Optimization of the Sliced Testis Steriodogenesis Assay

Unaudited
Final Draft Report

RTI International
Laboratory of Reproductive and Endocrine Toxicology
Chemistry and Life Sciences Group
Center for Life Sciences and Toxicology
Post Office Box 12194
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Battelle Memorial Institute
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1.0 INTRODUCTION

1.1 Background

In 1996, the Food Quality Protection Act (FQPA) amendments were enacted by Congress to authorize the Environmental Protection Agency (EPA) to implement an Endocrine Disruptor Screening Program (EDSP) on pesticides and other substances found in food or water sources for endocrine effects in humans (FQPA, 1996). In this program, comprehensive toxicological and ecotoxicological screens and tests are being developed for identifying and characterizing the endocrine effects of various environmental contaminants, industrial substances, and pesticides. A two-tiered approach will be utilized. Tier 1 employs a combination of *in vivo* and *in vitro* screens, and Tier 2 involves *in vivo* testing methods using two-generation reproductive studies. A steroidogenesis assay is proposed as one of the Tier 1 screening battery assays.

A detailed review paper (DRP) about steroidogenesis was prepared. The DRP: (1) summarized the state of the science of the *in vivo, ex vivo,* and *in vitro* methodologies available for measuring gonadal steroidogenesis; (2) for each methodology, presented a review of the individual assays and representative data generated by investigators who used the assay to evaluate a substance for steroidogenic-altering activity; (3) provided an evaluation of the various methodologies and the assays as tools for screening substances with suspected steroidogenic activity; (4) recommended a particular screening method and assay as a screening tool; and (5) described the strengths, weaknesses, and implications for further research associated with the recommended screening assay.

The *in vitro* sliced testis steroidogenesis assay was selected as the most promising screening tool for identifying substances with steroidogenic-altering activity. The sliced testis assay was recommended because it can be conducted at a minimal cost, quickly, and simply with standard laboratory equipment and basic laboratory training; the preparation is stable, and the parenchyma remains viable over a sufficient time period to measure changes in end-product hormone production; the assay is relatively sensitive and specific; the assay uses parenchyma that maintains the cytoarchitecture of the organ; the assay uses a reduced number of animals (up to quartered testis slices); the assay should be relatively easy to standardize (by optimization); and the assay has a well-defined endpoint in testosterone and, if desired, can be modified to include additional intermediate hormonal endpoints.

Although a promising tool, the sliced testis assay remains to be fully tested as an assay that can meet all the demands of an endocrine disruptor screening tool. Concerns raised by the EPA and Endocrine Disruptor Methods Validation Subcommittee (EDMVS) suggested that experiments be conducted to ensure the optimization of the assay prior to more rigorous

optimization was to describe in detail the experiments designed to provide data for setting in place the procedures and parameters that will optimize the performance of this assay.

1.2 Objectives

The study plan for testing the factors described in the previous section involved two phases and utilized single factor and factorial experimental designs. A diagram of the experimental design for this study is illustrated in Figure 1.

The study was divided into Phases I and II. In Phase I, the Preliminary Experimental Phase, the analytical assays planned for use were verified, storage containers and lengths of storage were selected, and three factors that may affect the performance of the assay were tested. The reasoning for including these 3 factors in the preliminary phase was to establish early whether a given level of each factor was going to affect assay performance. Although any factor listed in the study plan could have been rationalized to fit such a criteria, inclusion in the preliminary phase also required that the factor be unlikely to have an interaction or, at best, a minimal interaction with another experimental factor. Although subjective, these factors were believed to best fit these criteria. Furthermore, it was believed essential to establish the optimal level for each of these factors before proceeding with the factorial experiments since an effect of one of these would require additional verification experiments after sensitivity analysis. Finally, by establishing the media type early on in the experiment, the analytical assay verification testing (Phase I) and Optimization of Sample Testing (Phase II) could be initiated earlier in the study milestone schedule.

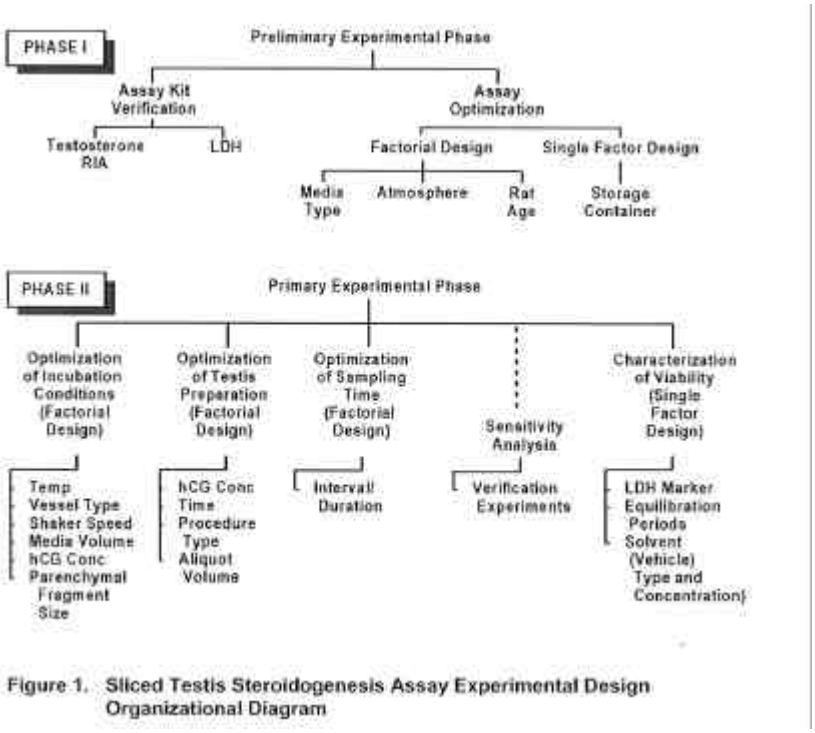


Figure 1. Sliced Testis Steroidogenesis Assay Experimental Design Organizational Diagram

The reasoning for including these 3 factors in the preliminary phase was to establish early whether a given level of each factor was going to affect assay performance. Although any factor listed in the study plan could have been rationalized to fit such a criteria, inclusion in the preliminary phase also required that the factor be unlikely to have an interaction or, at best, a minimal interaction with another experimental factor. Although subjective, these factors were believed to best fit these criteria. Furthermore, it was believed essential to establish the optimal level for each of these factors before proceeding with the factorial experiments since an effect of one of these would require additional verification experiments after sensitivity analysis. Finally, by establishing the media type early on in the experiment, the analytical assay verification testing (Phase I) and Optimization of Sample Testing (Phase II) could be initiated earlier in the study milestone schedule.

2.0 MATERIALS AND CHEMICALS

2.1 Reagents and Solutions

No test substances were used in this study. The chemicals used in this study were used to prepare reagents and solutions for the assay. All reagents and solutions had appropriate information documented, which included the identity, concentration, storage requirements, and expiration date. Reagents and solutions were prepared according to Standard Operating Procedures.

2.2 Standards

Verification of the analytical assays required testosterone for the radioimmunoassay (RIA) method and lactic dehydrogenase (LDH) for the spectrophotometric assay. In addition, hCG was used as a stimulant of the sliced testis bioassay. These substances were considered standards.

2.2.1 Testosterone

Chemical Name:	Testosterone
CAS No.:	58220
Molecular Weight:	288.4
Solubility:	Clear colorless to very faint yellow solution at 100 mg/mL in chloroform
Supplier:	Sigma-Aldrich Chemical Company
Lot No.:	6384-70-8
Purity:	NLT 98%
Storage Conditions:	2-year shelf life

A safety protocol exists for the use of the radioactive form of testosterone.

2.2.2 Human Chorionic Gonadotropin (hCG)

Chemical Name:	hCG
CAS No.:	9002-61-3
Molecular Weight:	36,700
Solubility:	H_2O
Supplier:	Calbiochem
Lot No.:	B11174, B51120

Purity:	Approx. 30% hCG by weight
Storage Conditions:	Freezer (-20°C). Following reconstitution, aliquot and freeze (-20°C). Stable for 2 years as supplied.

2.2.3 LDH

Chemical Name:	Lactate Dehydrogenase
CAS No.:	EC 1.1.127
Source:	Rabbit muscle
Solubility:	
Supplier:	Sigma-Aldrich Chemical Company
Lot No.:	99H7480
Purity:	NLT %
Storage Conditions:	2-8°C; 1-year shelf life

3.0 METHOD FOR PROTOTYPICAL ASSAY

The prototypical assay describes the sliced testis assay using the conditions that are believed to be the starting conditions of the assay. This section does not describe any experiments to be conducted; rather, it describes the settings of all factors, except for the one that is being tested in order to perform the optimization experiments described in the following sections. A run is defined as a single sample vessel with assay components.

The sliced testis assay prototype uses a 15-week-old male Sprague-Dawley rat, which is euthanized and its testes removed. The testes are decapsulated, weighed, and placed in cold (4°C) DPBS. The media is medium-199 (Gibco) that has added 0.71 g sodium bicarbonate, 2.1 g HEPES, 1.0 g/L BSA, and 0.025 g/L soybean trypsin inhibitor, and is adjusted to a pH of 7.4. The time from removal to the time of slicing is held to under 1 hour. Each testis is sliced along the longitudinal axis into approximately 4 slices. Each slice is placed in a 20 mL borosilicate scintillation vial (loosely capped) that contains 5 mL of media (Figure 2). The vials containing the testicular sections and media are incubated at 34°C on a shaker (at 135 rpm) in 5% CO_2/95% air. After the first period of incubation, the media is removed and discarded. Fresh media (5 mL) is added to the vial and an aliquot of media (0.5 mL) is collected. The sample is centrifuged and the supernatant transferred to a labeled vial and stored at approximately -70°C in a siliconized plastic container for no more than 1 month. This sample is the baseline sample. Next, 1/2 of the vials are challenged with a stimulant (e.g., hCG) and the other 1/2 are not. The final hCG concentration is 0.1 IU/mL. Additional media samples are collected from the vials at 1, 2, 3, and 4 hours postchallenge. These media samples are also stored frozen for later analysis. Samples are analyzed for testosterone using an RIA method. All samples for a given day's set of runs should be analyzed in the same testosterone RIA.

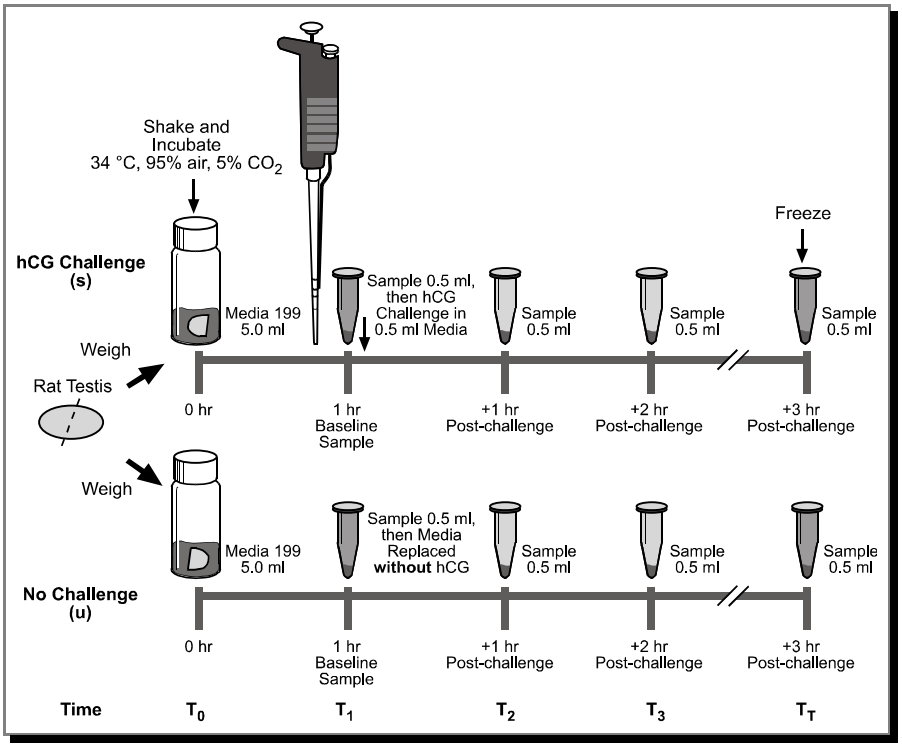


Figure 2. Technical Flow Illustration of the Sliced Testis Steroidogenesis Assay

4.0 METHODS FOR PHASE I—PRELIMINARY EXPERIMENTAL PHASE

Phase I is comprised of the verification experiments for the 2 analytical assay methods (testosterone RIA and LDH spectrophotometry), the determination of the preferred storage container and storage length, and the optimization experiments for the 3 factors to be tested (media type, incubation atmosphere, and animal age). The testosterone RIA method was verified prior to conducting any optimization assay since it was needed as an endpoint to determine optimization of the sliced testes assay. The storage container determination was also performed before any further optimization assays were run. The determination was made using standards of testosterone in the prototypical media, modified M-199 without phenol red.

4.1 Testosterone Radioimmunoassay

The objective of this experiment was to verify that testosterone can be measured in the sliced testis assay media. A RIA commercial kit (Diagnostic Products Corporation, Los Angeles, CA), that utilizes ^{125}I-testosterone and a testosterone-specific antibody affixed to polypropylene culture tubes, was used to measure testosterone. The assay was verified in all 3 of the potential assay media.

Testosterone (Sigma, St. Louis, MO; T-1500) was used for preparing the standard curve and was stored desiccated at room temperature in the RTI vault. A standard was prepared in ethanol (0.1 mg/mL). Up to an 8-point standard curve (but not less than a 4-point standard curve) was prepared using standards with concentrations of 0.07, 0.16, 0.41, 1.02 , 2.56, 6.4, 16, and 40 ng/ml in PBS gel buffer (0.1 M phosphate buffered saline with 0.1% [w/v] sodium azide and 0.1% [w/v] gelatin, pH 7.4). In addition, procedural controls were included in each run. The standard curve points and the procedural controls were prepared in quadruplicate; the bioassay unknowns and the internal standards (see below) were prepared in duplicate. The volume of all standards and controls (including bioassay unknowns) were adjusted to 50 µL by adding the PBS-Gel Buffer. Next, 1 mL ^{125}I-testosterone was added to each antibody-coated tube and mixed (vortexed). The tubes were incubated in a 37°C water bath for 3 hours, during which time testosterone, whether labeled or unlabeled, competed for testosterone-specific antibody binding sites. At the end of the incubation period, the free (unbound) testosterone in the supernatant fluid of all tubes was aspirated, and tubes were wiped clean of fluid. The bound testosterone was counted in a gamma counter for 1 minute. The concentration of testosterone was estimated against the standard curve. Values were reported as a mean concentration (ng/mL) of duplicate analyses, unless only a single value was available. Verification of the testosterone assay involved preparation of internal standards (at least 3) using spiked media, with concentrations ranging from 12.5 to 500 ng/mL. Each concentration was run at each of 3 volumes–10, 25, and 50 °L, to check for parallelism, and each sample was adjusted to 50°L by adding the PBS gel buffer. The

low and high standards were analyzed in at least duplicate. Verification was based on results determined for accuracy, precision, specificity, and linearity. Accuracy is expressed as the relative error, which was determined by comparing the measured to the target concentration. Relative errors within 15% were acceptable. Precision is expressed as the relative standard deviation (RSD) or coefficient of variance (CV), which was determined by calculating the mean and standard deviation (SD) of the low and high standards. A RSD or CV within 15% was acceptable. The sensitivity was acceptable if the means of the blanks and low standards were significantly different at the 5% significance level. Linear determinations of the standard curve line were made and a correlation coefficient (r) calculated. An r of 0.90 or greater was considered acceptable.

Inter- and intra-assay variability was determined. The intra-assay variability was determined from the precision results calculated from the results obtained by measuring the low and high standards in triplicate. The inter-assay variability was determined by repeated analyses of the standards by generating a standard curve on 3 different days.

4.2 LDH Spectrophotometric Assay

The objective of this experiment was to verify that LDH can be measured in the sliced testis assay media. The LDH assay measures the rate at which NADH is formed (when NAD is reduced) when it catalyzes the oxidation of lactate to pyruvate. NADH is measured at 340 nm using a kinetic-spectrophotometric method. The assay and samples are temperature sensitive. The samples should not be refrigerated or frozen. The assay has been characterized for assay conditions at 37ºC. LDH activity is expressed in U/L.

Qualification of the assay, using Media 199 without phenol red, consisted of determination of sensitivity and dilutional linearity. These were the only qualification tests specific to media use. Intra-assay and inter-assay imprecision were based on serum based controls normally used for instrument monitoring of quality control. Accuracy was based on the linear regression of a media sample spiked with purified LDH and diluted with media.

Intra-assay imprecision was measured by assaying 10 quality control samples within a single run. The mean SD and CV were calculated. The CV should be less than 10%. Inter-assay imprecision was measured by assaying 10 quality control samples over 10 separate runs. The mean SD and CV were calculated. The CV should be less than 10%.

Sensitivity of the assay or limit of detection was determined by analysis of 20 samples of media with no LDH present in the media. A low level of control was also analyzed to determine

the lowest level of detection. Two standard deviations from the mean of the media activity were used as the limit of detection.

Dilutional linearity was used to determine accuracy and linearity limits. A sample of media was spiked with purified LDH (Sigma #L1254). This was diluted with Media 199 without phenol red to within the linear limits of the assay. A series of dilutions by serial dilution of the spiked sample were conducted in triplicate. The linear regression of the line compared to the theoretical activity should be between 0.990 and 1.100.

All calculations were performed using EP Evaluator, Release 3.0 statistical analysis software from David Rhoads Associates, Inc., Kennett Square, PA.

4.3 Phase 1 Optimization Experiments for Media Type, Gaseous Atmosphere, Rat Age, and Storage Container Type

4.3.1 Media Type

The objective was to determine the effect of different types of media (with specified components) on testosterone production using the sliced testis assay. The prototypical assay conditions were used except with regard to the types of media.

The media types tested were:

- **RPMI-1640** media (without phenol red), 10% FCS, 50 ug/mL soybean trypsin inhibitor
- **Medium-199 (Gibco)**, 0.71 g Na bicarbonate, 2.1 g HEPES, 1.0 g/L BSA, 0.025 g / L soybean trypsin inhibitor, adjusted to pH 7.4
- **Eagles MEM**

4.3.2 Gaseous Atmosphere

The objective was to determine the effect of different types of gaseous atmospheres on testosterone production using the sliced testis assay. The prototypical assay conditions were used except with regard to the gaseous atmosphere.

The atmospheres tested were:

- **5% CO_2 / 95% air**
- **5% CO_2 / 95% O_2**

- **Air (3 gases)**

4.3.3 Rat Age

The objective will be to determine the effect of age on testosterone production using the sliced testis assay. The prototypical assay conditions will be used except with regard to the age of the rat used to obtain the testes.

The ages to be tested are:

- **11 weeks of age**
- **15 weeks of age**
- **22 weeks of age**

4.3.4 Storage Container Type

The objective was to determine the effect of storage-type containers on the stability of testosterone in media. The types of containers tested were: siliconized plastic and nonsiliconized plastic.

4.4 Phase 1 Experimental Design

4.4.1 Factorial Design Experiments

These experiments were conducted as a 3^3 full factorial design with 1 replicate per condition. The experimental factor levels are summarized in Table 1. The factorial test conditions are displayed in Table 2. The 27 factor level combinations were run in random order. Each combination was run with and without hCG stimulation for a total of 54 test runs. Each test run response (ng T/mL) were determined at 1, 2, 3, and 4 hours after media refreshment.

