.2341	27.24%	0.9137	0.128	14.01%
Factor 2	53.07	23.45	44.19%	49.56	6.554	13.22%
Factor 3	72.07	18.82	26.11%	69.74	9.714	13.93%
Factor 4	52.07	31.68	60.84%	40.51	6.94	17.13%
Factor 5	2234	588.1	26.32%	2320	621.8	26.80%
Factor 6	0.03157	0.00752	23.82%	0.03099	0.0098	31.64%

Table 1. Each factor's average concentration is ng per mg of total protein. Abbreviations: Avg: average; SD: standard deviation; %CV: percent coefficient of variation

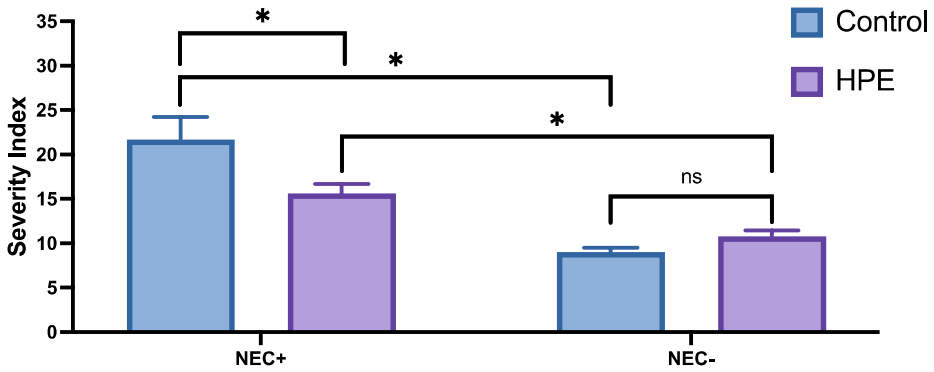

Figure 1 *Results are for all HPE dosed (0.5x HPE, 1x HPE, and 2x HPE) piglets. Data are mean and standard error mean assessed by 2-way ANOVA comparing Standard of Care (Control) to HPE across NEC status, p<0.05 was considered significant, p<0.05 = "*". Abbreviations: HPE: human placental extract; NEC: necrotizing enterocolitis; ns: not significant.*

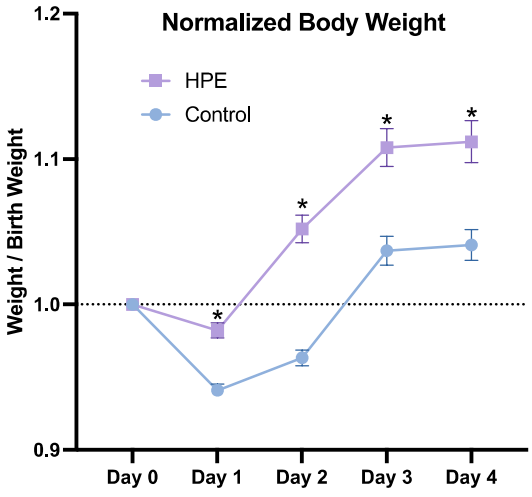

Figure 2 *Results are for all HPE dosed (0.5x HPE, 1x HPE, and 2x HPE) piglets. Data are mean and standard error mean assessed by 2-way ANOVA comparing Standard of Care (Control) to HPE doses and collective HPE therapy, p < 0.05 = "*" was considered significant. Abbreviations: HPE: human placental extract; NEC: necrotizing enterocolitis; ns: not significant.*

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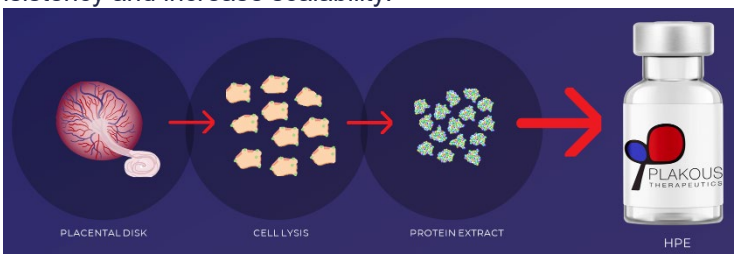
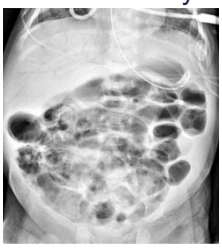
Human Placental Extract

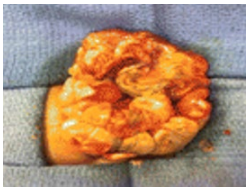
HPE is produced from pooled, donated term placental discs from n=10 donors. Plakous has received an exemption to pool product from the FDA to reduce donor variability, improve product consistency and increase scalability.



Background

- Necrotizing Enterocolitis (NEC) is an acute disorder of premature infants characterized by intestinal necrosis.
- An inflammatory condition of the GI tract with a combination of risk factors exaggerating the inflammatory response, leading to hemorrhagic necrosis.
- NEC is the most serious GI disease of premature babies.
- These neonates weigh <1.5kg and often suffer from a multifaceted array of medical issues.
- Occurs in 2.6k-3k premature babies annually.
- 30% mortality rate & 35% require surgery.

