Title: THREE DAYS OF ELEVATED PROGESTERONE IS A BETTER TEST THAN ONE DAY OF ELEVATED PROGESTERONE IN FRESH EMBRYO TRANSFER CYCLES

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Background: Prior literature has shown that a rise in serum progesterone on the day of trigger is associated with reduced likelihood of pregnancy outcomes following fresh embryo transfers (1,2). However, this rise is often gradual and there is a theoretical concern that cumulative progesterone exposure, even at modestly elevated values, may be negatively associated with embryo transfer outcomes. Thus, we tested the hypothesis that an elevated progesterone over 3 cumulative days is a better predictor of negative pregnancy outcomes than a 1 day progesterone level, putatively due to increased embryo-endometrial asynchrony.

Objective: To evaluate the association between persistently elevated serum progesterone prior to trigger (3 day cumulative measurement) with live birth following fresh embryo transfers. To compare progesterone on day of trigger and cumulative progesterone as predictive tests of failed live birth (1 versus 3 days progesterone levels).

Materials and Methods: We conducted a retrospective cohort study in a large REI network examining all fresh, autologous transfer cycles from 2013-2022. Cycles missing any progesterone values on the three days leading up to trigger were excluded. The primary outcome was live birth. Three day cumulative progesterone was defined as the sum for the three days ending on day of trigger. One day progesterone was defined as the serum progesterone on the day of trigger. Greater than efficiency curves were created to determine the threshold at which there was a decline in live birth for the 1 and 3 day progesterone values. Once thresholds were established, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for the two tests. Additionally, McNemar's test was performed to discriminate between the two tests ability to detect a failed live birth.

Results: In total, 13,374 cycles were included with an overall live birth of 37.1%. An efficiency curve using 1 day progesterone demonstrated a precipitous decline in live birth to 22.2% when P $\geq$2ng/ml. The efficiency curve of the 3 day cumulative progesterone showed a decrease in live birth to 23.6% when P $\geq$4.5ng/ml.

Sensitivity was poor for both tests (1 Day: 3.9%, 3 Day: 7.4%). The specificity (98.0% vs 96.1%), PPV (77.8% vs 76.4%), and positive likelihood ratios (2.0 vs 1.9) were similar between 1 day P ≥2ng/ml and cumulative 3 day P ≥4.5ng/ml, respectively (Table 1).

In analysis of only patients with disease (failure to achieve live birth), if both tests predicted the same outcome (i.e. live birth or no live birth each), then there was no difference in the ability to discriminate the two tests diagnostic ability. However, if there was a discrepancy in the predicted outcome such that the two tests predicted opposite outcomes, then 3 day cumulative P ≥4.5ng/ml was significantly better at predicting the negative outcome by identifying 318 cases of failed live birth versus 32 for the 1 day test (OR 9.9% CI 6.9-143, *P*<0.0001).

Conclusion: The 3 day cumulative serum P ≥4.5ng/ml is associated with failure to achieve live birth and detects more patients at risk of failed cycles than the 1 day progesterone level.

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Table 1: Sensitivity, Specificity, Positive and Negative Predictive Values of P thresholds

	1 day Progesterone ≥ 2.0 ng/ml	95% CI	3 day Cumulative Progesterone ≥ 4.5 ng/ml	95% CI
Sensitivity	3.9%	3.6 - 4.4%	7.4%	6.8 – 8.0%
Specificity	98.0%	97.6 - 98.4%	96.1%	95.6 – 96.7%
Positive Likelihood Ratio	2.0	1.59 – 2.49	1.91	1.63 – 2.23
Negative Likelihood Ratio	0.98	0.97 – 0.99	0.96	0.96 – 0.97
Positive Predictive Value	77.8	73.7 – 81.4%	76.4%	73.5 – 79.1%
Negative Predictive Value	36.7%	36.5 – 36.8%	37.9%	37.7 – 38.1%

Accuracy	38.0%	37.0 – 38.9%	40.3%	39.5 – 41.2%