Table 1. Summary of Experimental Factors for Phase 1 Optimization

Factor Identification	Units	Experimental Levels			Coded Experimental Levels		
		1	2	3	1	2	3
Media Type	NA	RPMI-1640	Medium-199	Eagles-MEM	-1	0	+1
Gaseous Atmosphere	NA	5% CO_2 ▾ 95% air	5% CO_2 ▾ 95% O_2	air	-1	0	+1
Rat Age	Wks	11	15	22	-1	0	+1

NA = not applicable.

Table 2. Factorial Test Conditions for Phase 1 Optimization Experiment

Media Type	Gaseous Atmosphere	Rat Age
-1	-1	-1
-1	-1	0
-1	-1	+1
-1	0	-1
-1	0	0
-1	0	+1
-1	+1	-1
-1	+1	0
-1	+1	+1
0	-1	-1
0	-1	0
0	-1	+1
0	0	-1
0	0	0
0	0	+1
0	+1	-1
0	+1	0
0	+1	+1
+1	-1	-1
+1	-1	0
+1	-1	+1
+1	0	-1

(Continued)

Table 2. Factorial Test Conditions for Phase 1 Optimization Experiment (Continued)

Media Type	Gaseous Atmosphere	Rat Age
+1	0	0
+1	0	+1
+1	+1	-1
+1	+1	0
+1	+1	+1

4.4.2 Phase 1 Single Factor Experimental Design

The objective of this set of experiments was to determine the effect of storage container type on the stability of testosterone in the media. This experimental series did not use the sliced testis assay. Stability was assessed as a function of sample handling factors. The incubation medium used will be determined in the factorial experiments (Phase I). To conduct the stability experiments, a known amount of testosterone was added to the media to achieve a specified target concentration. The target concentration was determined from the media type experiments in Phase I and was in the range of the lowest testosterone concentrations measured in the sliced testis assay. Using this target concentration, the measured concentration was compared to the target concentration. The stability was evaluated based on the difference between the measured and target concentrations. If no statistical difference existed at the 5% level, then the sample was determined to be stable under the conditions tested.

4.5 Phase I Data Evaluation

Upon completion of the Phase I optimization experiments, the results were reviewed for a possible change in the prototype conditions with regard to the 4 factors tested. A decision was made as to whether the Phase II optimization experiments would be conducted using the original prototype or modifications made to the media type, atmosphere, age of rat, and/or storage container type used for the remaining experiments. The endpoint was the amount of testosterone released into the media during the sliced testis assay. The test conditions resulting in the highest values for testosterone were used in Phase II.

5.0 RESULTS AND STATISTICAL ANALYSES

5.1 Testosterone RIA Verification

The testosterone RIA kit from Diagnostic Products was used to verify that the M-199 media without phenol red could be used for the sliced testis assay and provide accurate results for the RIA assay.

Table 3. Values of Standards on Testosterone RIA

Value of Standard	Factor	Reading
8 ng/ml	1	9.33
8 ng/ml	1	8.70
8 ng/ml	1	9.19
8 ng/ml	1	8.86
8 ng/ml	1	9.35
8 ng/ml	2	9.63
8 ng/ml	2	10.16
8 ng/ml	2	10.54
8 ng/ml	2	11.08
8 ng/ml	2	11.86
8 ng/ml	5	10.96
8 ng/ml	5	11.60
8 ng/ml	5	12.41
8 ng/ml	5	11.56
8 ng/ml	5	13.03
2 ng/ml	1	2.56
2 ng/ml	1	2.77
2 ng/ml	1	2.56
2 ng/ml	1	2.59
2 ng/ml	1	2.46
0.5 ng/ml	1	0.63
0.5ng/ml	1	0.80
0.5ng/ml	1	0.76
0.5ng/ml	1	0.74
0.5ng/ml	1	0.71
Unspiked Media		Below Detection Limits of 0.04 ng/mL

Table 4. Testosterone RIA Intra-assay CV

	Number	50 ▾l	25 ▾l	10 ▾l
Unspiked M199	2	Blanks		
+ 8 ng/ml	10	5.24%	8.64%	7.68%
+ 2 ng/ml	10	6.09%		
+ 0.5 ng/ml	10	13.34%		

Table 5. Testosterone RIA Percent Recovery

	50 ▾l	25 ▾l	10 ▾l
+ 8 ng/ml	113.8	133.3	149.0
+ 2 ng/ml	129.5		
0.5 ng/ml	146.4		

Table 6. Testosterone RIA Parallelism

	50 ▾l	25 ▾l	10 ▾l
+ 8 ng/mL*	9.10 ng/mL	10.66 ng/mL	11.92 ng/mL

*The Index between 50 and 10 ▾l was 117.1%, between 25 and 10 ▾l was 111.8%, and between 50 and 10 ▾l was 131.0%.

5.2 LDH Verification

See Appendix A.

5.3 Statistical Analysis of the Phase I Assay Optimization Experiment

5.3.1 Objectives

The assay optimization experiment involves 3 factors (media type, atmosphere, and rat age) which are run in a 3^3 factorial arrangement. Each of these 27 trials is run with and without hCG stimulation, and repeated measurements are taken at baseline (time 0) and at 1, 2, 3, and 4 hours after baseline. The conditions are identified in Table 7. Objectives of the experiment are:

1. To determine the set of conditions that yields the highest estimated testosterone level, and

2. To determine the set of conditions that yields the largest with-versus-without-hCG difference in testosterone levels.

5.3.2 Data

Two basic SAS data sets were constructed from the raw data, and 2 fundamental types of dependent variables were used in the analyses of each type:

- Date Set 1: Cases without hCG stimulation

  Dependent variables: testosterone concentrations
  Dependent variables: (natural) logarithm of testosterone concentrations

- Data Set 2: Cases with hCG stimulation

  Dependent variables: testosterone concentrations
  Dependent variables: (natural) logarithm of testosterone concentrations

Each data set can be viewed as consisting of 27 observations (rows). Each observation includes dependent variable values for 4 time points and a corresponding baseline level. Each observation also includes data identifying the levels of the pertinent factors. Data are listed in Table 8 for the unchallenged samples and in Table 9 for the challenged cases.

5.4 Statistical Analysis Methods

5.4.1 Objective 1

Several statistical analysis methods were used to address the first objective. Analysis of variance (ANOVA) and analysis of covariance (ANOCOVA) were used to analyze the data for each individual time point (including the baseline), and a mixed-model ANOCOVA method was used to jointly analyze the data (across time points 1 through 4). The ANOCOVA models utilized the baseline level (or log level) as a covariate. For each type of analysis, all main effects and two-factor interactions (2fi) of the 3 factors were initially included in the models. Tests for interactions were conducted and, where they were not detected as statistically significant ($p=0.05$), a reduced model was employed that retained the main effects, the baseline covariate (where applicable), and only those 2fi deemed to have significant effects. Additional details are provided in the Results section.

5.4.2 Objective 2

For each of the 27 trials, differences between the with-hCG and the without-hCG testosterone levels were computed for each hour (including baseline). These differences were computed on both the original and log scales. ANOVA was used to analyze these differences for each individual time point (including the baseline). For each model and type of data, all main effects and 2fi of the 3 factors were initially included in the models. Tests for interactions were conducted and where they were not detected as statistically significant ($p=0.05$), a reduced model

was employed that retained the main effects and only those 2fi deemed to have significant effects. Additional details are provided in the Results section.

5.4.3 Results

Overall Characterization of the Data. Table 10 provides summary statistics characterizing the testosterone levels in the non-hCG-stimulated data set. This summary ignores the particular experimental factors. The top portion of the table gives, by hour, the sample size (n), the mean, standard deviation, sum, minimum, and maximum. These variables are denoted as yJ, where J denotes the hour and takes on values of 0, 1, 2, 3, and 4. The lower portion of the table gives the correlations between the hourly data. The following trends are apparent:

- the means continue to increase over time
- the standard deviations also increase over time (i.e., as the mean level gets larger)
- the correlations are generally high and tend to be largest for adjacent hours

Table 11 provides a similar summary for the log-scaled data; these variables are denoted as lyJ, where J denotes the hour. Similar trends for the means and correlations are evident, but the standard deviations tend to be fairly stable across the various time points.

Table 7. Factor Levels in the Phase I Assay Optimization Experiment

Factor Identification	Units	Factor Name	Experimental Levels			Coded Experimental Levels		
			1	2	3	1	2	3
Media Type*		Z1	RPMI-1640	medium-199	Eagles-MEM	-1	0	+1
Atmosphere*		Z2	5% CO_2 / 95% air	5% CO_2 / 95% O_2	air	-1	0	+1
Rat Age*	wks	Z3	11	15	22	-1	0	+1

* Treated as a 3-level discrete factor.

Table 8. Data Listing for Samples Without hCG

Animal	Ear Tag	Set Number	Whole Testis Weight g	Testis Section g	z1	z2	z3	y0 = Testos. Conc. Baseline	y1 = Testos. Conc. Hour 1	y2 = Testos. Conc. Hour 2	y3 = Testos. Conc. Hour 3	y4 = Testos. Conc. Hour 4
3	303	A	RIGHT-1.5531	0.2331	-1	-1	-1	0.54	3.23	5.21	6.19	6.11
5	308	B	RIGHT-1.8189	0.2519	-1	-1	0	0.45	4.32	5.02	6.81	8.43
16	317	C	LEFT-2.0203	0.2626	-1	-1	1	0.13	1.17	1.96	2.54	2.94
3	303	D	LEFT-1.5632	0.2688	-1	0	-1	0.43	3.60	5.14	6.48	7.11
5	308	E	LEFT-1.7979	0.2750	-1	0	0	0.36	5.56	7.85	8.30	10.48
19	316	F	RIGHT-2.0218	0.2524	-1	0	1	0.18	2.10	3.77	5.11	6.26
2	304	G	RIGHT-1.7860	0.2692	-1	1	-1	0.58	3.73	4.99	5.72	6.06
7	315	H	RIGHT-1.7847	0.2449	-1	1	0	0.57	5.17	7.01	8.39	9.17
16	317	I	RIGHT-1.9804	0.2603	-1	1	1	0.17	1.70	2.67	3.64	4.79
3	303	J	RIGHT-1.5531	0.2742	0	-1	-1	0.63	5.35	6.27	7.52	8.64
5	308	K	RIGHT-1.8189	0.2349	0	-1	0	0.81	6.60	8.54	10.28	10.97
16	317	L	LEFT-2.0203	0.2502	0	-1	1	0.15	1.10	2.04	2.93	4.15
2	304	M	RIGHT-1.7860	0.2668	0	0	-1	0.86	6.60	9.81	11.35	13.48
5	308	N	LEFT-1.7979	0.2504	0	0	0	0.49	5.58	8.02	10.00	11.23
19	316	O	RIGHT-2.0218	0.2715	0	0	1	0.34	3.50	6.56	8.68	9.65
2	304	P	LEFT-1.7809	0.2577	0	1	-1	0.67	3.11	4.85	5.23	5.58
7	315	Q	RIGHT-1.7847	0.2419	0	1	0	0.78	5.23	6.54	7.29	7.85
16	317	R	RIGHT-1.9804	0.2563	0	1	1	0.13	1.31	1.82	2.46	2.83

Table 8. Data Listing for Samples Without hCG (Continued)

Animal	Ear Tag	Set Number	Whole Testis Teight g	Testis Section g	z1	z2	z3	y0 = Testos. Conc. Baseline	y1 = Testos. Conc. Hour 1	y2 = Testos. Conc. Hour 2	y3 = Testos. Conc. Hour 3	y4 = Testos. Conc. Hour 4
3	303	S	LEFT-1.5632	0.2651	1	-1	-1	0.21	3.98	6.10	7.37	10.16
5	308	T	RIGHT-1.8189	0.2371	1	-1	0	0.40	5.33	8.05	9.07	12.25
16	317	U	LEFT-2.0203	0.2586	1	-1	1	0.08	1.01	1.45	1.97	2.33
2	304	V	RIGHT-1.7860	0.2741	1	0	-1	0.48	3.44	5.53	6.98	8.12
5	308	W	LEFT-1.7979	0.2340	1	0	0	0.69	4.91	7.36	10.05	11.72
19	316	X	RIGHT-2.0218	0.2686	1	0	1	0.24	2.31	3.98	5.61	6.55
2	304	Y	LEFT-1.7809	0.2681	1	1	-1	0.45	4.44	5.49	6.71	6.86
7	315	Z	RIGHT-1.7847	0.2560	1	1	0	0.74	5.34	6.15	7.87	8.43
16	317	AA	RIGHT-1.9804	0.2408	1	1	1	0.09	0.84	1.18	1.73	1.74

Table 9. Data Listing for Samples With hCG

Animal	Ear Tag	Set Number	Whole Testis Weight g	Testis Section g	z1	z2	z3	yc0 = Testos. Conc. Baseline	yc1 = Testos. Conc. Hour 1	yc2 = Testos. Conc. Hour 2	yc3 = Testos. Conc. Hour 3	yc4 = Testos. Conc. Hour 4
3	303	AC	RIGHT-1.5531	0.2422	-1	-1	-1	0.54	6.56	16.16	28.30	42.09
5	308	BC	RIGHT-1.8189	0.2690	-1	-1	0	1.11	5.96	9.69	15.19	23.41
16	317	CC	LEFT-2.0203	0.2680	-1	-1	1	0.16	2.03	4.88	8.87	15.93
3	303	DC	LEFT-1.5632	0.2374	-1	0	-1	0.73	5.91	20.96	41.76	51.74
5	308	EC	LEFT-1.7979	0.2398	-1	0	0	0.43	4.39	10.84	22.92	33.45
19	316	FC	RIGHT-2.0218	0.2515	-1	0	1	0.26	3.80	9.94	18.38	24.77
2	304	GC	RIGHT-1.7860	0.2494	-1	1	-1	1.29	4.71	7.14	10.26	13.39
7	315	HC	RIGHT-1.7847	0.2401	-1	1	0	0.62	6.35	8.09	11.90	14.64
16	317	IC	RIGHT-1.9804	0.2642	-1	1	1	0.10	1.30	2.74	3.63	5.13
3	303	JC	RIGHT-1.5531	0.2613	0	-1	-1	0.72	8.26	18.51	31.30	39.20
5	308	KC	RIGHT-1.8189	0.2386	0	-1	0	0.47	7.84	15.75	28.83	43.60
16	317	LC	LEFT-2.0203	0.2672	0	-1	1	0.11	2.19	6.70	11.23	18.38
2	304	MC	RIGHT-1.7860	0.2631	0	0	-1	0.86	9.73	31.45	55.27	77.68
5	308	NC	LEFT-1.7979	0.2461	0	0	0	0.89	7.33	18.38	33.23	59.89
19	316	OC	RIGHT-2.0218	0.2516	0	0	1	0.42	4.15	10.45	18.93	27.32
2	304	PC	LEFT- 1.7809	0.2461	0	1	-1	0.61	5.57	9.65	12.75	15.73

Table 9. Data Listing for Samples With hCG (Continued)

Animal	Ear Tag	Set Number	Whole Testis Weight g	Testis Section g	z1	z2	z3	yc0 = Testos. Conc. Baseline	yc1 = Testos. Conc. Hour 1	yc2 = Testos. Conc. Hour 2	yc3 = Testos. Conc. Hour 3	yc4 = Testos. Conc. Hour 4
7	315	QC	RIGHT-1.7847	0.2618	0	1	0	1.38	7.03	9.69	12.35	13.47
16	317	RC	RIGHT-1.9804	0.2607	0	1	1	0.15	1.03	2.14	2.92	4.01
3	303	SC	LEFT-1.5632	0.2375	1	-1	-1	0.34	6.43	18.23	26.69	38.80
5	308	TC	RIGHT-1.8189	0.2668	1	-1	0	0.65	8.79	18.07	29.25	46.72
16	317	UC	LEFT-2.0203	0.2683	1	-1	1	0.11	1.81	4.44	7.96	12.42
2	304	VC	RIGHT-1.7860	0.2593	1	0	-1	0.66	5.91	15.48	24.92	32.07
5	308	WC	LEFT-1.7979	0.2515	1	0	0	0.90	9.38	25.90	45.21	67.45
19	316	XC	RIGHT-2.0218	0.2595	1	0	1	0.18	3.98	10.13	18.33	27.01
2	304	YC	LEFT-1.7809	0.2591	1	1	-1	0.43	5.17	10.59	16.60	25.20
7	315	ZC	RIGHT-1.7847	0.2684	1	1	0	0.52	7.09	12.48	20.01	27.96
16	317	AAC	RIGHT-1.9804	0.2459	1	1	1	0.12	2.44	5.53	9.16	13.76

Table 10. Summary of Data -- Original Scale, Without hCG

Simple Statistics							
Variable	**N**	**Mean**	**Std Dev**	**Sum**	**Minimum**	**Maximum**	**Label**
y0	27	0.43148	0.24012	11.65000	0.08000	0.86000	y0 =_Testos._Conc._Baseline
y1	27	3.72444	1.77928	100.56000	0.84000	6.60000	y1 =_Testos._Conc._Hour 1
y2	27	5.30963	2.33770	143.36000	1.18000	9.81000	y2 =_Testos._Conc._Hour 2
y3	27	6.52889	2.67986	176.28000	1.73000	11.35000	y3 =_Testos._Conc._Hour 3
y4	27	7.55148	3.16645	203.89000	1.74000	13.48000	y4 =_Testos._Conc._Hour 4

Pearson Correlation Coefficients, N = 27 Prob > |r| under H0: Rho=0					
	y0	**y1**	**y2**	**y3**	**y4**
y0 y0 =_Testos._Conc._Baseline	1.00000	0.83359 <.0001	0.77555 <.0001	0.75779 <.0001	0.64894 0.0003
y1 y1 =_Testos._Conc._Hour 1	0.83359 <.0001	1.00000	0.96006 <.0001	0.93735 <.0001	0.89924 <.0001
y2 y2 =_Testos._Conc._Hour 2	0.77555 <.0001	0.96006 <.0001	1.00000	0.98329 <.0001	0.96280 <.0001
y3 y3 =_Testos._Conc._Hour 3	0.75779 <.0001	0.93735 <.0001	0.98329 <.0001	1.00000	0.97499 <.0001
y4 y4 =_Testos._Conc._Hour 4	0.64894 0.0003	0.89924 <.0001	0.96280 <.0001	0.97499 <.0001	1.00000

Table 11. Summary of Data -- Log Scale, Without hCG

Simple Statistics						
Variable	**N**	**Mean**	**Std Dev**	**Sum**	**Minimum**	**Maximum**
ly0	27	-1.04291	0.70964	-28.15853	-2.52573	-0.15082
ly1	27	1.15995	0.62490	31.31878	-0.17435	1.88707
ly2	27	1.53822	0.57738	41.53197	0.16551	2.28340
ly3	27	1.76591	0.52363	47.67970	0.54812	2.42922
ly4	27	1.90916	0.52876	51.54720	0.55389	2.60121

Pearson Correlation Coefficients, N = 27 Prob > |r| under H0: Rho=0					
	ly0	**ly1**	**ly2**	**ly3**	**ly4**
ly0	1.00000	0.90646 <.0001	0.88113 <.0001	0.86395 <.0001	0.79479 <.0001
ly1	0.90646 <.0001	1.00000	0.97732 <.0001	0.96441 <.0001	0.93255 <.0001
ly2	0.88113 <.0001	0.97732 <.0001	1.00000	0.99193 <.0001	0.97266 <.0001
ly3	0.86395 <.0001	0.96441 <.0001	0.99193 <.0001	1.00000	0.98411 <.0001
ly4	0.79479 <.0001	0.93255 <.0001	0.97266 <.0001	0.98411 <.0001	1.00000

Tables 12 and 13 furnish comparable information for the hCG-stimulated samples. Similar trends are evident for these data. Mean levels tend to be much higher than for the nonstimulated samples.

