

# Does age matter?: An Investigation of the Variation in Vitellogenin Production Compared to Age of Fathead Minnow (*Pimephales promelas*) Using Historical Control Data



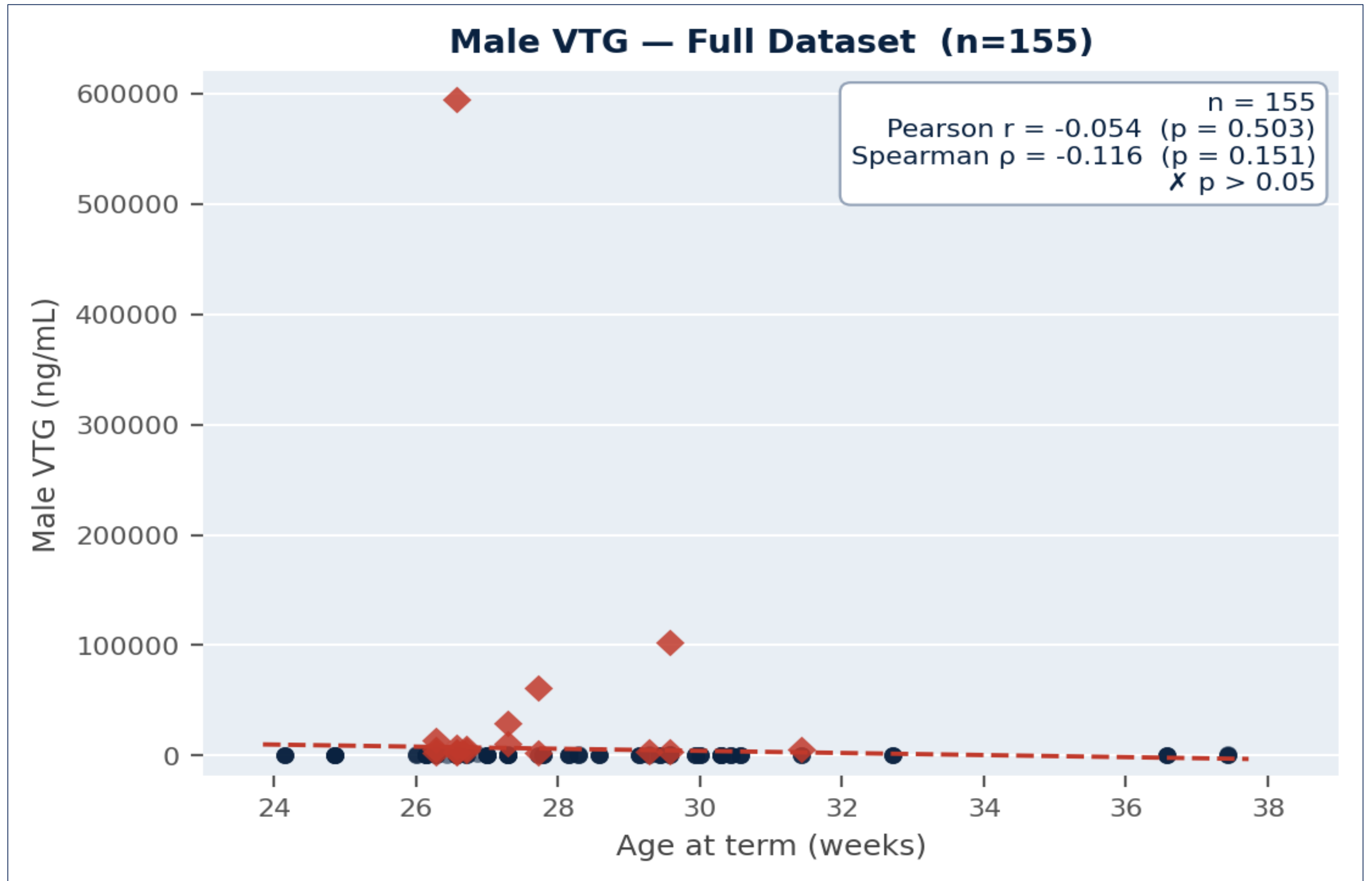
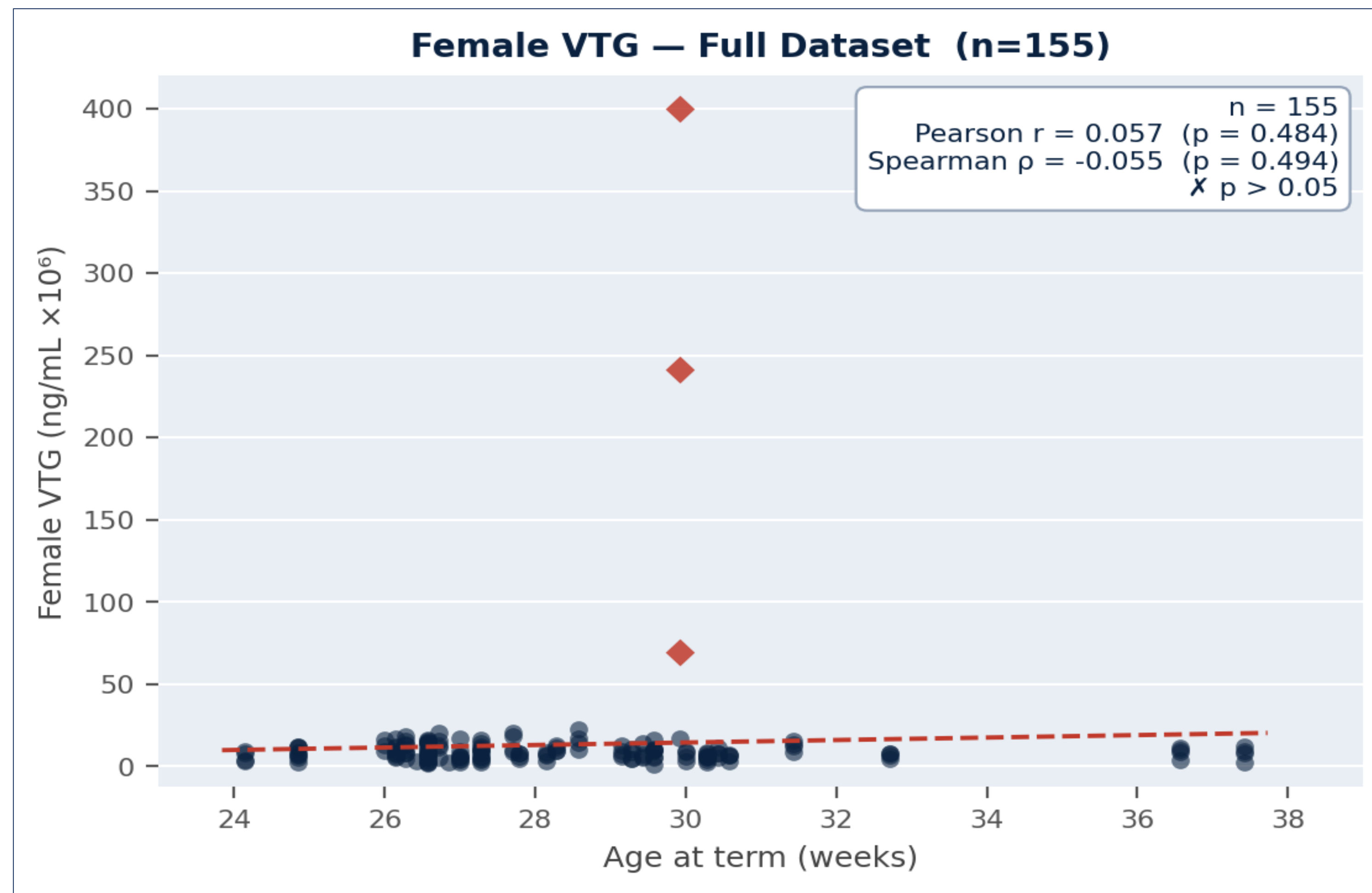
SETAC 2026 | *Pimephales promelas* | Female & Male Vitellogenin