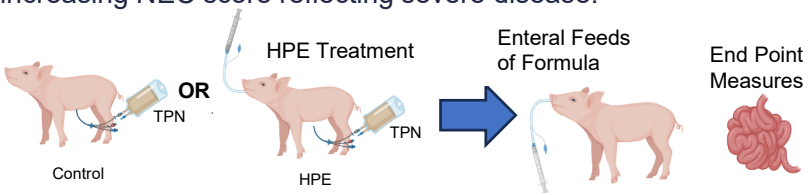




X-ray of Neonatal Intestinal Distension & photo of surgical resection.

Methods

To determine a direct effect of HPE on injured neonatal gut epithelial cells, scratch wound assays were performed on confluent monolayers derived from ~1-day old piglet ileal crypts over 24 hours. Next, NEC was induced in piglets through premature (90%) cesarean delivery followed by 48 hours of fasting then hyperosmolar formula feeding. Prior to formula feeding, piglets were nil per os or enterally supplemented with HPE. GI tissues were assessed grossly and histologically, with increasing NEC score reflecting severe disease.



Conclusions

Plakous has successfully scaled HPE production by pooling multiple placental tissues per lot, this decreases donor variability and increases yield, and in turn produces a more consistent HPE product across lots. Plakous has evaluated preclinical bioactivity of pooled lots of HPE, demonstrated the products' effectiveness to decrease NEC severity and improve gut healing, and in the future believes patients will benefit from a more consistent product with lower lot to lot variability of the constituent protein concentrations.

Acknowledgements

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Preliminary Results

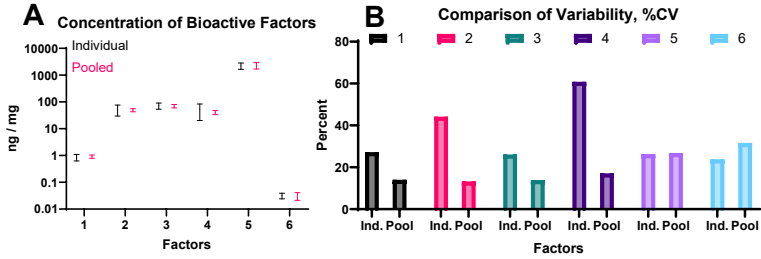


Figure 1. The average **(A)** and corresponding %CV results **(B)** from individual versus pooled batches for each of the lot release factors indicate reduced or equal variation for the three pooled lots when compared to the thirty individual tissues.

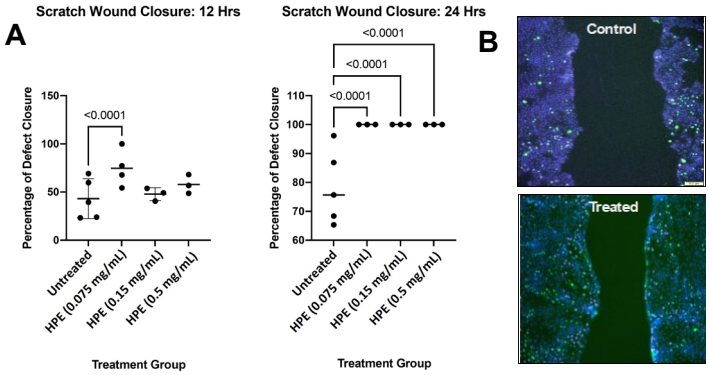
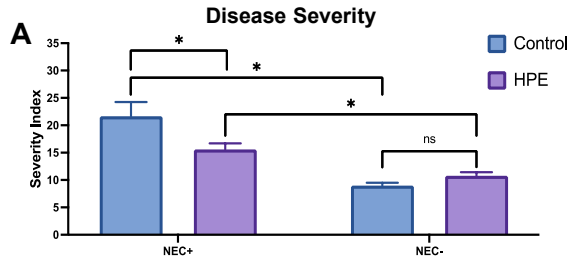
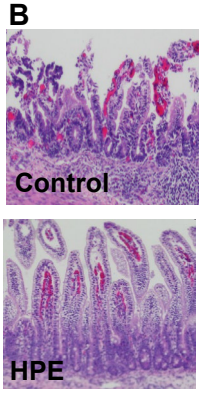


Figure 2. HPE increased the closure of scratch wounds compared to untreated wounds in neonatal porcine ileal epithelial monolayers **(A)**. HPE also expanded the proportion of proliferative cells in scratch wound leading edges compared to untreated wounds **(B)**.





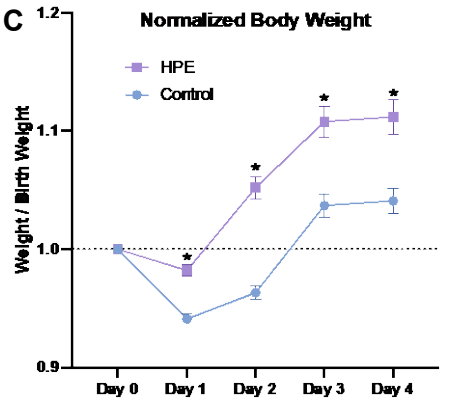


Figure 3. In Vivo Assessment of HPE Therapy on NEC. In vivo administration of HPE reduced overall disease severity by 28% in treated compared to untreated piglets **(A)**, representative images of ileum **(B)**, and HPE increased weight gained **(C)**. (*p<0.05; ns = not significant)