Analysis of Baseline Data. Since we intend to adjust for baseline (time 0) levels for subsequent analyses of the hourly (nonbaseline) data, it is important to understand how the experimental factors affect the baseline levels. For instance, if one of the factors does impact the baseline levels, then adjusting for baseline levels in those subsequent analyses may obscure the effect of the experimental factor. Table14 presents the results that summarize the ANOVA results for the baseline data. Initially, we fit an ANOVA model that included all mean effects (denoted as z1, z2, and z3) and all 2-factor interactions (denoted as z1*z2, z1*z3, and z2*z3). We examined the statistical significance of each of the interactions and reduced the model to contain only main effects and the pertinent 2fi. For 3 of the 4 cases considered, only the main effects were retained; for the log-scale, without hCG case, 2 of the interaction effects were deemed significant. Among the 3 experimental factors, it is clear that the rat age (z3) has the most pronounced effect on the baseline levels (estimated testosterone levels are 0.54, 0.58, and 0.17 for 11-week, 15-week, and 22-week old animals). For the non-hCG-stimulated case, media type also impacted the baseline levels, with the highest level occurring for the z1=0 case (0.54 versus 0.38 for the other two media). The lower portion of Table 14 furnishes statistics characterizing the model fit:

- R^2 = the proportion of variability accounted for by the model,
- RMSE = root mean squared error = the square root of the residual variance,
- C.V. = the coefficient of variation = the RMSE divided by the mean testosterone level (times 100%).

Analyses Directed at Objective 1. Two fundamental types of statistical analysis were used to address Objective 1 (assessing the effects of the experimental factors on the testosterone levels) – separate analyses for each hour and a combined mixed-model approach.

Individual-hour analyses. These analyses involved:

- fitting the testosterone data for a given hour as a function of the experimental factors, their 2-factor interactions, and the baseline level
- examining the significance of the 2-factor interactions (2fi)
- choosing (and fitting) a reduced model form by eliminating any 2fi that was not satistically significant in any of the four hourly models.

Table 12. Summary of Data -- Original Scale, With hCG

Simple Statistics							
Variable	**N**	**Mean**	**Std Dev**	**Sum**	**Minimum**	**Maximum**	**Label**
yc0	27	0.54667	0.36060	14.76000	0.10000	1.38000	yc0 =_Testos._Conc._Baseline
yc1	27	5.37556	2.48467	145.14000	1.03000	9.73000	yc1 =_Testos._Conc._Hour 1
yc2	27	12.37074	7.01613	334.01000	2.14000	31.45000	yc2 =_Testos._Conc._Hour 2
yc3	27	20.96852	12.82440	566.15000	2.92000	55.27000	yc3 =_Testos._Conc._Hour 3
yc4	27	30.19333	18.74161	815.22000	4.01000	77.68000	yc4 =_Testos._Conc._Hour 4

Pearson Correlation Coefficients, N = 27 Prob > |r| under H0: Rho=0					
	yc0	**yc1**	**yc2**	**yc3**	**yc4**
yc0 yc0 =_Testos._Conc._Baseline	1.00000	0.66941 0.0001	0.44159 0.0211	0.38125 0.0497	0.34634 0.0768
yc1 yc1 =_Testos._Conc._Hour 1	0.66941 0.0001	1.00000	0.86213 <.0001	0.80157 <.0001	0.78474 <.0001
yc2 yc2 =_Testos._Conc._Hour 2	0.44159 0.0211	0.86213 <.0001	1.00000	0.98464 <.0001	0.96305 <.0001
yc3 yc3 =_Testos._Conc._Hour 3	0.38125 0.0497	0.80157 <.0001	0.98464 <.0001	1.00000	0.98016 <.0001
yc4 yc4 =_Testos._Conc._Hour 4	0.34634 0.0768	0.78474 <.0001	0.96305 <.0001	0.98016 <.0001	1.00000

Table 13. Summary of Data—Log Scale, With hCG

Simple Statistics						
Variable	**N**	**Mean**	**Std Dev**	**Sum**	**Minimum**	**Maximum**
lyc0	27	-0.86822	0.80586	-23.44183	-2.30259	0.32208
lyc1	27	1.53650	0.61019	41.48556	0.02956	2.27521
lyc2	27	2.34072	0.64378	63.19938	0.76081	3.44840
lyc3	27	2.83766	0.70489	76.61684	1.07158	4.01223
lyc4	27	3.19518	0.71702	86.26981	1.38879	4.35260

Pearson Correlation Coefficients, N = 27 Prob > |r| under H0: Rho=0					
	lyc0	**lyc1**	**lyc2**	**lyc3**	**lyc4**
lyc0	1.00000	0.84699 <.0001	0.68581 <.0001	0.61367 0.0007	0.54138 0.0035
lyc1	0.84699 <.0001	1.00000	0.92139 <.0001	0.87024 <.0001	0.82124 <.0001
lyc2	0.68581 <.0001	0.92139 <.0001	1.00000	0.98599 <.0001	0.95954 <.0001
lyc3	0.61367 0.0007	0.87024 <.0001	0.98599 <.0001	1.00000	0.98703 <.0001
lyc4	0.54138 0.0035	0.82124 <.0001	0.95954 <.0001	0.98703 <.0001	1.00000

Table 14. Summary of Statistical Analysis of Baseline Data

		Original-Scale Models		Log-Scale Models	
		Without hCG Stimulation	**With hCG Stimulation**	**Without hCG Stimulation**	**With hCG Stimulation**
Dependent Variable:		Y0	Y0	log(Y0)	log(Y0)
Dependent Variable Mean:		0.431	0.547	-1.043	0.868
Significance of Model Terms:	z1	xx		xxx	
	z2			xx	
	z3	xxx	xxx	xxx	xxx
	z1*z2	na	na	xx	na
	z1*z3	na	na	na	na
	z2*z3	na	na	xx	na
R^2		0.766	0.628	0.950	0.822
RMSE		0.132	0.251	0.233	0.388
C.V.		30.7	45.9	--	--

x = statistically significant effect at 0.10 level of significance.
xx = statistically significant effect at 0.05 level of significance.
xxx = statistically significant effect at 0.01 level of significance.
na = not applicable (effect not included in the model).

Table 15. Summary of ANOCOVA Results for Individual-Hour Original-Scale Models

Dependent Variable:		Without hCG Stimulation				With hCG Stimulation			
		Y1	Y2	Y3	Y4	Y1	Y2	Y3	Y4
Dependent Variable Mean:		3.72	5.31	6.53	7.55	5.38	12.37	20.97	30.19
Significance of Model Terms:	Y0	xx	xx	xx					
	z1					xx	x		
	z2	x	xxx	xxx	xxx	xx	xxx	xxx	xxx
	z3	xxx	xx	xxx	xx	xxx	xxx	xxx	xxx
	z1*z2	xx	xx	xx	x	na	na	na	na
	z1*z3	na	na	na	na	x		x	
R^2		0.931	0.913	0.917	0.877	0.904	0.862	0.865	0.847
RMSE		0.62	0.91	1.02	1.46	1.01	3.43	6.21	9.65
C.V.		16.6	17.1	15.5	19.3	18.9	27.7	29.6	32.0

x = statistically significant effect at 0.10 level of significance.
xx = statistically significant effect at 0.05 level of significance.
xxx = statistically significant effect at 0.01 level of significance.
na = not applicable (effect not included in the model).

Table 16. Adjusted Mean Levels (Based on Original-Scale Models)

Level of Independent Variable			Mean Levels of Dependent Variables: Without hCG Stimulation				Mean Levels of Dependent Variables: With hCG Stimulation			
z1	**z2**	**z3**	**Y1 Mean**	**Y2 Mean**	**Y3 Mean**	**Y4 Mean**	**Y1 Mean**	**Y2 Mean**	**Y3 Mean**	**Y4 Mean**
-1			3.55	5.05	6.14	6.99	4.52*	10.08*	18.11	25.30
0			3.95	5.64	6.83	7.92	5.83	13.70	23.41	34.01
+1			3.67	5.24	6.62	7.75	5.77	13.34	21.38	31.26
	-1		3.72	5.16*	6.31**	7.50*	5.62	12.43*	20.40**	30.39**
	0		4.12	6.37	7.97	9.33	6.02	17.10	31.25	45.05
	+1		3.34*	4.40**	5.30**	5.82**	4.49**	7.59**	11.25**	15.14**
		-1	3.86**	5.53*	6.59*	7.67*	6.34	16.58	28.33	38.71
		0	4.89	6.58	7.99	9.56	6.91	14.50	25.61	38.99
		+1	2.43**	3.83**	5.01**	5.43**	2.88**	6.03**	8.97**	12.88**
-1	-1		3.07**	4.28**	5.44**	6.01**				
-1	0		4.06	6.00	7.11*	8.30				
-1	+1		3.51*	4.86**	5.88**	6.65**				
0	-1		4.07	5.24**	6.48**	7.60*				
0	0		4.85	7.63	9.43	11.03				
0	+1		2.94**	4.04**	4.57**	5.11**				
+1	-1		4.02	5.96	7.02*	8.89				
+1	0		3.44*	5.48*	7.38*	8.67				
+1	+1		3.55*	4.29**	5.46**	5.69**				
-1		-1					5.43**	15.00	28.51	38.78
-1		0					5.40**	9.68**	17.65**	25.55**
-1		+1					2.73**	5.55**	8.18**	11.58**
0		-1					7.68	20.02	34.14	46.02
0		0					7.05	14.90	26.88	42.62
0		+1					2.76**	6.17**	9.21**	13.40**
+1		-1					5.90*	14.71	22.34*	31.33*
+1		0					8.28	18.93	32.30	48.80
+1		+1					3.13**	6.37**	9.50**	13.67**

Shaded cell indicates highest mean estimated level.
* Indicates that the mean level is significantly lower than the cell with the maximum estimated level, p=0.05.
** Indicates that the mean level is significantly lower than the cell with the maximum estimated level, p=0.01.

Results for the original-scale data are summarized in Tables 15 and 16. Table 15 provides an indication of which effects were retained in the reduced model and which of those terms were statistically significant. The lower portion of the table gives statistics characterizing the fit of the models.

Table 15 indicates that mean concentration levels increase with time. The models for the without and with-hCG stimulation data are somewhat different, but both indicate statistical significance for z2 (atmosphere type) and z3 (rat age). For the without-hCG case, the model also shows a significant effect of the covariate and of the z1*z2 (media type by atmosphere type) interaction. For the with-hCG case, the covariate was not statistically significant and there was some (weak) indication of a z1*z3 (media type by rat age) interaction. The RMSE values tend to increase with increasing concentration levels (i.e., with time). The C.V.s, on the other hand, tend to be fairly stable, suggesting that a log-tranform of the concentrations should result in data with approximately homogeneous variances over the various time points.

Adjusted means based on the models that were indicated in Table 15 are presented in Table 16. The means are those that are estimated to occur for a given level of a factor (or given combination of factors) when other effects in the model (e.g., the baseline level covariate) are fixed at their mean values. The 3 first columns of the table identify the factor levels (see Table 7). Within each set of the levels (e.g., the three rows with z1= -1, 0, and +1), the estimated adjusted mean that is largest is highlighted. Asterisks beside the other nonhighlighted means indicate if that particular mean is deemed to be statistically significant from the one that is highlighted. For the nonstimulated data, for instance, the table indicates no significant difference among the z1 levels, although the zero level is consistently estimated to be the largest. For the atmosphere and rat age factors, the zero levels generally have the highest estimated mean testosterone concentrations and the other levels typically have significantly lower means. An exception is the rat age (z3) factor for the hCG-stimulated case, where the 11-week-old and the 15-week-old rats had similar adjusted mean levels. Even when interactions are considered (lower portion of Table 16), the zero levels of all 3 factors are either estimated to have the highest adjusted means or to have adjusted means that are not significantly different from the factor combination having the highest estimated mean level.

Tables 17 and 18 show results for the log-transformed data. These tables are analogous to Tables 15 and 16, respectively. The models are somewhat different than those indicated in Table 15. On the log scale, the rat age factor does not appear to be as prominent. Also, the Eagle-MEM (z1=1) medium yields the highest mean levels, although the 199 medium (z1=0) levels are not significantly smaller. The air atmosphere consistently produces lower mean levels.

As with the original-scale data, the zero levels of all 3 factors are either estimated to have the highest adjusted means or to have

Table 17. Summary of ANOCOVA Results for Individual-Hour Log-Scale Models

Dependent Variable:		Without hCG Stimulation				With hCG Stimulation			
		log(Y1)	log(Y2)	log(Y3)	log(Y4)	log(Y1)	log(Y2)	log(Y3)	log(Y4)
Dependent Variable Mean:		1.160	1.538	1.766	1.909	1.537	2.341	2.838	3.195
Significance of Model Terms:	log(Y0)	xxx	xxx	xxx	xxx	xx			
	z1					x	xx	x	x
	z2	x	xxx	xxx	xxx	xx	xxx	xxx	xxx
	z3						x	x	x
	z1*z2	xx	xx	xx	xx			x	x
	z1*z3		x	x	xx	na	na	na	na
R^2		0.963	0.964	0.962	0.951	0.894	0.900	0.917	0.924
RMSE		0.185	0.167	0.156	0.181	0.261	0.268	0.268	0.260

x = statistically significant effect at 0.10 level of significance.
xx = statistically significant effect at 0.05 level of significance.
xxx = statistically significant effect at 0.01 level of significance.
na = not applicable (effect not included in the model)

Table 18. Adjusted Mean Levels Based on Log-Scale Models

Level of Independent Variable			Mean Levels of Dependent Variables: Without hCG Stimulation				Mean Levels of Dependent Variables: With hCG Stimulation			
z1	z2	z3	log(Y1) Mean	log(Y2) Mean	log(Y3) Mean	log(Y4) Mean	log(Y1) Mean	log(Y2) Mean	log(Y3) Mean	log(Y4) Mean
-1			1.162	1.551	1.764	1.903	1.381*	2.147*	2.669*	3.021*
0			1.085	1.456	1.679	1.834	1.527	2.362	2.836	3.190
+1			1.232	1.608	1.855	1.990	1.702	2.513	3.008	3.374
	-1		1.208	1.591	1.810	1.98	1.628	2.431	2.949*	3.356*
	0		1.240	1.690	1.935	2.10	1.644	2.696	3.305	3.687
	+1		1.031*	1.334**	1.552**	1.64**	1.337*	1.895**	2.259**	2.542**
		-1	1.096	1.442	1.640	1.780	1.651	2.610	3.098	3.444
		0	1.279	1.550	1.774	1.956	1.698	2.463	2.982	3.408
		+1	1.105	1.623	1.884	1.991	1.261	1.949*	2.433*	2.734*
-1	-1		1.021*	1.406**	1.645**	1.752**	1.419	2.192*	2.730*	3.211*
-1	0		1.371	1.805	1.993	2.157	1.515	2.566	3.251	3.552
-1	+1		1.094*	1.440*	1.654*	1.801*	1.208*	1.682**	2.026**	2.302**
0	-1		1.066*	1.403*	1.659*	1.857*	1.755	2.582	3.124	3.477
0	0		1.292	1.740	1.981	2.144	1.675	2.782	3.379	3.864
0	+1		0.898**	1.225**	1.396**	1.501**	1.151*	1.723**	2.005**	2.229**
+1	-1		1.539	1.963	2.127	2.341	1.711	2.520	2.991	3.381
+1	0		1.057*	1.524*	1.833	2.001	1.743	2.740	3.285	3.645
+1	+1		1.102*	1.338**	1.606**	1.628**	1.651	2.281	2.747*	3.097**
-1		-1	0.946*	1.307*	1.512*	1.587**				
-1		0	1.401	1.658	1.855	2.053				
-1		+1	1.139	1.686	1.925	2.070				
0		-1	0.982*	1.288*	1.470*	1.650*				
0		0	1.213	1.470	1.685	1.821				
0		+1	1.061	1.609	1.882	2.031				
+1		-1	1.359	1.730	1.939	2.104				
+1		0	1.221	1.521	1.781	1.996				
+1		+1	1.117	1.572	1.846	1.871				

Shaded cell indicates highest mean estimated level.
* Indicates that the mean level is significantly lower than the cell with the maximum estimated level, p=0.05.
** Indicates that the mean level is significantly lower than the cell with the maximum estimated level, p=0.01.

adjusted means that are not significantly different from the factor combination having the highest estimated mean level.

Mixed-model analyses. For each of 2 data sets and 2 types of dependent variables indicated above (see "Data"), these analyses involved several steps. First, we employed a mixed model that included:

- the main effects of the experimental factor,
- two-factor interactions,
- the baseline testosterone level,
- a linear and a quadratic time component,
- cross products of the linear and quadratic time components with the main effects, and
- cross products of the linear and quadratic time components with the 2fi.

For this "full" model, we utilized the SAS PROC MIXED procedure to determine a relevant covariance structure for the data set; in particular, we examined 10 different possible covariance structures, using maximum likelihood estimation, and selected one that appeared to be optimal or near optimal. Using that structure, we estimated fixed effects for all of the above model terms. We then reduced the model by eliminating nonsignificant higher-order terms. We then re-examined the covariance structure (again selecting from among 10 possible structures) for this reduced model. Using the selected structure, we estimated the fixed effects in the reduced model (using restricted maximum likelihood estimation) and computed adjusted means for the various factor levels, along with approximate 95% confidence intervals for the means.

The adjusted means for the various cases and factors are given in Tables 19 through 22. These are denoted as EST., where J = 1, 2, 3, or 4 denotes hour. Approximate 95% confidence limits are given in the right portion of the table. The lower and upper limits are denoted as LOWJ and HIJ, respectively, where J = 1, 2, 3, or 4 denotes hour. Unlike the individual-hour analyses, these estimates (and the interval estimates) rely on data from all 4 hours and also reflect a smoothing over time (due to the assumed quadratic time dependence).

Analyses Directed at Objective 2. Differences in hCG-stimulated and nonstimulated testosterone levels were computed for the 27 trials on an hour-by-hour basis. These differences were then analyzed by hour using an initial ANOVA model that included all main effects and 2fi.

A reduced ANOVA model was then selected by eliminating those 2fi that were not statistically significant. The original-scale models are summarized in Table 23; no 2fi were deemed necessary, so that the model only includes the main effects. Both atmosphere type and rat age were judged to have impact on the testosterone concentration levels. Table 24 shows the adjusted means derived from the model. The estimated adjusted mean difference that is largest is highlighted. Asterisks beside the other nonhighlighted mean differences indicate if that particular difference is deemed to be statistically significant from the one that is highlighted. The media type appears to have little effect, but rat age and atmosphere type are significant factors affecting the with-minus-without-hCG differences.