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The OCSPP 890.1350 FSTRA guideline recommends the use of reproductively active fathead minnow between the ages of 4.5 to 6 months (18-24 weeks) old for testing. The OECD 229 guideline states fathead minnow should be approximately  $20 \pm 2$  weeks of age for use in FSTRA testing if reared at 25°C. Do these windows of time dictate any difference of VTG concentration at test termination? In this investigation, the fathead minnow from 39 studies were compared between the ages of 24 to 37 weeks at termination to compare the vitellogenin output against age.

**Study Goal:** Evaluate whether fish age is a significant variable of female vitellogenin and male vitellogenin concentration in *Pimephales promelas* using historical control data. Both analyses were run with the full termination data set and with statistical outliers removed from the data.

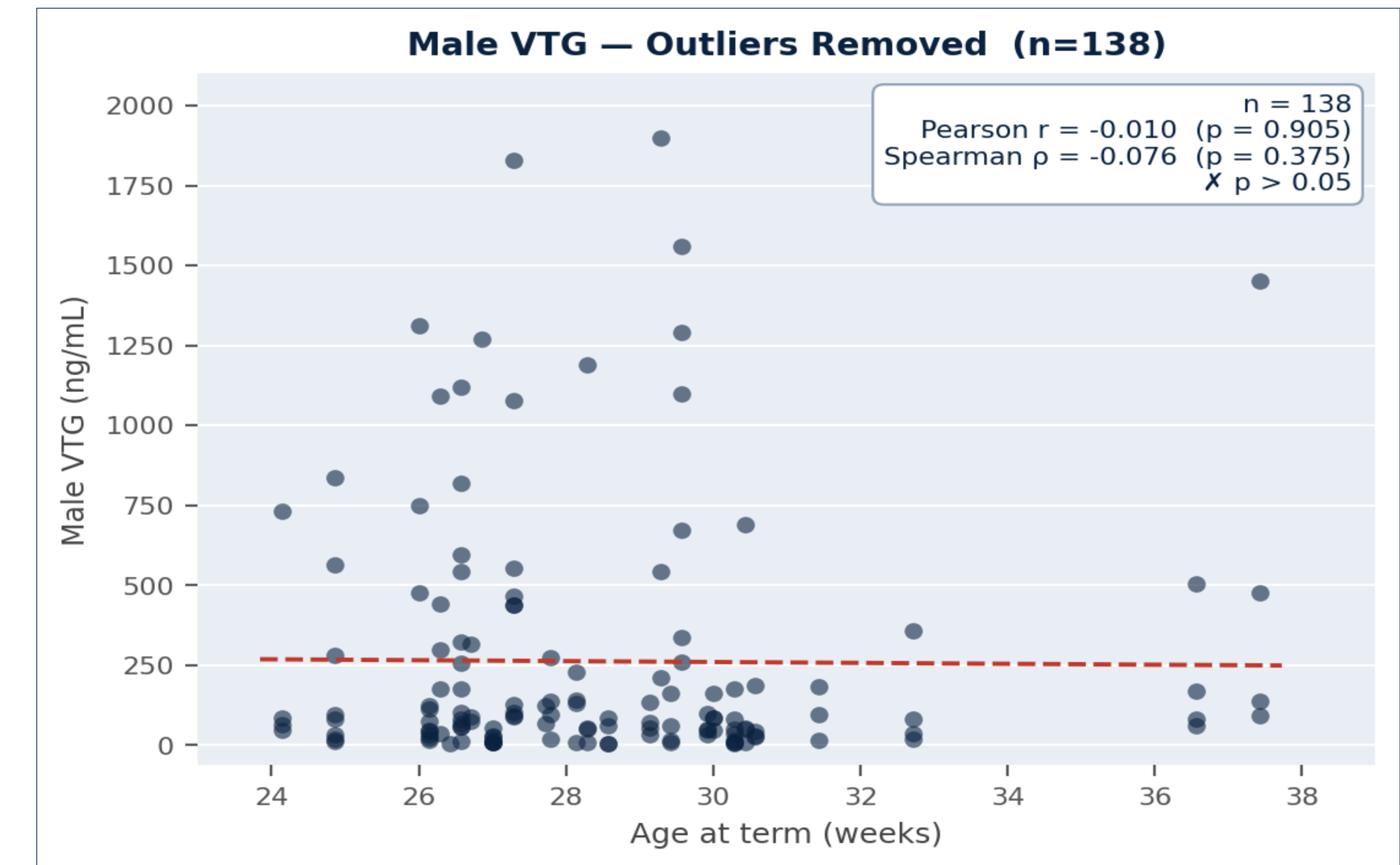
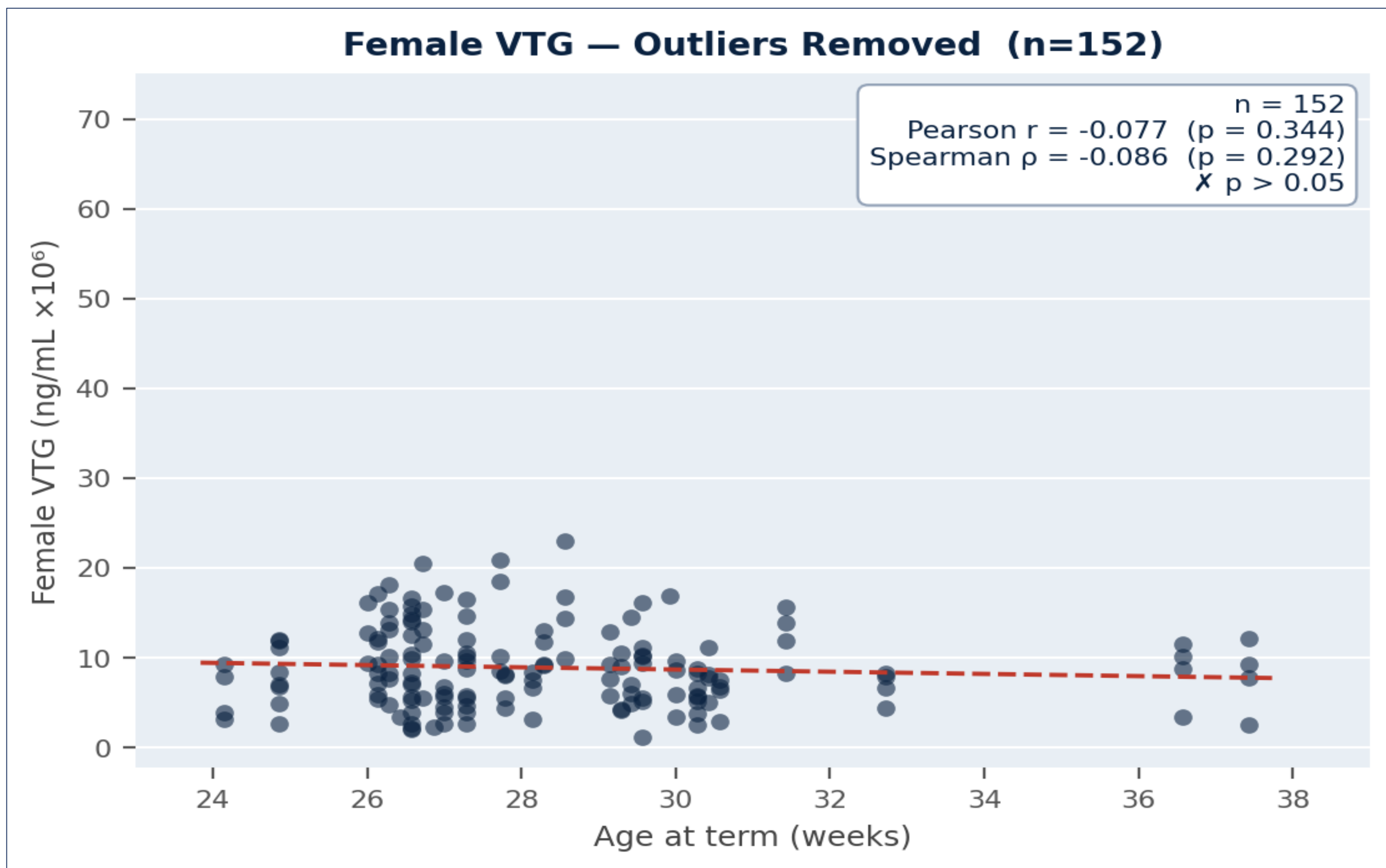