Table 19. Least Squares Means for Reduced Log-scale Model: Without hCG

Mean covariate value: ly0=-1.043

x1	x2	x3	EST1	EST2	EST3	EST4	LOW1	HI1	LOW2	HI2	LOW3	HI3	LOW4	HI4
-1	_	_	1.163	1.527	1.773	1.901	1.059	1.267	1.424	1.629	1.670	1.875	1.797	2.005
0	_	_	1.116	1.471	1.708	1.828	0.942	1.290	1.298	1.644	1.536	1.881	1.655	2.002
1	_	_	1.211	1.587	1.846	1.988	1.064	1.357	1.442	1.733	1.701	1.992	1.842	2.134
_	-1	_	1.184	1.563	1.824	1.968	1.039	1.330	1.418	1.708	1.680	1.969	1.823	2.114
_	0	_	1.278	1.672	1.949	2.109	1.146	1.409	1.542	1.802	1.819	2.080	1.978	2.240
_	1	_	1.028	1.350	1.554	1.640	0.928	1.128	1.251	1.448	1.455	1.652	1.540	1.741
_	_	-1	1.137	1.472	1.683	1.771	0.901	1.374	1.236	1.708	1.448	1.919	1.534	2.007
_	_	0	1.327	1.606	1.813	1.949	1.031	1.622	1.311	1.901	1.518	2.108	1.654	2.245
_	_	1	1.026	1.507	1.831	1.998	0.523	1.529	1.004	2.010	1.328	2.334	1.494	2.501

Reduced model was fit using compound symmetry covariance structure.
Effects retained in the reduced model were the following (tL denote the linear time effect, tQ denotes the quadratic): ly0, z1, z2, z3, z1*z2, z1*z3, tL, tL*z1, tL*z2, tL*z3, tL*z1*z3, tQ, tQ*z3.

Table 20. Least Squares Means for Reduced Original-scale Model: Without hCG

Mean covariate value: y0=0.43

x1	x2	x3	EST1	EST2	EST3	EST4	LOW1	HI1	LOW2	HI2	LOW3	HI3	LOW4	HI4
-1	_	_	3.70	5.11	6.24	7.09	2.99	4.40	4.41	5.80	5.55	6.94	6.39	7.79
0	_	_	3.74	5.35	6.67	7.72	2.82	4.66	4.43	6.27	5.75	7.59	6.79	8.64
1	_	_	3.76	5.40	6.75	7.82	3.05	4.48	4.69	6.10	6.04	7.46	7.11	8.53
_	-1	_	3.78	5.30	6.54	7.50	3.07	4.48	4.60	6.00	5.84	7.24	6.80	8.21
_	0	_	4.17	6.18	7.91	9.36	3.54	4.81	5.56	6.81	7.29	8.54	8.73	9.99
_	1	_	3.25	4.37	5.21	5.77	2.59	3.90	3.72	5.01	4.56	5.85	5.12	6.42
_	_	-1	3.65	5.20	6.47	7.46	2.74	4.57	4.29	6.12	5.56	7.38	6.54	8.38
_	_	0	4.45	6.30	7.87	9.15	3.29	5.62	5.14	7.46	6.70	9.03	7.99	10.32
_	_	1	3.09	4.35	5.32	6.02	1.31	4.87	2.57	6.13	3.55	7.10	4.24	7.80

Reduced model was fit using compound symmetry covariance structure.
Effects retained in the reduced model were the following (tL denote the linear time effect, tQ denotes the quadratic): y0, z1, z2, z3, z1*z2, z1*z3, z2*z3, tL, tL*z1, tL*z2, tL*z3, tL*z1*z2, tL*z1*z3, tL*z2*z3, tQ.

Table 21. Least Squares Means for Reduced Log-scale Model: With hCG
Mean covariate value: lyc0=-0.868

x1	x2	x3	EST1	EST2	EST3	EST4	LOW1	HI1	LOW2	HI2	LOW3	HI3	LOW4	HI4
-1	_	_	1.388	2.153	2.694	3.012	1.199	1.576	1.970	2.335	2.512	2.876	2.823	3.200
0	_	_	1.554	2.314	2.852	3.166	1.360	1.748	2.126	2.503	2.664	3.040	2.972	3.360
1	_	_	1.668	2.471	3.051	3.408	1.469	1.867	2.278	2.665	2.858	3.244	3.209	3.607
_	-1	_	1.598	2.400	2.996	3.385	1.402	1.795	2.206	2.595	2.801	3.190	3.188	3.581
_	0	_	1.682	2.662	3.319	3.651	1.481	1.884	2.463	2.862	3.119	3.518	3.449	3.852
_	1	_	1.329	1.876	2.283	2.550	1.141	1.518	1.690	2.062	2.097	2.469	2.361	2.738
_	_	-1	1.726	2.560	3.109	3.372	1.489	1.963	2.325	2.795	2.873	3.344	3.135	3.609
_	_	0	1.793	2.443	2.951	3.317	1.530	2.056	2.182	2.704	2.690	3.212	3.054	3.580
_	_	1	1.091	1.935	2.537	2.896	0.713	1.470	1.558	2.313	2.159	2.914	2.518	3.275

Reduced model was fit using first-order autoregressive covariance structure.
Effects retained in the reduced model were the following (tL denote the linear time effect, tQ denotes the quadratic): lyc0, z1, z2, z3, z1*z2, tL, tL*z1, tL*z2, tL*z3, tL*z1*z2, tQ, tQ*z2, tQ*z3.

Table 22. Least Squares Means for Reduced Original-scale Model: With hCG
Mean covariate value: yc0=0.55

x1	x2	x3	EST 1	EST 2	EST 3	EST 4	LOW 1	HI 1	LOW 2	HI 2	LOW 3	HI 3	LOW 4	HI 4
-1	_	_	5.11	12.20	20.55	30.17	4.43	5.79	10.49	13.90	17.76	23.34	26.03	34.31
0	_	_	5.52	12.60	20.96	30.57	4.83	6.21	10.90	14.31	18.17	23.75	26.43	34.71
1	_	_	5.59	12.68	21.03	30.65	4.89	6.29	10.97	14.39	18.24	23.82	26.51	34.79
_	-1	_	5.68	12.69	20.97	30.52	4.71	6.64	9.80	15.59	16.09	25.85	23.51	37.52
_	0	_	6.14	17.64	30.40	44.43	5.18	7.09	14.75	20.53	25.52	35.28	37.43	51.44
_	1	_	4.40	7.15	11.16	16.44	3.45	5.36	4.26	10.04	6.28	16.04	9.43	23.45
_	_	-1	6.32	16.54	27.02	37.76	5.36	7.29	13.66	19.42	22.24	31.81	30.62	44.90
_	_	0	6.78	14.01	23.91	36.48	5.79	7.78	11.12	16.90	19.12	28.70	29.34	43.63
_	_	1	3.11	6.92	11.60	17.15	2.04	4.17	4.01	9.84	6.80	16.41	9.99	24.30

Reduced model was fit using first-order autoregressive covariance structure with heterogeneous variances. Effects retained in the reduced model were the following (tL denote the linear time effect, tQ denotes the quadratic): y0, z1, z2, z3, tL, tL*z2, tL*z3, tQ, tQ*z3.

Table 23. Analysis of Differences in Levels for With and Without hCG Stimulation: Original-Scale Models

Dependent Variable:		diff(Y0)	diff(Y1)	diff(Y2)	diff(Y3)	diff(Y4)
Dependent Variable Mean:		0.12	1.65	7.06	14.44	22.64
Significance of Model Terms:	z1					
	z2			xxx	xxx	xxx
	z3		xx	xxx	xxx	xxx
R^2		0.175	0.457	0.687	0.722	0.712
RMSE		0.25	1.05	3,49	6,57	10.04

x = statistically significant effect at 0.10 level of significance.
xx = statistically significant effect at 0.05 level of significance.
xxx = statistically significant effect at 0.01 level of significance.

Table 24. Adjusted Mean Differences (With-Without hCG), Based on Original-Scale Models

Level of Independent Variable			Mean Levels of Dependent Variables				
z1	z2	z3	Mean diff (Y0)	Mean diff (Y1)	Mean diff (Y2)	Mean diff (Y3)	Mean diff (Y4)
-1			0.20	1.16	5.20	12.00	18.13
0			0.08	1.64	7.59	15.67	24.99
+1			0.06	2.16	8.40	15.64	24.80
	-1		0.09	1.98	7.53	14.77*	23.84*
	0		0.14	1.89	10.61	22.93	35.20
	+1		0.12	1.09	3.04**	5.62**	8.89**
		-1	0.15	2.31	10.53	20.48	29.31
		0	0.19	1.79	7.15	15.65	26.67
		+1	0.01	0.85**	3.50**	7.19**	11.94**

Shaded cell indicates highest mean estimated level.
* Indicates that the mean level is significantly lower than the cell with the maximum estimated level, p=0.05.
** Indicates that the mean level is significantly lower than the cell with the maximum estimated level, p=0.01.

Tables 25 and 26 show comparable results for the differences of the log-transformed data. In this case, rat age appears less important and atmosphere type is the most dominant factors.

Table 25. Analysis of Differences in Levels for With and Without hCG Stimulation: Log-Scale Models

Dependent Variable:		diff (log(Y0))	diff (log(Y1))	diff (log(Y2))	diff (log(Y3))	diff (log(Y4))
Dependent Variable Mean:		0.175	0.377	0.802	1.072	1.286
Significance of Model Terms:	z1			x		
	z2			xx	xxx	xxx
	z3					
R^2		0.142	0.305	0.528	0.585	0.503
RMSE		0.393	0.289	0.313	0.335	0.405

x = statistically significant effect at 0.10 level of significance.
xx = statistically significant effect at 0.05 level of significance.
xxx = statistically significant effect at 0.01 level of significance.

Table 26. Adjusted Mean Differences (With-Without hCG), Based on Log-Scale Models

Level of Independent Variable			Mean Levels of Dependent Variables				
z1	z2	z3	Mean diff log(Y0)	Mean diff log(Y1)	Mean diff log(Y2)	Mean diff log(Y3)	Mean diff log(Y4)
-1			0.282	0.289	0.659**	0.963	1.166
0			0.079	0.307	0.732	0.995	1.197
+1			0.164	0.533	1.017	1.278	1.496
	-1		0.186	0.493	0.956	1.248	1.486
	0		0.242	0.380	0.931	1.298	1.503
	+1		0.096	0.257	0.520**	0.670**	0.869**
		-1	0.236	0.446	0.957	1.259	1.447
		0	0.243	0.274	0.637*	0.948	1.170
		+1	0.045	0.410	0.814	1.008	1.241

Shaded cell indicates highest mean estimated level.
* Indicates that the mean level is significantly lower than the cell with the maximum estimated level, p=0.05.
** Indicates that the mean level is significantly lower than the cell with the maximum estimated level, p=0.01.

6.0 Statistical Analysis of the Phase IB Assay Optimization Experiment

6.1 Objectives

Since the gaseous atmosphere of 5% CO_2 / 95% O_2 was optimal, and it was thought that most laboratories would not have incubators to accommodate this mixture, a comparison was made between incubated samples and those in media that had be gassed with the mixture.

The Phase IB assay optimization experiment involved assessing the effect of a single experimental factor—using gassed or incubated samples. All other factors were held fixed. Ten trials of each condition were run both with and without hCG stimulation. For each trial, repeated measurements are taken at baseline (time 0) and at 1, 2, 3, and 4 hours after baseline. The objective of the experiment was to assess whether the treatments differed in terms of the resultant testosterone levels.

6.2 Data

Two basic SAS data sets were constructed from the raw data ,and 2 fundamental types of dependent variables were used in the analyses of each type:

- Date Set 1: Cases without hCG stimulation
  Dependent variables: testosterone concentrations
  Dependent variables: (natural) logarithm of testosterone concentrations

- Data Set 2: Cases with hCG stimulation
  Dependent variables: testosterone concentrations
  Dependent variables: (natural) logarithm of testosterone concentrations

Each data set can be viewed as consisting of 20 observations (rows). Each observation includes dependent variable values for 4 time points and a corresponding baseline level. Each observation also includes data identifying the levels of the pertinent factors. Data are listed in Table 27 for the unchallenged samples and in Table 28 for the challenged cases.

6.3 Statistical Analysis Methods

ANOVA was used to analyze the data for each individual time point (including the baseline). ANOVAs were performed for both original-scale data and log-scaled data (natural logarithm). ANOCOVA models utilizing the baseline level (or log-level) as a covariate were

also employed.

6.3.1 Results

Overall Characterization of the Data. Table 29 provides summary statistics characterizing the testosterone levels in the non-hCG-stimulated data set. This summary ignores the experimental factor. The top portion of the table gives, by hour, the sample size (n), the mean, standard deviation, sum, minimum, and maximum. These variables are denoted as yJ, where J denotes the hour and takes on values of 0, 1, 2, 3, and 4. The lower portion of the table gives the correlations between the hourly data. The following trends are apparent:

- the means continue to increase over time,
- the standard deviations also increase over time (i.e., as the mean level gets larger), and
- the correlations are generally high and tend to be largest for adjacent hours.

Table 30 provides a similar summary for the log-scaled data; these variables are denoted as lyJ, where J denotes the hour. Similar trends for the means and correlations are evident, but the standard deviations tend to be fairly stable across the various time points.

Tables 31 and 32 furnish comparable information for the hCG-stimulated samples. Similar trends are evident for these data. Mean levels tend to be much higher than for the nonstimulated samples.

Analysis of Variance and Covariance. Means, by hour and sample condition, are presented in Table 33 for the non-hCG-stimulated original-scale data. The table gives the number of observations, the approximate 95% confidence limits for the mean, the estimated mean, and standard deviation. The last column gives the mean (for hours 1, 2, 3, and 4) adjusted for the baseline level, as determined from the ANOCOVA. Tables 34, 35, and 36 give corresponding results for the log-scaled data and the hCG-stimulated cases. The table below summarizes the findings detailed in Tables 33 through 36. For the most part, the ANOVAs and ANOCOVAs of the hourly data did not detect significant differences between the testosterone levels of the gassed and incubated samples. If the 0.05 significance level is used to judge statistical significance, then only the baseline case of Table 33 yielded a significant difference.

Source	Type Data Analyzed	ANOVA Results	ANOCOVA Results
Table 33	Original scale, non hCG stimulated	Baseline gassed samples have lower testosterone mean (p=0.05); all other hours not significantly different at 0.10 level.	Hour-1 and hour-4 gassed samples have higher testosterone means (p=0.08 and 0.07, respectively); other hours not significantly different at 0.10 level.
Table 34	Log scale, non hCG stimulated	Baseline gassed samples have lower testosterone mean (p=0.06); all other hours not significantly different at 0.10 level.	No significant differences at 0.10 level.
Table 35	Original scale, hCG stimulated	No significant differences at 0.10 level.	No significant differences at 0.10 level.
Table 36	Log scale, hCG stimulated	No significant differences at 0.10 level.	No significant differences at 0.10 level.

Table 27. Phase IB Data: Sample Without hCG

Ear Tag	Body Weight g	Testis Wt g	Section Wt g	Run Number	x0	y0 =Testos. Conc. Baseline	y1 =Testos. Conc. Hour 1	y2 =Testos. Conc. Hour 2	y3 =Testos. Conc. Hour 3	y4 =Testos. Conc. Hour 4
320	361.74	RIGHT - 1.5641	0.2413	1	1	0.35	3.49	5.46	6.66	8.05
321	361.74	LEFT - 1.5303	0.2657	2	1	0.38	3.92	4.95	6.88	8.27
321	365.84	RIGHT - 1.8176	0.2623	3	1	0.36	4.66	6.52	7.88	8.53
321	365.84	LEFT - 1.8273	0.2463	4	1	0.34	3.94	4.94	6.30	7.71
321	365.84	LEFT - 1.8273	0.2389	5	1	0.32	4.53	6.36	7.56	7.73
322	372.13	RIGHT - 1.5835	0.2341	6	1	0.14	3.30	4.34	5.65	6.40
322	372.13	LEFT - 1.5263	0.2436	7	1	0.18	1.67	2.47	3.16	3.63
323	365.46	RIGHT - 1.7040	0.2722	8	1	0.45	3.87	5.41	6.06	7.90
324	356.94	RIGHT - 1.4908	0.2626	9	1	0.42	5.32	7.49	9.71	11.01
323	365.46	LEFT - 1.6760	0.2711	10	1	0.35	5.32	7.07	8.83	10.17
320	361.74	RIGHT - 1.5641	0.2713	11	2	0.49	5.06	7.37	9.13	10.97
320	361.74	LEFT - 1.5303	0.2543	12	2	0.38	2.94	4.36	5.44	6.45
321	365.84	RIGHT - 1.8176	0.2610	13	2	0.38	4.76	6.78	8.51	9.21
321	365.84	LEFT - 1.8273	0.2408	14	2	0.36	3.64	5.37	6.34	6.87
322	372.13	RIGHT - 1.5835	0.2472	15	2	0.33	3.38	4.87	5.53	6.41
322	372.13	LEFT - 1.5263	0.2499	16	2	0.46	4.45	5.56	6.85	8.15
323	365.46	RIGHT - 1.7040	0.2474	17	2	0.40	4.26	6.30	7.99	9.13
323	365.46	LEFT - 1.6760	0.2630	18	2	0.39	3.56	5.63	6.78	7.71
324	356.94	RIGHT - 1.4908	0.2365	19	2	0.37	2.62	3.69	4.82	5.84
324	356.94	LEFT - 1.4136	0.2684	20	2	0.49	4.56	5.85	6.55	9.02

x0 = sample condition: 1 = gassed, 2 = incubated

Table 28. Phase IB Data: Samples With hCG

Ear Tag	Body Weight g	Testis Wt g	Section Wt g	Run Number	x0	y0 =Testos. Conc. Baseline	y1 =Testos. Conc. Hour 1	y2 =Testos. Conc. Hour 2	y3 =Testos. Conc. Hour 3	y4 =Testos. Conc. Hour 4
320	361.74	RIGHT - 1.5641	0.2387	1	1	0.36	9.56	20.60	32.84	41.13
320	361.74	LEFT - 1.5303	0.2727	2	1	0.52	6.31	13.49	22.25	29.13
321	365.84	RIGHT - 1.8176	0.2518	3	1	0.40	5.12	13.29	18.32	25.33
321	365.84	LEFT - 1.8273	0.2748	4	1	0.48	7.57	14.92	23.79	30.83
321	365.84	LEFT - 1.8273	0.2681	5	1	0.43	4.84	9.66	13.47	18.59
322	372.13	RIGHT - 1.5835	0.2429	6	1	0.28	8.73	18.74	30.71	45.84
322	372.13	LEFT - 1.5263	0.2523	7	1	0.23	4.18	9.38	12.68	18.38
323	365.46	RIGHT - 1.7040	0.2550	8	1	0.42	5.95	12.41	21.00	30.28
324	356.94	RIGHT - 1.4908	0.2489	9	1	0.12	5.09	11.99	17.25	28.43
323	365.46	LEFT - 1.6760	0.2513	10	1	0.21	3.40	8.04	13.78	19.14
320	361.74	RIGHT - 1.5641	0.2451	11	2	0.34	6.71	17.75	29.37	39.76
320	361.74	LEFT - 1.5303	0.2409	12	2	0.38	5.07	12.01	17.63	23.46
321	365.84	RIGHT - 1.8176	0.2471	13	2	0.24	7.90	17.49	27.99	39.46
321	365.84	LEFT - 1.8273	0.2695	14	2	0.41	7.52	19.79	30.29	43.69
322	372.13	RIGHT - 1.5835	0.2452	15	2	0.37	4.24	10.43	14.70	19.41
322	372.13	LEFT - 1.5263	0.2635	16	2	0.31	5.06	11.70	19.98	29.90
323	365.46	RIGHT - 1.7040	0.2615	17	2	0.44	5.71	13.87	21.35	30.16
323	365.46	LEFT - 1.6760	0.2328	18	2	0.72	6.93	21.00	31.05	40.41
324	356.94	RIGHT - 1.4908	0.2632	19	2	0.81	5.73	13.45	23.01	34.28
324	356.94	LEFT - 1.4136	0.2491	20	2	0.31	6.14	16.68	26.65	33.43

x0 = sample condition: 1 = gassed, 2 = incubated

Table 29. Summary of Data—Original Scale, Without hCG

Simple Statistics							
Variable	**N**	**Mean**	**Std Dev**	**Sum**	**Minimum**	**Maximum**	**Label**
Y0	20	0.36700	0.08652	7.34000	0.14000	0.49000	y0 =_Testos._Conc._Baseline
Y1	20	3.96250	0.92905	79.25000	1.67000	5.32000	y1 =_Testos._Conc._Hour 1
Y2	20	5.53950	1.26145	110.79000	2.47000	7.49000	y2 =_Testos._Conc._Hour 2
Y3	20	6.83150	1.57510	136.63000	3.16000	9.71000	y3 =_Testos._Conc._Hour 3
Y4	20	7.95800	1.76957	159.16000	3.63000	11.01000	y4 =_Testos._Conc._Hour 4

Pearson Correlation Coefficients, N = 20Prob > |r| under H0: Rho=0					
	Y0	**Y1**	**Y2**	**Y3**	**Y4**
Y0y0 =_Testos._Conc._Baseline	1.00000	0.56839 0.0089	0.57435 0.0081	0.51050 0.0215	0.65502 0.0017
Y1y1 =_Testos._Conc._Hour 1	0.56839 0.0089	1.00000	0.95710 <.0001	0.94357 <.0001	0.94257 <.0001
Y2y2 =_Testos._Conc._Hour 2	0.57435 0.0081	0.95710 <.0001	1.00000	0.97439 <.0001	0.94352 <.0001
Y3y3 =_Testos._Conc._Hour 3	0.51050 0.0215	0.94357 <.0001	0.97439 <.0001	1.00000	0.95462 <.0001
Y4y4 =_Testos._Conc._Hour 4	0.65502 0.0017	0.94257 <.0001	0.94352 <.0001	0.95462 <.0001	1.00000

Table 30. Summary of Data—Log Scale, Without hCG

Simple Statistics						
Variable	**N**	**Mean**	**Std Dev**	**Sum**	**Minimum**	**Maximum**
ly0	20	-1.03875	0.30294	-20.77491	-1.96611	-0.71335
ly1	20	1.34511	0.27439	26.90217	0.51282	1.67147
ly2	20	1.68265	0.26153	33.65299	0.90422	2.01357
ly3	20	1.89300	0.25530	37.86004	1.15057	2.27316
ly4	20	2.04705	0.25043	40.94100	1.28923	2.39880

Pearson Correlation Coefficients, N = 20Prob > |r| under H0: Rho=0					
	ly0	**ly1**	**ly2**	**ly3**	**ly4**
ly0	1.00000	0.5842 20.0068	0.60978 0.0043	0.55761 0.0106	0.65998 0.0015
ly1	0.58422 0.0068	1.00000	0.96741 <.0001	0.95797 <.0001	0.95831 <.0001
ly2	0.60978 0.0043	0.96741 <.0001	1.00000	0.97796 <.0001	0.95576 <.0001
ly3	0.55761 0.0106	0.95797 <.0001	0.97796 <.0001	1.00000	0.96540 <.0001
ly4	0.65998 0.0015	0.95831 <.0001	0.95576 <.0001	0.96540 <.0001	1.00000

Table 31. Summary of Data—Original Scale, With hCG

Simple Statistics							
Variable	N	Mean	Std Dev	Sum	Minimum	Maximum	Label
YC0	20	0.38900	0.16189	7.78000	0.12000	0.81000	yc0 =_Testos._Conc._Baseline
YC1	20	6.08800	1.58725	121.76000	3.40000	9.56000	yc1 =_Testos._Conc._Hour 1
YC2	20	14.33450	3.87932	286.69000	8.04000	21.00000	yc2 =_Testos._Conc._Hour 2
YC3	20	22.40550	6.48074	448.11000	12.68000	32.84000	yc3 =_Testos._Conc._Hour 3
YC4	20	31.05200	8.66685	621.04000	18.38000	45.84000	yc4 =_Testos._Conc._Hour 4

Pearson Correlation Coefficients, N = 20Prob > |r| under H0: Rho=0					
	YC0	**YC1**	**YC2**	**YC3**	**YC4**
YC0yc0 =_Testos._Conc._Baseline	1.00000	0.14785 0.5339	0.24875 0.2903	0.25182 0.2841	0.19761 0.4037
YC1yc1 =_Testos._Conc._Hour 1	0.14785 0.5339	1.00000	0.88953 <.0001	0.90763 <.0001	0.87874 <.0001
YC2yc2 =_Testos._Conc._Hour 2	0.24875 0.2903	0.88953 <.0001	1.00000	0.97501 <.0001	0.93343 <.0001
YC3yc3 =_Testos._Conc._Hour 3	0.25182 0.2841	0.90763 <.0001	0.97501 <.0001	1.00000	0.96812 <.0001
YC4yc4 =_Testos._Conc._Hour 4	0.19761 0.4037	0.87874 <.0001	0.93343 <.0001	0.96812 <.0001	1.00000

Table 32. Summary of Data—Log Scale, With hCG

Simple Statistics						
Variable	**N**	**Mean**	**Std Dev**	**Sum**	**Minimum**	**Maximum**
lyc0	20	-1.02614	0.42653	-20.52279	-2.12026	-0.21072
lyc1	20	1.77407	0.26184	35.48142	1.22378	2.25759
lyc2	20	2.62709	0.27611	52.54184	2.08443	3.04452
lyc3	20	3.06710	0.30327	61.34207	2.54003	3.49165
lyc4	20	3.39608	0.29444	67.92156	2.91126	3.82516

Pearson Correlation Coefficients, N = 20Prob > |r| under H0: Rho=0					
	lyc0	**lyc1**	**lyc2**	**lyc3**	**lyc4**
lyc0	1.00000	0.2590 10.2702	0.29545 0.2060	0.3062 40.1891	0.21198 0.3696
lyc1	0.2590 10.2702	1.00000	0.91838 <.0001	0.91443 <.0001	0.89007 <.0001
lyc2	0.29545 0.2060	0.91838 <.0001	1.00000	0.96817 <.0001	0.93546 <.0001
lyc3	0.30624 0.1891	0.91443 <.0001	0.96817 <.0001	1.00000	0.97426 <.0001
lyc4	0.21198 0.3696	0.89007 <.0001	0.93546 <.0001	0.97426 <.0001	1.00000

Table 33. Summary of Results by Sample Condition—Original Scale, Without hCG

x0	N Obs	Variable	Lower 95%CL for Mean	Upper 95%CL for Mean	Mean	Std Dev	ANOCOVA Adjusted Mean
1	10	Y0	0.26	0.40	**0. 33**	0.101.08	**4.30**
		Y1	3.23	4.77	4.00	1.47	5.89
		Y2	4.45	6.55	5.50	1.82	7.32
		Y3	5.57	8.17	6.87	2.00	**8.57**
		Y4	6.51	9.37	7.94		
2	10	Y0	0.37	0.44	**0.41**	0.06	**3.62**
		Y1	3.34	4.51	3.92	0.81	5.19
		Y2	4.79	6.36	5.58	1.10	6.34
		Y3	5.80	7.79	6.79	1.39	**7.34**
		Y4	6.82	9.13	7.98	1.62	

x0 = sample condition: 1 = gassed, 2 = incubated
Bolded entries are statistically significant at the 0.10 level.
Bolded and underlined entries are statistically significant at the 0.05 level.

Table 34. Summary of Results by Sample Condition—Log Scale, Without hCG

x0	N Obs	Variable	Lower 95%CL for Mean	Upper 95%CL for Mean	Mean	Std Dev	ANOCOVA Adjusted Mean
1	10	ly0	-1.433	-0.898	**-1.165**	0.374	
		ly1	1.105	1.583	1.344	0.334	1.426
		ly2	1.437	1.893	1.665	0.318	1.743
		ly3	1.667	2.110	1.889	0.309	1.961
		ly4	1.820	2.252	2.036	0.302	2.118
2	10	ly0	-1.008	-0.816	**-0.912**	0.134	
		ly1	1.190	1.502	1.346	0.218	1.264
		ly2	1.553	1.848	1.700	0.206	1.623
		ly3	1.751	2.044	1.897	0.205	1.825
		ly4	1.914	2.202	2.058	0.202	1.976

x0 = sample condition: 1 = gassed, 2 = incubated

Bolded entries are statistically significant at the 0.10 level.

Bolded and underlined entries are statistically significant at the 0.05 level.

Table 35. Summary of Results by Sample Condition—Original Scale, With hCG

x0	N Obs	Variable	Lower 95%CL for Mean	Upper 95%CL for Mean	Mean	Std Dev	ANOCOVA Adjusted Mean
1	10	YC0	0.25	0.44	0.35	0.13	
		YC1	4.65	7.50	6.08	1.99	6.14
		YC2	10.39	16.12	13.25	4.00	13.44
		YC3	15.60	25.62	20.61	7.00	20.94
		YC4	22.10	35.31	28.71	9.23	29.02
2	10	YC0	0.30	0.57	0.43	0.18	
		YC1	5.27	6.93	6.10	1.16	6.03
		YC2	12.82	18.01	15.42	3.62	15.22
		YC3	20.13	28.28	24.20	5.69	23.87
		YC4	27.80	38.99	33.40	7.82	33.09

x0 = sample condition: 1 = gassed, 2 = incubated
Bolded entries are statistically significant at the 0.10 level.
Bolded and underlined entries are statistically significant at the 0.05 level.

Table 36. Summary of Results by Sample Condition—Log Scale, With hCG

x0	N Obs	Variable	Lower 95%CL for Mean	Upper 95%CL for Mean	Mean	Std Dev	ANOCOVA Adjusted Mean
1	10	lyc0	-1.474	-0.818	-1.146	0.458	
		lyc1	1.524	1.989	1.757	0.325	1.776
		lyc2	2.331	2.757	2.544	0.297	2.562
		lyc3	2.737	3.214	2.975	0.334	2.995
		lyc4	3.085	3.538	3.312	0.317	3.323
2	10	lyc0	-1.176	-0.637	-0.906	0.377	
		lyc1	1.652	1.931	1.792	0.195	1.772
		lyc2	2.539	2.881	2.710	0.239	2.693
		lyc3	2.978	3.340	3.159	0.253	3.139
		lyc4	3.295	3.665	3.480	0.259	3.469

x0 = sample condition: 1 = gassed, 2 = incubated
Bolded entries are statistically significant at the 0.10 level.
Bolded and underlined entries are statistically significant at the 0.05 level.

7.0 DISCUSSION

The optimization of the sliced testis assay is necessary in order to proceed to the prevalidation and validation stages of the testing of the assay for use in the Tier I tests for screening of substances for potential as endocrine disruptors. The Phase I studies have contributed to the initial portion of this optimization. These factors will be used for the rest of the optimization in Part II. Without these initial studies, we would not have used the best gaseous atmosphere for optimal testosterone concentrations from the testicular tissues.

8.0 CONCLUSIONS FOR PHASE I

The testosterone RIA and the LDH assay can both be verified with M-199 without phenol red. Both were validated and show the characteristics necessary for use to optimize the sliced testis assay.

There were certain factors in the initial Phase I experiments that definitely were not beneficial to use in the assay. For instance, 22-week-old rats do not show the responsiveness in their testicular tissue that is necessary for an optimal assay. The air atmosphere was also not a favorable condition for the assay. The prototypical assay media, Media 199 without phenol red, was equal to any of the others tested. Statistical analysis was necessary to show that the atmosphere of 5% CO_2/ 95% O_2 was optimal, and that rats of the 11- to 15-week-old range could be used for the assay. From these conclusions, we were ready to advance to the Phase II experiments.

Media 199 without phenol red will be used after it is gassed with the 5 % CO_2/ 95% O_2 mixture and pH adjusted to 7.4 for testicular tissues from 11- to 15-week-old rats for the Phase II experimental studies.

PHASE II

Phase II Experiments consisted of several groups of studies. The multifactorial studies were for the incubation conditions of the assay and the testes slice preparation factors of the assay. After the optimal factors were determined, the LDH experiments were performed to determine LDH and testosterone concentrations under various conditions.

9.0 INCUBATION EXPERIMENT

9.1 Statistical Analysis of Phase II Incubation Optimization Experiment

9.1.1 Objectives

The objectives of the incubation optimization experiment were to determine the effect of various incubation factor settings on testosterone production using the sliced testis assay and to identify the incubation factor settings that maximize the amount of testosterone production. These experiments tested the effect(s) of six factors: incubation temperature, incubation vessel type, incubation shaker speed, incubation media volume, hCG concentration, and testicular fragment size. Table 37 presents the incubation factor settings used in this experiment. Each trial in the experiment was run with and without hCG stimulation, repeated measurements were taken at baseline (time 0) and at 1, 2, 3, and 4 hours after baseline.

9.1.2 Data

A SAS data set was constructed from the raw Excel data files, and 2 fundamental types of dependent variables were used in the analyses: the original testosterone concentrations and the (natural) logarithm of the testosterone concentrations. Each observation includes dependent variable values for 4 time points and a corresponding baseline level. Each observation also includes data identifying the levels of the pertinent factors. The data used in the analysis are displayed in Tables 38 and 39.

9.1.3 Statistical Analysis Methods

In order to assess the effects of each incubation factor on the amount of testosterone production, ANOVA models were fit to the data separately for the trials with and without hCG stimulation. For each hCG stimulation type, the ANOVA models were fit to each individual time point. All main effects and 2-factor interactions (2fi) of the 6 factors were initially included in the models. Tests for interactions were conducted, and where they were not detected as statistically significant (p=0.10), a reduced model was employed that retained the main effects,

the baseline covariate, and only those 2fi's deemed to have significant effects. Once the final ANOVA model was determined, the data were fit using a response surface regression analysis to determine the maximum predicted value of testosterone and the incubation factor levels associated with the maximum. Additional details are provided in the Results portion of this section.

9.1.4 Results

To assess the effects of each incubation factor on testosterone production, an ANOVA model was fit separately to the set of data for each hCG stimulation type and each time point beyond the baseline.

Table 37. Incubation Factor Settings and Coded Values

Factor Identification	Units	Variable Name	Factor Name	Experimental Levels					Coded Experimental Levels				
				1	2	3	4	5	1	2	3	4	5
Incubation Temperature	°C	Temp	X1	--	34*	--	37	--	--	-1	--	+1	--
Incubation Vessel Type	NA[a]	Vessel	X2	scintillation vial*	test tube	--	--	--	-1	+1	--	--	--
Incubation Shaker Speed	NA	Speed	X3	--	none	low	high*	--	--	-1	0	+1	--
Incubation Media Volume	ml	Inc_vol	X4	--	2.5	5*	10	--	--	-1	0	+1	--
hCG Concentration	IU/ml	hCG_conc	X5	0.001	0.01	0.1*	1	10	-2	-1	0	+1	+2
Fragment Size	mg	mg_tissue	X6	25	50	125	250*	--	-0.8	-0.6	0	+1	--

*** Prototypical value.**
[a] NA - not applicable.
Note: Shaker speed was treated as a continuous factor.

The ANOVA models containing all main effects and 2fi's were fit to the original testosterone concentrations and the logarithms of testosterone concentrations using PROC GLM. The logarithm of the baseline testosterone concentration (T0) was also added to the model as a dependent variable. Upon examining the residuals from fitting the full ANOVA models, the residuals for the original testosterone concentrations showed signs of heteroskedasticity. The residuals for the logarithms of testosterone concentrations appear homoskedastic and randomly distributed around zero, thereby satisfying the assumptions necessary for valid ANOVA modeling results. The logarithms of the testosterone concentrations were therefore chosen as the more appropriate dependent variables for the analysis of the incubation experiment data.

For the full ANOVA models using the logarithm of testosterone concentration as the dependent variable, we examined the t-tests for the significance of each factor in the model. In the first round of model evaluation, we examined the t-test results for the 2fis to see if any of those terms could be removed from the models. Any 2fis with p-values greater than 0.1 were removed from the models. The reduced models, one model for the data without hCG stimulation and one model for the data with hCG stimulation, were fit to the logarithms of testosterone concentration again and the t-tests of the model terms were re-evaluated for significance. After 2 rounds of removing insignificant 2fis, we arrived at the final reduced models containing only significant 2fis and first order factors.

To obtain the optimal combination of factors for each time point, the data were fit using response surface regression models and the SAS procedure RSREG. (The PROC GLM modeling results were used to determine which factors and 2fis needed to remain in the models. Any factors which did not have any associated 2fis in the final models were treated as covariates in the response surface regression models.) The ANOVA results for the response surface regressions are summarized in Table 40. The parameter estimates from the response surface regression models for the data without hCG stimulation are presented in Table 41, and the parameter estimates for the data with hCG stimulation are presented in Table 42. Because the vessel type is a categorical variable, we needed to run separate models for each vessel type in PROC RSREG. Differentiation of the prediction equation is used to determine the location of the stationary point; in most cases the stationary point was not a point of maximal response. Under these circumstances, the RIDGE technique in SAS is used to find a point of maximal response within the experimental region. Starting at the center of the experimental region, a hypersphere having a given radius is considered, and the point on the hypersphere with the maximal response is determined. The radius is then incremented and the maximum on that hypersphere is determined. This process, when repeated, traces out a "ridge of maximal response." The iterations are terminated when one of the factors reaches the extremity of its range, as defined by the levels used in the experiment. We determined the factor combination that produced the maximum predicted value of logarithm of testosterone concentration for each time point at each combination of with/without hCG stimulation and for each vessel type. The optimal value factor combinations were plugged back into the response surface regression models to obtain predicted values for the optimal factor combinations at all time points. For example, we used the RIDGE regression results to determine the optimal factor combinations for the first time point (T1) for the scintillation vial without hCG stimulation. We then used the optimal T1 combination to obtain the predicted values for logarithm of testosterone concentration and their standard errors for the scintillation vial without hCG stimulation for that time point and for all the other time points (T2, T3 and T4). The optimal factor results, presented in Table 43, show the effects of the optimal factors for 1 time point on the other time points in evaluating the best combination of factors to use for further experimentation.

After examining the possible optimal factors for each time point, vessel type, and hCG stimulation, the chosen optimal values and their predicted values and standard errors are presented in Table 44.

In order to assess the effects of the baseline testosterone concentrations on the effects of factors, the same analysis steps that were performed above were also repeated on the logarithms of testosterone concentration but with the baseline concentration removed from the models. The ANOVA results for the final reduced model from PROC RSREG are shown in Table 45.