n	Pearson r	p (Pearson)	Spearman ρ	p (Spearman)
155	0.057	0.484	-0.055	0.494

n	Pearson r	p (Pearson)	Spearman ρ	p (Spearman)
155	-0.054	0.503	-0.116	0.151

Outliers Removed (n = 152, -3 pts removed | 3×IQR fence: 30.7 x10<sup>6</sup> ng/mL)

Outliers Removed (n = 138, -17 pts removed | 3×IQR fence: 2,034 ng/mL)



n	Pearson r	p (Pearson)	Spearman ρ	p (Spearman)
152	-0.077	0.344	-0.086	0.292

n	Pearson r	p (Pearson)	Spearman ρ	p (Spearman)
138	-0.01	0.905	-0.076	0.375

## Comparative Summary

Statistic	Female VTG – Full	Female VTG – Outliers Removed	Male VTG – Full	Male VTG – Outliers Removed
n	155	152 (-3)	155	138 (-17)
Outlier fence	30.7 x10 <sup>6</sup> ng/mL	—	2,034 ng/mL	—
Pearson r	0.057	-0.077	-0.054	-0.010
Pearson p-value	0.484	0.344	0.503	0.905
Spearman ρ	-0.055	-0.086	-0.116	-0.076
Spearman p-value	0.494	0.292	0.151	0.375
Mean VTG	13.3 x10 <sup>6</sup> ng/mL	8.9 x10 <sup>6</sup> ng/mL	5,777 ng/mL	262 ng/mL
SD VTG	37.0 x10 <sup>6</sup> ng/mL	4.4 x10 <sup>6</sup> ng/mL	48,643 ng/mL	394 ng/mL
Result (α = 0.05)	Not significant	Not significant	Not significant	Not significant



### Conclusions:

- No statistically significant correlation was detected between age at termination and female vitellogenin concentration in either the full dataset ( $r = 0.057$ ,  $p = 0.484$ ) or with outliers removed ( $r = -0.077$ ,  $p = 0.344$ ).
- No statistically significant correlation was detected between age at termination and male vitellogenin concentration in either the full dataset ( $r = -0.054$ ,  $p = 0.503$ ) or with outliers removed ( $r = -0.010$ ,  $p = 0.905$ ).
- Outlier removal substantially reduced VTG variance (female SD: 37.0 → 4.4 M ng/mL; male SD: 48,643 → 394 ng/mL) but did not change the null result in either endpoint. This is important to note as the OCSPP guideline recommends the use of outlier analysis but the OECD 229 guideline does not.
- These results suggest that age at initiation of the study is not a significant confounding variable for VTG response in *Pimephales promelas* under these test conditions. Therefore, the sexual dimorphism of the population should be a greater indicator of appropriate use, not only age.
- Next Steps: Future studies may focus on the VTG production during different stages of fish reproduction (i.e. prior to egg production or immediately following) as another variable to understand the irregularity of vitellogenin output amongst the species.

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