Comparing the results in Table 45 with those in Table 40 (results for models with baseline concentration in the models) indicates that there are many more significant terms for the models without the baseline concentrations.[1] The root mean square errors (RMSEs) in Table 45 are higher than Table 40 for the cases with no hCG stimulation, whereas the RMSEs in Table 45 are lower than Table 40 for the cases with hCG stimulation.[2] The response surface regression results from PROC RSREG are presented in Tables 46 and 47. The optimal values for each time point are shown in Table 48. The factor combinations that produced the optimal values for each time point in Table 48 are very similar to the combinations in Table 43. The standard errors of the predicted values for Table 48 are higher for the data without hCG stimulation than Table 43, whereas the standard errors for Table 48 are lower than Table 43 for the data with hCG stimulation. The differences in the standard errors between the two types of models (with and without baseline concentrations) are consistent with the results of the RMSEs in Tables 40 and 45. The predicted values for the chosen optimal combination of factors are presented in Table 49. The predicted values in Table 49 are very similar to the predicted values in Table 44. The standard errors for the cases without hCG stimulation are similar between the two model types; however the standard errors for the cases with hCG are lower in Table 13 than those in Table 8.

[1] This suggests that the baseline levels are affected by (some of) the factors and that their resultant effect on later times is diminished if adjustments for baseline levels are made.

[2] This indicates that baseline adjustments for the latter cases were unnecessary, as was also apparent from Table 4 (since baseline effect was not significant).

Table 38. Data Listing for Incubation Experiment

Run	Temp	Vessel	Speed	Inc Vol	hCG Conc	mg Tissue	Weight	Test 0	Test 1	Test 2	Test 3	Test 4
M	37	test tube	high	2.5	0.010	250	0.2606	7.061	24.751	30.890	65.349	87.145
MC	37	test tube	high	2.5	0.010	250	0.2666	7.877	65.791			272.168
N	37	test tube	high	2.5	1.000	50	0.0569	5.800	22.671	25.308	30.404	33.919
NC	37	test tube	high	2.5	1.000	50	0.0494	7.490	18.016	44.939	59.717	78.138
O	37	test tube	high	10.0	0.010	50	0.0554	3.971	12.274	14.079	18.412	18.051
OC	37	test tube	high	10.0	0.010	50	0.0480		16.667	35.833	57.500	75.000
P	37	test tube	high	10.0	1.000	250	0.2736	0.365	9.064	11.367	13.852	16.228
PC	37	test tube	high	10.0	1.000	250	0.2654	0.414	24.265	65.825	93.331	109.570
13	37	vial	high	2.5	0.010	50	0.0509	30.452	69.745	59.332	55.010	50.098
13C	37	vial	high	2.5	0.010	50	0.0506		59.486	103.557	206.522	175.692
13*	37	vial	high	2.5	0.010	50	0.0488	38.934	45.082	51.639	47.746	50.205
13C*	37	vial	high	2.5	0.010	50	0.0492			19.715	24.390	26.829
14	37	vial	high	2.5	1.000	250	0.2653	35.733	46.702	41.425	40.709	39.540
14C	37	vial	high	2.5	1.000	250	0.2569	24.251	240.132	378.786	407.513	635.072
14*	37	vial	high	2.5	1.000	250	0.2621	27.890	90.729	70.927	59.634	51.354
14C*	37	vial	high	2.5	1.000	250	0.2512	39.411	238.774	245.900	502.906	458.718
15	37	vial	high	10.0	0.010	250	0.2580		27.209	28.605	28.488	31.085
15C	37	vial	high	10.0	0.010	250	0.2411	4.977	42.555	110.162	134.343	216.715
15*	37	vial	high	10.0	0.010	250	0.2606		8.711	8.941	15.349	15.503
15C*	37	vial	high	10.0	0.010	250	0.2569		34.761	88.945	134.877	156.637
16	37	vial	high	10.0	1.000	50	0.0490	3.265	3.469	2.245	2.041	1.837
16C	37	vial	high	10.0	1.000	50	0.0535	1.682	2.617	4.486	5.421	6.729
16*	37	vial	high	10.0	1.000	50	0.0506	2.767	5.138	5.336	5.138	5.336
16C*	37	vial	high	10.0	1.000	50	0.0566	2.473	6.184	13.428	17.668	24.205
S	37	test tube	low	5.0	0.100	125	0.1236	1.375	6.068	9.709	14.968	16.100
SC	37	test tube	low	5.0	0.100	125	0.1282		4.134	7.098	12.637	15.679
19	37	vial	low	5.0	0.100	125	0.1296	6.096	44.830	59.182	72.068	75.540

Table 38. Data Listing for Incubation Experiment (Continued)

Run	Temp	Vessel	Speed	Inc Vol	hCG Conc	mg Tissue	Weight	Test 0	Test 1	Test 2	Test 3	Test 4
19C	37	vial	low	5.0	0.100	125	0.1158	5.534	43.178	61.658	74.180	94.732
I	37	test tube	none	2.5	0.010	50	0.0503	4.374	11.332	16.700	21.272	23.658
IC	37	test tube	none	2.5	0.010	50	0.0544	4.044	19.669	39.338	40.625	57.169
J	37	test tube	none	2.5	1.000	250	0.2430	2.469	5.802	7.284	10.329	13.951
JC	37	test tube	none	2.5	1.000	250	0.2592	2.160	8.642	12.539	13.927	18.519
K	37	test tube	none	10.0	0.010	250	0.2405	2.204	7.318	9.813	11.268	10.894
KC	37	test tube	none	10.0	0.010	250	0.2496	1.482	4.888	7.973	7.652	9.014
L	37	test tube	none	10.0	1.000	50	0.0473		3.383	7.400	9.091	8.457
LC	37	test tube	none	10.0	1.000	50	0.0569		1.933	3.163	4.042	4.394
9	37	vial	none	2.5	0.010	250	0.2510	3.705	14.980	20.478	24.462	27.410
9C	37	vial	none	2.5	0.010	250	0.2499	3.601	15.846	23.609		35.214
10	37	vial	none	2.5	1.000	50	0.0504	8.929	55.556	58.532	70.437	77.579
10C	37	vial	none	2.5	1.000	50	0.0554	14.801	73.466	96.570	129.422	172.924
11	37	vial	none	10.0	0.010	50	0.0474	3.797	13.502	16.245	19.198	21.730
11C	37	vial	none	10.0	0.010	50	0.0515		2.330	1.553	1.748	
12	37	vial	none	10.0	1.000	250	0.2467	0.365	4.945	7.053	9.360	12.282
12C	37	vial	none	10.0	1.000	250	0.2341		5.083	6.835	10.423	17.044
E	34	test tube	high	2.5	0.010	50	0.0499	9.619	9.018	10.621	9.018	10.220
EC	34	test tube	high	2.5	0.010	50	0.0514	12.257	41.829	57.004	82.101	97.082
F	34	test tube	high	2.5	1.000	250	0.2463	9.013	18.270	23.752	30.694	46.041
FC	34	test tube	high	2.5	1.000	250	0.2440	8.893	17.254	22.131	30.574	36.352
G	34	test tube	high	10.0	0.010	250	0.2534	8.011	13.062	15.746	16.338	17.285
GC	34	test tube	high	10.0	0.010	250	0.2503	2.797	5.473	8.670	10.228	10.907
H	34	test tube	high	10.0	1.000	50	0.0506	3.755	7.708	12.451	13.636	16.206
HC	34	test tube	high	10.0	1.000	50	0.0493	6.085	19.270	21.704	19.675	20.081
U	34	test tube	high	5.0	0.100	125	0.1225	8.000	13.551	16.653	20.898	22.286
UC	34	test tube	high	5.0	0.100	125	0.1269	4.649	13.002	14.106	20.016	24.665
U*	34	test tube	high	5.0	0.100	125	0.1254	6.380	16.667	21.372	27.193	22.488
UC*	34	test tube	high	5.0	0.100	125	0.1282	6.786	12.871	18.487	20.827	7.878

Table 38. Data Listing for Incubation Experiment (Continued)

Run	Temp	Vessel	Speed	Inc Vol	hCG Conc	mg Tissue	Weight	Test 0	Test 1	Test 2	Test 3	Test 4
5	34	vial	high	2.5	0.010	50	0.0546	11.172	11.538	11.722	12.271	9.890
5C	34	vial	high	2.5	0.010	50	0.0457	13.786	10.284	15.536	15.536	12.473
5*	34	vial	high	2.5	0.010	50	0.0520	4.615	4.615	3.654	4.808	6.154
5C*	34	vial	high	2.5	0.010	50	0.0505	7.723	11.485	11.683	8.713	7.921
6	34	vial	high	2.5	1.000	50	0.0481	15.800	12.266	12.474	13.721	16.632
6C	34	vial	high	2.5	1.000	50	0.0508	34.449	91.142	153.346	206.693	197.441
6*	34	vial	high	2.5	1.000	50	0.0485	14.639	32.371	36.907	42.474	50.928
6C*	34	vial	high	2.5	1.000	50	0.0454	16.520	14.978	20.044	20.264	26.432
7	34	vial	high	10.0	0.010	50	0.0498	3.614	7.028	9.438	10.241	15.462
7C	34	vial	high	10.0	0.010	50	0.0541		2.218	2.403	1.664	1.848
7*	34	vial	high	10.0	0.010	50	0.0530	2.642	3.396	4.340	4.717	5.094
7C*	34	vial	high	10.0	0.010	50	0.0472			2.331	2.542	1.907
8	34	vial	high	10.0	1.000	250	0.2520	2.341	12.143	13.373	13.651	14.921
8C	34	vial	high	10.0	1.000	250	0.2685	4.469	22.868	37.877	54.562	70.689
8*	34	vial	high	10.0	1.000	250	0.2441	6.145	7.948	8.726	9.586	10.651
8C*	34	vial	high	10.0	1.000	250	0.2455	5.662	31.772	53.931	98.737	142.729
21	34	vial	high	5.0	0.100	125	0.1196	8.528	8.612	9.448	6.773	9.532
21C	34	vial	high	5.0	0.100	125	0.1289	4.888	40.962	86.656	209.930	324.438
21*	34	vial	high	5.0	0.100	125	0.1227	7.742	8.883	7.661	7.579	9.128
21C*	34	vial	high	5.0	0.100	125	0.1266	8.610	24.329	38.468	48.578	68.325
Q	34	test tube	low	5.0	0.100	125	0.1256	0.717	4.697	8.599	9.475	11.226
QC	34	test tube	low	5.0	0.100	125	0.1214	6.096	6.260	9.967	9.720	10.626
Q*	34	test tube	low	5.0	0.100	125	0.1288	8.463	9.084	8.929	11.102	10.093
QC*	34	test tube	low	5.0	0.100	125	0.1320	5.909	13.712	15.833	18.561	21.136
R	34	test tube	low	5.0	0.100	125	0.1302	2.995	4.378	9.908	15.131	14.132
RC	34	test tube	low	5.0	0.100	125	0.1256	5.494	6.210	11.306	13.217	12.341
V	34	test tube	low	2.5	0.100	125	0.1232	1.948	7.143	13.555	16.883	19.643
VC	34	test tube	low	2.5	0.100	125	0.1269	2.837	11.348	20.331	31.678	49.724
W	34	test tube	low	10.0	0.100	125	0.1237	7.842	10.024	12.369	16.006	18.432

Table 38. Data Listing for Incubation Experiment (Continued)

Run	Temp	Vessel	Speed	Inc Vol	hCG Conc	mg Tissue	Weight	Test 0	Test 1	Test 2	Test 3	Test 4
WC	34	test tube	low	10.0	0.100	125	0.1270	2.441	3.858	5.669	7.559	8.583
X	34	test tube	low	5.0	0.001	125	0.1262	7.369	11.094	14.659	16.640	18.463
XC	34	test tube	low	5.0	0.001	125	0.1258	6.200	12.480	13.752	17.647	19.873
Y	34	test tube	low	5.0	10.000	125	0.1263	2.692	6.809	8.947	11.718	12.827
YC	34	test tube	low	5.0	10.000	125	0.1247	2.165	8.340	14.354	17.562	21.010
Z	34	test tube	low	5.0	0.100	25	0.0223		3.587			
ZC	34	test tube	low	5.0	0.100	25	0.0235	12.766	23.404	31.064		49.787
AA	34	test tube	low	5.0	0.100	250	0.2450	1.143	4.082	6.898	8.286	10.490
AAC	34	test tube	low	5.0	0.100	250	0.2530	1.502	3.162	4.862	7.747	8.814
17	34	vial	low	5.0	0.100	125	0.1254	20.335	24.402	21.132	50.718	53.907
17C	34	vial	low	5.0	0.100	125	0.1275	45.333	28.941	40.314		
17*	34	vial	low	5.0	0.100	125	0.1223	20.687	28.373	21.096	50.286	54.538
17C*	34	vial	low	5.0	0.100	125	0.1323	11.413	13.983	12.472	34.618	46.712
18	34	vial	low	5.0	0.100	125	0.1294	27.821	36.012	23.416	56.260	62.906
18C	34	vial	low	5.0	0.100	125	0.1290	17.674	20.388	18.527	48.372	63.256
22	34	vial	low	2.5	0.100	125	0.1262	2.853	22.979	30.349	40.254	49.366
22C	34	vial	low	2.5	0.100	125	0.1288	4.581	37.112	55.745	86.568	125.000
23	34	vial	low	10.0	0.100	125	0.1298	2.234	9.014	11.325	13.482	14.946
23C	34	vial	low	10.0	0.100	125	0.1255	0.637	9.004	14.821	19.602	26.056
24	34	vial	low	5.0	0.001	125	0.1226	3.507	23.002	31.403	37.847	43.312
24C	34	vial	low	5.0	0.001	125	0.1238	1.858	12.278	15.670	20.679	24.717
25	34	vial	low	5.0	10.000	125	0.1285	0.623	12.918	19.377	22.957	24.358
25C	34	vial	low	5.0	10.000	125	0.1235	2.429	18.462	21.862	31.741	50.526
26	34	vial	low	5.0	0.100	25	0.0274		7.664	6.204	7.299	7.299
26C	34	vial	low	5.0	0.100	25	0.0272					
27	34	vial	low	5.0	0.100	250	0.2647	3.060	12.694	17.265	22.289	22.818
27C	34	vial	low	5.0	0.100	250	0.2537	2.877	25.778	46.078	90.579	121.167
A	34	test tube	none	2.5	0.010	250	0.2435	7.885	18.070	22.300	27.556	36.468
AC	34	test tube	none	2.5	0.010	250	0.2606	7.866	14.275	19.916	22.909	26.592

Table 38. Data Listing for Incubation Experiment (Continued)

Run	Temp	Vessel	Speed	Inc Vol	hCG Conc	mg Tissue	Weight	Test 0	Test 1	Test 2	Test 3	Test 4
B	34	test tube	none	2.5	1.000	50	0.0523	13.767	15.488	17.400		19.503
BC	34	test tube	none	2.5	1.000	50	0.0533	18.949	30.769	32.645	35.835	37.148
C	34	test tube	none	10.0	0.010	50	0.0452	9.513	15.708	18.142	16.372	19.027
CC	34	test tube	none	10.0	0.010	50	0.0516	12.984	24.225	34.884	45.543	48.062
D	34	test tube	none	10.0	1.000	250	0.2408	7.766	7.226	9.053	9.925	12.209
DC	34	test tube	none	10.0	1.000	250	0.2406	6.151	7.606	11.887	14.630	16.417
T	34	test tube	none	5.0	0.100	125	0.1254	7.257	16.188	18.979	16.108	27.193
TC	34	test tube	none	5.0	0.100	125	0.1287	4.895	15.540		28.594	36.752
T*	34	test tube	none	5.0	0.100	125	0.1253	6.305	11.333	19.154	24.900	18.037
TC*	34	test tube	none	5.0	0.100	125	0.1296	3.858	11.960	17.515	19.444	23.843
1	34	vial	none	2.5	0.010	50	0.0476	15.336	86.134	120.588	135.294	150.420
1C	34	vial	none	2.5	0.010	50	0.0448	18.750	41.071	55.580	62.500	78.125
2	34	vial	none	2.5	1.000	250	0.2439	12.423	30.217	53.424	61.337	71.628
2C	34	vial	none	2.5	1.000	250	0.2345	19.446	49.595	88.913	134.371	
3	34	vial	none	10.0	0.010	250	0.2314	1.642	6.785	14.866	11.841	16.854
3C	34	vial	none	10.0	0.010	250	0.2462	3.249	7.433	13.891	11.779	16.288
4	34	vial	none	10.0	1.000	50	0.0521	2.495	7.294	9.405	10.749	10.940
4C	34	vial	none	10.0	1.000	50	0.0528	4.167	10.795	16.288	20.644	24.621
20	34	vial	none	5.0	0.100	125	0.1239	4.358	18.402	27.603	32.849	37.530
20C	34	vial	none	5.0	0.100	125	0.1263	1.504	30.404	37.609	42.439	47.348
20*	34	vial	none	5.0	0.100	125	0.1242		18.438	23.108	31.481	33.253
20C*	34	vial	none	5.0	0.100	125	0.1224		26.389	34.967	42.157	44.444

Table 39. Data Listing of Coded Factor Values and Logarithm of Testosterone Concentrations

Run	hCG Stimulation	Temp Code	Vessel Code	Speed Code	Inc Vol Code	hCG Conc Code	mg Tissue Code	L Test 0	L Test 1	L Test 2	L Test 3	L Test 4
M	**0**	**1**	**1**	**1**	**-1**	**-1**	**1.0**	**1.95**	**3.21**	**3.43**	**4.18**	**4.47**
MC	**1**	**1**	**1**	**1**	**-1**	**-1**	**1.0**	**2.06**	**4.19**			**5.61**
N	**0**	**1**	**1**	**1**	**-1**	**1**	**-0.6**	**1.76**	**3.12**	**3.23**	**3.41**	**3.52**
NC	**1**	**1**	**1**	**1**	**-1**	**1**	**-0.6**	**2.01**	**2.89**	**3.81**	**4.09**	**4.36**
O	**0**	**1**	**1**	**1**	**1**	**-1**	**-0.6**	**1.38**	**2.51**	**2.64**	**2.91**	**2.89**
OC	**1**	**1**	**1**	**1**	**1**	**-1**	**-0.6**		**2.81**	**3.58**	**4.05**	**4.32**
P	**0**	**1**	**1**	**1**	**1**	**1**	**1.0**	**-1.01**	**2.20**	**2.43**	**2.63**	**2.79**
PC	**1**	**1**	**1**	**1**	**1**	**1**	**1.0**	**-0.88**	**3.19**	**4.19**	**4.54**	**4.70**
13	**0**	**1**	**-1**	**1**	**-1**	**-1**	**-0.6**	**3.42**	**4.24**	**4.08**	**4.01**	**3.91**
13C	**1**	**1**	**-1**	**1**	**-1**	**-1**	**-0.6**		**4.09**	**4.64**	**5.33**	**5.17**
13*	**0**	**1**	**-1**	**1**	**-1**	**-1**	**-0.6**	**3.66**	**3.81**	**3.94**	**3.87**	**3.92**
13C*	**1**	**1**	**-1**	**1**	**-1**	**-1**	**-0.6**			**2.98**	**3.19**	**3.29**
14	**0**	**1**	**-1**	**1**	**-1**	**1**	**1.0**	**3.58**	**3.84**	**3.72**	**3.71**	**3.68**
14C	**1**	**1**	**-1**	**1**	**-1**	**1**	**1.0**	**3.19**	**5.48**	**5.94**	**6.01**	**6.45**
14*	**0**	**1**	**-1**	**1**	**-1**	**1**	**1.0**	**3.33**	**4.51**	**4.26**	**4.09**	**3.94**
14C*	**1**	**1**	**-1**	**1**	**-1**	**1**	**1.0**	**3.67**	**5.48**	**5.50**	**6.22**	**6.13**
15	**0**	**1**	**-1**	**1**	**1**	**-1**	**1.0**		**3.30**	**3.35**	**3.35**	**3.44**
15C	**1**	**1**	**-1**	**1**	**1**	**-1**	**1.0**	**1.60**	**3.75**	**4.70**	**4.90**	**5.38**
15*	**0**	**1**	**-1**	**1**	**1**	**-1**	**1.0**		**2.16**	**2.19**	**2.73**	**2.74**
15C*	**1**	**1**	**-1**	**1**	**1**	**-1**	**1.0**		**3.55**	**4.49**	**4.90**	**5.05**
16	**0**	**1**	**-1**	**1**	**1**	**1**	**-0.6**	**1.18**	**1.24**	**0.81**	**0.71**	**0.61**
16C	**1**	**1**	**-1**	**1**	**1**	**1**	**-0.6**	**0.52**	**0.96**	**1.50**	**1.69**	**1.91**
16*	**0**	**1**	**-1**	**1**	**1**	**1**	**-0.6**	**1.02**	**1.64**	**1.67**	**1.64**	**1.67**
16C*	**1**	**1**	**-1**	**1**	**1**	**1**	**-0.6**	**0.91**	**1.82**	**2.60**	**2.87**	**3.19**
S	**0**	**1**	**1**	**0**	**0**	**0**	**0.0**	**0.32**	1.80	2.27	2.71	2.78
SC	1	1	1	0	0	0	0.0		1.42	1.96	2.54	2.75
19	0	1	-1	0	0	0	0.0	1.81	3.80	4.08	4.28	4.32
19C	1	1	-1	0	0	0	0.0	1.71	3.77	4.12	4.31	4.55
I	0	1	1	-1	-1	-1	-0.6	1.48	2.43	2.82	3.06	3.16

Table 39. Data Listing of Coded Factor Values and Logarithm of Testosterone Concentrations (Continued)

Run	hCG Stimulation	Temp Code	Vessel Code	Speed Code	Inc Vol Code	hCG Conc Code	mg Tissue Code	L Test 0	L Test 1	L Test 2	L Test 3	L Test 4
IC	1	1	1	-1	-1	-1	-0.6	1.40	2.98	3.67	3.70	4.05
J	0	1	1	-1	-1	1	1.0	0.90	1.76	1.99	2.33	2.64
JC	1	1	1	-1	-1	1	1.0	0.77	2.16	2.53	2.63	2.92
K	0	1	1	-1	1	-1	1.0	0.79	1.99	2.28	2.42	2.39
KC	1	1	1	-1	1	-1	1.0	0.39	1.59	2.08	2.03	2.20
L	0	1	1	-1	1	1	-0.6		1.22	2.00	2.21	2.13
LC	1	1	1	-1	1	1	-0.6		0.66	1.15	1.40	1.48
9	0	1	-1	-1	-1	-1	1.0	1.31	2.71	3.02	3.20	3.31
9C	1	1	-1	-1	-1	-1	1.0	1.28	2.76	3.16		3.56
10	0	1	-1	-1	-1	1	-0.6	2.19	4.02	4.07	4.25	4.35
10C	1	1	-1	-1	-1	1	-0.6	2.69	4.30	4.57	4.86	5.15
11	0	1	-1	-1	1	-1	-0.6	1.33	2.60	2.79	2.95	3.08
11C	1	1	-1	-1	1	-1	-0.6		0.85	0.44	0.56	
12	0	1	-1	-1	1	1	1.0	-1.01	1.60	1.95	2.24	2.51
12C	1	1	-1	-1	1	1	1.0		1.63	1.92	2.34	2.84
E	0	-1	1	1	-1	-1	-0.6	2.26	2.20	2.36	2.20	2.32
EC	1	-1	1	1	-1	-1	-0.6	2.51	3.73	4.04	4.41	4.58
F	0	-1	1	1	-1	1	1.0	2.20	2.91	3.17	3.42	3.83
FC	1	-1	1	1	-1	1	1.0	2.19	2.85	3.10	3.42	3.59
G	0	-1	1	1	1	-1	1.0	2.08	2.57	2.76	2.79	2.85
GC	1	-1	1	1	1	-1	1.0	1.03	1.70	2.16	2.33	2.39
H	0	-1	1	1	1	1	-0.6	1.32	2.04	2.52	2.61	2.79
HC	1	-1	1	1	1	1	-0.6	1.81	2.96	3.08	2.98	3.00
U	0	-1	1	1	0	0	0.0	2.08	2.61	2.81	3.04	3.10
UC	1	-1	1	1	0	0	0.0	1.54	2.57	2.65	3.00	3.21
U*	0	-1	1	1	0	0	0.0	1.85	2.81	3.06	3.30	3.11
UC*	1	-1	1	1	0	0	0.0	1.91	2.55	2.92	3.04	2.06
5	0	-1	-1	1	-1	-1	-0.6	2.41	2.45	2.46	2.51	2.29
5C	1	-1	-1	1	-1	-1	-0.6	2.62	2.33	2.74	2.74	2.52
5*	0	-1	-1	1	-1	-1	-0.6	1.53	1.53	1.30	1.57	1.82
5C*	1	-1	-1	1	-1	-1	-0.6	2.04	2.44	2.46	2.16	2.07
6	0	-1	-1	1	-1	1	-0.6	2.76	2.51	2.52	2.62	2.81
6C	1	-1	-1	1	-1	1	-0.6	3.54	4.51	5.03	5.33	5.29

Table 39. Data Listing of Coded Factor Values and Logarithm of Testosterone Concentrations (Continued)

Run	hCG Stimulation	Temp Code	Vessel Code	Speed Code	Inc Vol Code	hCG Conc Code	mg Tissue Code	L Test 0	L Test 1	L Test 2	L Test 3	L Test 4
6*	0	-1	-1	1	-1	1	-0.6	2.68	3.48	3.61	3.75	3.93
6C*	1	-1	-1	1	-1	1	-0.6	2.80	2.71	3.00	3.01	3.27
7	0	-1	-1	1	1	-1	-0.6	1.28	1.95	2.24	2.33	2.74
7C	1	-1	-1	1	1	-1	-0.6		0.80	0.88	0.51	0.61
7*	0	-1	-1	1	1	-1	-0.6	0.97	1.22	1.47	1.55	1.63
7C*	1	-1	-1	1	1	-1	-0.6			0.85	0.93	0.65
8	0	-1	-1	1	1	1	1.0	0.85	2.50	2.59	2.61	2.70
8C	1	-1	-1	1	1	1	1.0	1.50	3.13	3.63	4.00	4.26
8*	0	-1	-1	1	1	1	1.0	1.82	2.07	2.17	2.26	2.37
8C*	1	-1	-1	1	1	1	1.0	1.73	3.46	3.99	4.59	4.96
21	0	-1	-1	1	0	0	0.0	2.14	2.15	2.25	1.91	2.25
21C	1	-1	-1	1	0	0	0.0	1.59	3.71	4.46	5.35	5.78
21*	0	-1	-1	1	0	0	0.0	2.05	2.18	2.04	2.03	2.21
21C*	1	-1	-1	1	0	0	0.0	2.15	3.19	3.65	3.88	4.22
Q	0	-1	1	0	0	0	0.0	-0.33	1.55	2.15	2.25	2.42
QC	1	-1	1	0	0	0	0.0	1.81	1.83	2.30	2.27	2.36
Q*	0	-1	1	0	0	0	0.0	2.14	2.21	2.19	2.41	2.31
QC*	1	-1	1	0	0	0	0.0	1.78	2.62	2.76	2.92	3.05
R	0	-1	1	0	0	0	0.0	1.10	1.48	2.29	2.72	2.65
RC	1	-1	1	0	0	0	0.0	1.70	1.83	2.43	2.58	2.51
V	0	-1	1	0	-1	0	0.0	0.67	1.97	2.61	2.83	2.98
VC	1	-1	1	0	-1	0	0.0	1.04	2.43	3.01	3.46	3.91
W	0	-1	1	0	1	0	0.0	2.06	2.31	2.52	2.77	2.91
WC	1	-1	1	0	1	0	0.0	0.89	1.35	1.74	2.02	2.15
X	0	-1	1	0	0	-2	0.0	2.00	2.41	2.69	2.81	2.92
XC	1	-1	1	0	0	-2	0.0	1.82	2.52	2.62	2.87	2.99
Y	0	-1	1	0	0	2	0.0	0.99	1.92	2.19	2.46	2.55
YC	1	-1	1	0	0	2	0.0	0.77	2.12	2.66	2.87	3.05
Z	0	-1	1	0	0	0	-0.8		1.28			
ZC	1	-1	1	0	0	0	-0.8	2.55	3.15	3.44		3.91
AA	0	-1	1	0	0	0	1.0	0.13	1.41	1.93	2.11	2.35

Table 39. Data Listing of Coded Factor Values and Logarithm of Testosterone Concentrations (Continued)

Run	hCG Stimulation	Temp Code	Vessel Code	Speed Code	Inc Vol Code	hCG Conc Code	mg Tissue Code	L Test 0	L Test 1	L Test 2	L Test 3	L Test 4
AAC	1	-1	1	0	0	0	1.0	0.41	1.15	1.58	2.05	2.18
17	0	-1	-1	0	0	0	0.0	3.01	3.19	3.05	3.93	3.99
17C	1	-1	-1	0	0	0	0.0	3.81	3.37	3.70		
17*	0	-1	-1	0	0	0	0.0	3.03	3.35	3.05	3.92	4.00
17C*	1	-1	-1	0	0	0	0.0	2.43	2.64	2.52	3.54	3.84
18	0	-1	-1	0	0	0	0.0	3.33	3.58	3.15	4.03	4.14
18C	1	-1	-1	0	0	0	0.0	2.87	3.01	2.92	3.88	4.15
22	0	-1	-1	0	-1	0	0.0	1.05	3.13	3.41	3.70	3.90
22C	1	-1	-1	0	-1	0	0.0	1.52	3.61	4.02	4.46	4.83
23	0	-1	-1	0	1	0	0.0	0.80	2.20	2.43	2.60	2.70
23C	1	-1	-1	0	1	0	0.0	-0.45	2.20	2.70	2.98	3.26
24	0	-1	-1	0	0	-2	0.0	1.25	3.14	3.45	3.63	3.77
24C	1	-1	-1	0	0	-2	0.0	0.62	2.51	2.75	3.03	3.21
25	0	-1	-1	0	0	2	0.0	-0.47	2.56	2.96	3.13	3.19
25C	1	-1	-1	0	0	2	0.0	0.89	2.92	3.08	3.46	3.92
26	0	-1	-1	0	0	0	-0.8		2.04	1.83	1.99	1.99
26C	1	-1	-1	0	0	0	-0.8					
27	0	-1	-1	0	0	0	1.0	1.12	2.54	2.85	3.10	3.13
27C	1	-1	-1	0	0	0	1.0	1.06	3.25	3.83	4.51	4.80
A	0	-1	1	-1	-1	-1	1.0	2.06	2.89	3.10	3.32	3.60
AC	1	-1	1	-1	-1	-1	1.0	2.06	2.66	2.99	3.13	3.28
B	0	-1	1	-1	-1	1	-0.6	2.62	2.74	2.86		2.97
BC	1	-1	1	-1	-1	1	-0.6	2.94	3.43	3.49	3.58	3.61
C	0	-1	1	-1	1	-1	-0.6	2.25	2.75	2.90	2.80	2.95
CC	1	-1	1	-1	1	-1	-0.6	2.56	3.19	3.55	3.82	3.87
D	0	-1	1	-1	1	1	1.0	2.05	1.98	2.20	2.30	2.50
DC	1	-1	1	-1	1	1	1.0	1.82	2.03	2.48	2.68	2.80
T	0	-1	1	-1	0	0	0.0	1.98	2.78	2.94	2.78	3.30
TC	1	-1	1	-1	0	0	0.0	1.59	2.74		3.35	3.60
T*	0	-1	1	-1	0	0	0.0	1.84	2.43	2.95	3.21	2.89
TC*	1	-1	1	-1	0	0	0.0	1.35	2.48	2.86	2.97	3.17

Table 39. Data Listing of Coded Factor Values and Logarithm of Testosterone Concentrations (Continued)

Run	hCG Stimulation	Temp Code	Vessel Code	Speed Code	Inc Vol Code	hCG Conc Code	mg Tissue Code	L Test 0	L Test 1	L Test 2	L Test 3	L Test 4
1	0	-1	-1	-1	-1	-1	-0.6	2.73	4.46	4.79	4.91	5.01
1C	1	-1	-1	-1	-1	-1	-0.6	2.93	3.72	4.02	4.14	4.36
2	0	-1	-1	-1	-1	1	1.0	2.52	3.41	3.98	4.12	4.27
2C	1	-1	-1	-1	-1	1	1.0	2.97	3.90	4.49	4.90	
3	0	-1	-1	-1	1	-1	1.0	0.50	1.91	2.70	2.47	2.82
3C	1	-1	-1	-1	1	-1	1.0	1.18	2.01	2.63	2.47	2.79
4	0	-1	-1	-1	1	1	-0.6	0.91	1.99	2.24	2.37	2.39
4C	1	-1	-1	-1	1	1	-0.6	1.43	2.38	2.79	3.03	3.20
20	0	-1	-1	-1	0	0	0.0	1.47	2.91	3.32	3.49	3.63
20C	1	-1	-1	-1	0	0	0.0	0.41	3.41	3.63	3.75	3.86
20*	0	-1	-1	-1	0	0	0.0		2.91	3.14	3.45	3.50
20C*	1	-1	-1	-1	0	0	0.0		3.27	3.55	3.74	3.79

Table 40. ANOVA results for PROC RSREG

hCG Stimulation	Vessel Type	Error df	Time	R^2	hCG Conc.	Incub. Vol.	Shaker Speed	Frag. Size	Incub. Temp.	Baseline	RMSE
No	scintillation vial	19	T1	87	NA	**	***		*	***	0.443
			T2	77	NA	*	**				0.585
			T3	83	NA	*	***			***	0.530
			T4	80	NA		***			**	0.570
	test tube	12	T1	86	NA		***		*	**	0.267
			T2	82	NA		***	*			0.250
			T3	81	NA		**	*	*		0.296
			T4	86	NA	**	**	**	*		0.278
Yes	scintillation vial	10	T1	91		**		***			0.506
			T2	85		**		**			0.649
			T3	83		*		**			0.740
			T4	85		*		**			0.727
	test tube	8	T1	91		**	**	**	*		0.334
			T2	91		**	**	**	*		0.339
			T3	93	*	***	***	***	**		0.308
			T4	87		***		*	*		0.482

*Statistically significant F test at 0.10 level of significance.
**Statistically significant F test at 0.05 level of significance.
***Statistically significant F test at 0.01 level of significance.

Note: R^2 is the percentage of variation accounted for by the model.
RMSE = square root of the residual (error) mean square
NA = not applicable

Table 41. Response Surface Regression Results for No hCG Stimulation

Vessel Type	Model Terms	L Test 1		L Test 2		L Test 3		L Test 4	
		Beta	P Value	Beta	P Value	Beta	P Value	Beta	P Value
Scintillation Vial	Increasing volume code	-0.47	0.003	-0.56	0.007	-0.43	0.020	-0.40	0.038
	Speed code	-0.29	0.014	-0.42	0.007	-0.55	0.000	-0.59	0.000
	mg tissue code	0.05	0.863	0.07	0.848	-0.02	0.962	0.10	0.786
	Temperature code	0.21	0.035	0.12	0.334	0.11	0.337	0.07	0.557
	Increasing volume code*Increasing volume code	0.02	0.958	0.06	0.886	0.03	0.942	0.07	0.856
	Speed code*Increasing volume code	0.09	0.436	0.07	0.621	0.12	0.378	0.12	0.414
	Speed code*Speed code	-0.46	0.093	-0.37	0.299	-0.85	0.014	-0.74	0.041
	mg tissue code*Increasing volume code	0.27	0.102	0.25	0.237	0.31	0.116	0.32	0.124
	mg tissue code*Speed code	0.36	0.017	0.29	0.131	0.25	0.155	0.18	0.326
	mg tissue code*mg tissue code	-0.06	0.908	-0.04	0.954	0.19	0.773	-0.03	0.967
	Temperature code*Increasing volume code	-0.09	0.382	-0.17	0.245	-0.11	0.411	-0.11	0.419
	Temperature code*Speed code	0.16	0.151	0.19	0.194	0.12	0.381	0.07	0.631
	Temperature code*mg tissue code	0.06	0.683	-0.01	0.979	0.12	0.537	0.11	0.582
	L test 0	0.36	0.005	0.22	0.158	0.39	0.010	0.39	0.015
Test Tube	Increasing volume code	-0.07	0.426	-0.10	0.223	-0.12	0.214	-0.17	0.062
	Speed code	0.11	0.147	0.07	0.320	0.13	0.136	0.12	0.155
	mg tissue code	0.23	0.255	0.24	0.196	0.48	0.040	0.50	0.026
	Temperature code	0.12	0.153	0.06	0.472	0.19	0.047	0.14	0.108
	Increasing volume code*Increasing volume code	0.14	0.438	0.20	0.253	0.22	0.289	0.32	0.107
	Speed code*Increasing volume code	-0.01	0.919	-0.05	0.544	-0.06	0.588	-0.08	0.396
	Speed code*Speed code	0.50	0.016	0.53	0.008	0.44	0.045	0.45	0.031
	mg tissue code*Increasing volume code	-0.06	0.587	-0.11	0.268	-0.22	0.075	-0.30	0.017
	mg tissue code*Speed code	0.21	0.047	0.22	0.028	0.23	0.052	0.25	0.028
	mg tissue code*mg tissue code	-0.52	0.177	-0.69	0.066	-0.97	0.034	-0.88	0.038
	Temperature code*Increasing volume code	0.04	0.595	0.01	0.899	-0.04	0.675	-0.07	0.388
	Temperature code*Speed code	0.16	0.041	0.12	0.105	0.13	0.143	0.14	0.081
	Temperature code*mg tissue code	-0.06	0.564	-0.13	0.229	-0.15	0.226	-0.17	0.157
	LTest_0	0.31	0.013	0.11	0.282	0.13	0.283	0.07	0.543

Note: L Test J = log testosterone concentration at time J
Beta = regression coefficient, as applied to coded factors
P Value = significance level for t-test of hypothesis that the true Beta = 0

Table 42. Response Surface Regression Results for hCG Stimulation

Vessel Type	Model Terms	L Test 1		L Test 2		L Test 3		L Test 4	
		Beta	P Value	Beta	P Value	Beta	P Value	Beta	P Value
Scintillation Vial	hCG concentration code	0.08	0.718	-0.02	0.942	0.12	0.710	0.11	0.729
	Speed code	-0.28	0.321	-0.16	0.660	0.15	0.717	0.22	0.593
	mg tissue code	1.05	0.012	1.00	0.045	1.11	0.050	1.23	0.031
	Temperature code	0.29	0.230	0.33	0.294	0.07	0.832	0.06	0.852
	hCG concentration code*hCGconcentration code	-0.07	0.502	-0.11	0.428	-0.16	0.324	-0.16	0.327
	Speed code*hCG concentration code	0.26	0.255	0.34	0.247	0.23	0.481	0.27	0.399
	Speed code*Speed code	0.34	0.331	0.49	0.277	0.18	0.712	0.19	0.704
	mg tissue code*hCG concentration code	-0.22	0.492	-0.46	0.284	-0.69	0.162	-0.88	0.079
	mg tissue code*Speed code	0.65	0.086	0.81	0.095	1.21	0.037	1.41	0.017
	mg tissue code*mg tissue code	-0.72	0.206	-0.52	0.466	-0.98	0.239	-1.21	0.146
	Temperature code*hCG concentration code	-0.07	0.773	-0.13	0.676	0.00	0.998	-0.03	0.931
	Temperature code*Speed code	-0.30	0.348	-0.31	0.436	-0.09	0.834	-0.05	0.914
	Temp code*mg tissue code	0.36	0.397	0.31	0.568	-0.03	0.964	-0.16	0.791
	L test 0	0.04	0.853	-0.12	0.624	0.11	0.714	0.08	0.765
	Increasing volume code	-0.74	0.027	-0.82	0.048	-0.79	0.087	-0.86	0.063
Test Tube	hCG concentration code	-0.22	0.195	-0.27	0.122	-0.33	0.049	-0.34	0.166
	Speed code	0.33	0.071	0.44	0.026	0.55	0.006	0.48	0.075
	mg tissue code	-0.35	0.224	-0.43	0.150	-0.50	0.076	-0.77	0.081
	Temperature code	-0.16	0.359	0.04	0.825	-0.04	0.806	-0.07	0.784
	hCG concentration code*hCG concentration code	0.10	0.156	0.08	0.250	0.08	0.231	0.10	0.333
	Speed code*hCG concentration code	0.09	0.451	0.07	0.565	0.05	0.650	0.06	0.734
	Speed code*Speed code	0.67	0.018	0.62	0.026	0.59	0.023	0.47	0.190
	mg tissue code*hCG concentration code	0.27	0.066	0.32	0.037	0.42	0.007	0.45	0.039
	mg tissue code*Speed code	0.07	0.609	0.06	0.657	0.05	0.714	0.02	0.923
	mg tissue code*mg tissue code	0.28	0.448	0.30	0.419	0.44	0.211	0.80	0.153
	Temperature code*hCG concentration code	-0.14	0.371	-0.20	0.232	-0.21	0.178	-0.20	0.391
	Temperature code*Speed code	0.34	0.054	0.50	0.012	0.59	0.003	0.61	0.024
	Temperature code*mg tissue code	0.42	0.021	0.32	0.063	0.31	0.053	0.26	0.250
	L test 0	0.04	0.818	-0.07	0.714	-0.18	0.322	-0.35	0.220
	Increasing volume code	-0.38	0.012	-0.38	0.012	-0.46	0.003	-0.57	0.01

Note: L Test J = log testosterone concentration at time J
Beta = regression coefficient, as applied to coded factors
P Value = significance level for t-test of hypothesis that the true Beta = 0

Table 43. Maximum Values for Each Time Point

hCG Stimu-lation	Vessel Type	Time	Predicted Log(Testosterone Concentration)*								Media Volume	hCG Conc	Fragment Size	Temp	Shaker Speed Code	Shaker Speed[a]	log (T0)
			T1		T2		T3		T4								
No	Scintillation vial	T1 opt	3.992	(0.289)	4.139	(0.382)	4.446	(0.346)	4.503	(0.372)	2.5		98.1	36.1	-0.251	low	1.8108
No	Scintillation vial	T2 opt	3.945	(0.293)	4.105	(0.387)	4.403	(0.351)	4.484	(0.377)	2.5		108.6	35.9	-0.310	low	1.8108
No	Scintillation vial	T3 opt	4.064	(0.348)	4.212	(0.460)	4.671	(0.417)	4.605	(0.449)	2.5 = 98		42.0	35.7	-0.339	low	1.8108
No	Scintillation vial	T4 opt	3.945	(0.282)	4.110	(0.373)	4.418	(0.338)	4.495	(0.363)	2.5		102.3	35.8	-0.343	low	1.8108
No	Test tube	T1 opt	2.870	(0.175)	3.121	(0.163)	3.536	(0.193)	3.543	(0.182)	4.7		162.1	35.8	0.998	high	1.4189
No	Test tube	T2 opt	2.856	(0.175)	3.135	(0.164)	3.542	(0.194)	3.568	(0.182)	4.3		159.5	35.7	0.998	high	1.4189
No	Test tube	T3 opt	2.913	(0.180)	3.190	(0.168)	3.634	(0.199)	3.685	(0.187)	3.9		164.8	35.9	0.991	high	1.4189
No	Test tube	T4 opt	2.990	(0.204)	3.317	(0.191)	3.794	(0.226)	3.927	(0.213)	3.1		173.5	35.8	0.993	high	1.4189
Yes	Scintillation vial	T1 opt	5.327	(0.864)	5.703	(1.108)	4.876	(1.263)	5.048	(1.240)	2.5	0.053	172.5	36.6	-0.994	none	1.8142
Yes	Scintillation vial	T2 opt	5.087	(0.543)	5.919	(0.696)	6.421	(0.794)	6.919	(0.779)	2.5	0.085	234.8	35.8	0.990	high	1.8142
Yes	Scintillation vial	T3 opt	4.961	(0.574)	5.766	(0.736)	6.377	(0.840)	6.904	(0.824)	2.5	0.077	223.3	35.5	0.994	high	1.8142
Yes	Scintillation vial	T4 opt	4.937	(0.588)	5.743	(0.754)	6.374	(0.860)	6.909	(0.844)	2.5	0.071	220.7	35.4	0.999	high	1.8142
Yes	Test tube	T1 opt	3.169	(0.355)	3.876	(0.360)	4.163	(0.328)	4.101	(0.513)	2.5	0.083	135.2	35.7	0.991	high	1.4847
Yes	Test tube	T2 opt	3.242	(0.403)	4.025	(0.409)	4.323	(0.372)	4.267	(0.583)	2.5	0.071	130.3	36.0	0.999	high	1.4847
Yes	Test tube	T3 opt	3.276	(0.398)	4.075	(0.404)	4.392	(0.368)	4.375	(0.576)	2.5	0.061	115.6	36.0	0.998	high	1.4847
Yes	Test tube	T4 opt	2.945	(0.403)	3.693	(0.409)	4.140	(0.372)	4.711	(0.582)	2.5	0.046	25.3	35.5	0.350	low	1.4847

*Values in parentheses are standard errors

[a] If the coded value for shaker speed was between -1 and -0.5 then the shaker speed was defined as “none”. If the coded value for shaker speed was between -0.5 and 0.5 then the shaker speed was defined as “low”. If the coded value for shaker speed was between 0.5 and 1 then the shaker speed was defined as “high”.

Table 44. Predicted values for Specified Optimum Factor Combinations

hCG Stimu-lation	Vessel Type	Time	Predicted Log(Testosterone Concentration)*				Media Volume	hCG Concent	Fragment Size	Temp	Shaker Speed Code	Shaker Speed[a]	log (T0)
			T1	T2	T3	T4							
No	Scintillation vial	Optimal	3.368 (0.204)	3.333 (0.270)	3.800 (0.245)	3.861 (0.263)	5.0		175.0	36.0	0.0	low	1.8108
No	Scintillation vial	Optimal	2.820 (0.305)	2.728 (0.403)	2.538 (0.365)	2.624 (0.392)	5.0		175.0	36.0	1.0	high	1.8108
Yes	Scintillation vial	Optimal	4.496 (0.358)	4.810 (0.459)	5.120 (0.524)	5.448 (0.515)	2.5	0.100	175.0	36.0	0.0	low	1.8142
Yes	Scintillation vial	Optimal	4.715 (0.590)	5.363 (0.757)	5.906 (0.864)	6.398 (0.848)	2.5	0.100	175.0	36.0	1.0	high	1.8142

*Values in parentheses are standard errors

[a] If the coded value for shaker speed was between -1 and -0.5 then the shaker speed was defined as “none”. If the coded value for shaker speed was between -0.5 and 0.5 then the shaker speed was defined as “low”. If the coded value for shaker speed was between 0.5 and 1 then the shaker speed was defined as “high”.

Table 45. ANOVA results for PROC RSREG with the Baseline Concentration Removed from Model

hCG Stimulation	Vessel Type	Error df	Time	R^2	hCG Conc.	Incub. Vol.	Shaker Speed	Frag. Size	Incub. Temp.	RMSE
No	scintillation vial	24	T1	77	NA	***	***	**	**	0.532
			T2	72	NA	***	**	*	*	0.600
			T3	72	NA	***	***	*		0.625
			T4	69	NA	***	**	*		0.653
	test tube	14	T1	71	NA		***		*	0.390
			T2	76	NA	*	***		*	0.279
			T3	76	NA	**	***	**	*	0.316
			T4	82	NA	***	***	**	**	0.300
Yes	scintillation vial	16	T1	91		***	***	***		0.455
			T2	88	*	***	***	***	*	0.552
			T3	85		***	*	***	*	0.681
			T4	85		***	*	***	*	0.693
	test tube	12	T1	93	***	***	***	***	***	0.278
			T2	92	**	***	***	***	***	0.315
			T3	94	***	***	***	***	***	0.288
			T4	87	*	***	***	**	***	0.447

*Statistically significant F test at 0.10 level of significance.
**Statistically significant F test at 0.05 level of significance.
***Statistically significant F test at 0.01 level of significance.

Note: R^2 is the percentage of variation accounted for by the model.
RMSE = square root of the residual (error) mean square
NA = not applicable

Table 46. Response Surface Regression Results for No hCG Stimulation with Baseline Concentration Removed

Vessel Type	Model Terms	L Test 1		L Test 2		L Test 3		L Test 4	
		Beta	P Value	Beta	P Value	Beta	P Value	Beta	P Value
Scintillation Vial	Inc_vol_code	-0.78	<.0001	-0.75	<.0001	-0.75	<.0001	-0.72	<.0001
	Speed_code	-0.14	0.206	-0.30	0.022	-0.38	0.008	-0.41	0.006
	mg_tissue_code	0.18	0.409	0.32	0.194	0.36	0.163	0.44	0.106
	Temp_code	0.22	0.047	0.15	0.218	0.15	0.239	0.12	0.378
	Inc_vol_code*Inc_vol_code	0.10	0.734	0.26	0.430	0.26	0.447	0.29	0.414
	Speed_code*Inc_vol_code	0.04	0.722	0.06	0.675	0.10	0.491	0.11	0.477
	Speed_code*Speed_code	-0.23	0.385	-0.17	0.573	-0.46	0.144	-0.39	0.229
	mg_tissue_code*Inc_vol_code	0.18	0.243	0.22	0.208	0.27	0.145	0.30	0.126
	mg_tissue_code*Speed_code	0.48	0.005	0.37	0.043	0.40	0.038	0.34	0.086
	mg_tissue_code*mg_tissue_code	-0.52	0.226	-0.62	0.196	-0.73	0.151	-0.86	0.107
	Temp_code*Inc_vol_code	-0.15	0.196	-0.18	0.179	-0.13	0.343	-0.13	0.361
	Temp_code*Speed_code	0.22	0.062	0.23	0.076	0.21	0.124	0.16	0.244
	Temp_code*mg_tissue_code	-0.09	0.555	-0.10	0.542	-0.02	0.888	-0.01	0.947
Test Tube	Inc_vol_code	-0.19	0.079	-0.18	0.031	-0.19	0.037	-0.25	0.006
	Speed_code	0.21	0.051	0.13	0.078	0.19	0.032	0.18	0.029
	mg_tissue_code	0.41	0.151	0.33	0.107	0.58	0.020	0.58	0.014
	Temp_code	-0.06	0.542	-0.04	0.596	0.10	0.225	0.06	0.451
	Inc_vol_code*Inc_vol_code	0.29	0.264	0.25	0.185	0.28	0.188	0.35	0.089
	Speed_code*Inc_vol_code	-0.03	0.762	-0.03	0.668	-0.05	0.584	-0.04	0.610
	Speed_code*Speed_code	0.79	0.002	0.63	0.001	0.56	0.005	0.51	0.007
	mg_tissue_code*Inc_vol_code	-0.04	0.783	-0.07	0.458	-0.19	0.087	-0.24	0.031
	mg_tissue_code*Speed_code	0.09	0.487	0.15	0.125	0.16	0.151	0.17	0.099
	mg_tissue_code*mg_tissue_code	-0.89	0.095	-0.81	0.038	-1.12	0.015	-0.95	0.025
	Temp_code*Inc_vol_code	-0.12	0.226	-0.07	0.312	-0.12	0.145	-0.15	0.070
	Temp_code*Speed_code	0.23	0.025	0.17	0.022	0.17	0.039	0.20	0.013
	Temp_code*mg_tissue_code	-0.07	0.572	-0.10	0.310	-0.13	0.219	-0.12	0.261

Note: Ltest_J = log testosterone concentration at time J
Beta = regression coefficient, as applied to coded factors
Pvalue = significance level for t-test of hypothesis that the true Beta = 0

Table 47. Response Surface Regression Results for hCG Stimulation with Baseline Concentration Removed

Vessel Type	Model Terms	L Test 1		L Test 2		L Test 3		L Test 4	
		Beta	P Value	Beta	P Value	Beta	P Value	Beta	P Value
Scintillation Vial	hCG_conc_code	-0.09	0.534	-0.17	0.333	-0.11	0.609	-0.07	0.734
	Speed_code	-0.09	0.545	-0.03	0.870	0.02	0.933	-0.04	0.869
	mg_tissue_code	0.80	0.007	0.87	0.013	1.05	0.016	1.18	0.008
	Temp_code	0.33	0.035	0.40	0.034	0.46	0.048	0.50	0.034
	hCG_conc_code*hCG_conc_code	-0.06	0.485	-0.07	0.509	-0.13	0.334	-0.13	0.372
	Speed_code*hCG_conc_code	0.20	0.266	0.23	0.281	0.19	0.473	0.24	0.363
	Speed_code*Speed_code	0.41	0.136	0.57	0.091	0.35	0.380	0.30	0.467
	mg_tissue_code*hCG_conc_code	-0.09	0.606	-0.25	0.228	-0.20	0.428	-0.27	0.293
	mg_tissue_code*Speed_code	0.70	0.001	0.71	0.003	0.78	0.006	0.82	0.005
	mg_tissue_code*mg_tissue_code	-0.71	0.098	-0.67	0.192	-0.89	0.163	-1.03	0.116
	Temp_code*hCG_conc_code	-0.28	0.043	-0.35	0.038	-0.41	0.048	-0.41	0.055
	Temp_code*Speed_code	-0.03	0.825	-0.04	0.806	-0.02	0.927	-0.10	0.656
	Temp_code*mg_tissue_code	-0.06	0.679	-0.14	0.457	-0.26	0.277	-0.23	0.327
	Inc_vol_code	-1.00	<.0001	-0.96	<.0001	-1.02	<.0001	-0.95	0.000
Test Tube	hCG_conc_code	-0.20	0.011	-0.19	0.028	-0.21	0.011	-0.23	0.055
	Speed_code	0.31	0.001	0.38	0.001	0.46	<.000		
	mg_tissue_code	-0.36	0.043	-0.34	0.083	-0.36	0.053	-0.47	0.093
	Temp_code	-0.24	0.004	-0.05	0.499	0.00	0.978	0.07	0.507
	hCG_conc_code*hCG_conc_code	0.11	0.074	0.09	0.159	0.08	0.194	0.09	0.300
	Speed_code*hCG_conc_code	0.13	0.117	0.16	0.088	0.12	0.176	0.12	0.372
	Speed_code*Speed_code	0.70	0.001	0.62	0.006	0.50	0.013	0.28	0.301
	mg_tissue_code*hCG_conc_code	0.27	0.017	0.30	0.018	0.37	0.003	0.40	0.023
	mg_tissue_code*Speed_code	0.07	0.509	0.09	0.413	0.12	0.248	0.12	0.463
	mg_tissue_code*mg_tissue_code	0.31	0.295	0.32	0.329	0.37	0.229	0.66	0.169
	Temp_code*hCG_conc_code	-0.12	0.096	-0.13	0.122	-0.11	0.156	-0.13	0.268
	Temp_code*Speed_code	0.33	0.001	0.42	0.000	0.48	<.0001	0.53	0.001
	Temp_code*mg_tissue_code	0.44	0.001	0.38	0.004	0.33	0.006	0.32	0.057
	Inc_vol_code	-0.41	0.000	-0.39	0.000	-0.41	0.000	-0.47	0.002

Note: Ltest_J = log testosterone concentration at time J
Beta = regression coefficient, as applied to coded factors
Pvalue = significance level for t-test of hypothesis that the true Beta = 0

Table 48. Maximum Values for Models with Baseline Concentration Removed

hCG Stimu-lation	Vessel Type		Predicted Log(Testosterone Concentration)								Incubation Media Volume	hCG Conc	Fragment Size	Temp	Shaker Speed Code	Shaker Speed
			T1		T2		T3		T4							
No	Scintillation vial	T1 opt	4.201	(0.347)	4.322	(0.391)	4.735	(0.407)	4.802	(0.425)	2.5		127.3	36.0	-0.069	low
No	Scintillation vial	T2 opt	4.151	(0.341)	4.302	(0.384)	4.726	(0.400)	4.805	(0.418)	2.5		132.2	35.8	-0.177	low
No	Scintillation vial	T3 opt	4.146	(0.341)	4.301	(0.385)	4.726	(0.400)	4.807	(0.418)	2.5		131.6	35.8	-0.188	low
No	Scintillation vial	T4 opt	4.135	(0.339)	4.298	(0.383)	4.724	(0.398)	4.809	(0.416)	2.5		133.4	35.7	-0.221	low
No	Test tube	T1 opt	2.959	(0.247)	3.164	(0.177)	3.570	(0.201)	3.567	(0.190)	4.4		155.1	35.6	0.996	high
No	Test tube	T2 opt	2.976	(0.248)	3.180	(0.178)	3.590	(0.201)	3.594	(0.190)	4.2		156.7	35.6	0.996	high
No	Test tube	T3 opt	3.062	(0.256)	3.254	(0.184)	3.696	(0.208)	3.720	(0.197)	3.9		161.5	35.8	1.000	high
No	Test tube	T4 opt	3.209	(0.274)	3.387	(0.196)	3.872	(0.222)	3.946	(0.210)	3.2		169.3	35.9	0.996	high
Yes	Scintillation vial	T1 opt	5.334	(0.284)	5.883	(0.345)	6.458	(0.426)	6.654	(0.433)	2.5	0.098	207.4	35.8	0.999	high
Yes	Scintillation vial	T2 opt	5.328	(0.291)	5.891	(0.353)	6.462	(0.436)	6.654	(0.444)	2.5	0.079	206.8	35.8	1.000	high
Yes	Scintillation vial	T3 opt	5.355	(0.288)	5.923	(0.350)	6.492	(0.432)	6.683	(0.439)	2.5	0.078	209.4	35.9	1.000	high
Yes	Scintillation vial	T4 opt	5.355	(0.283)	5.920	(0.343)	6.491	(0.423)	6.684	(0.431)	2.5	0.080	211.7	35.9	0.990	high
Yes	Test tube	T1 opt	3.105	(0.203)	3.662	(0.230)	3.967	(0.210)	3.971	(0.326)	2.5	0.091	132.7	35.6	0.997	high
Yes	Test tube	T2 opt	3.112	(0.221)	3.726	(0.251)	4.049	(0.229)	4.069	(0.355)	2.5	0.099	146.8	35.9	0.998	high

Table 48. Maximum Values for Models with Baseline Concentration Removed (Continued)

hCG Stimu-lation	Vessel Type		Predicted Log(Testosterone Concentration)				Incubation Media Volume	hCG Conc	Fragment Size	Temp	Shaker Speed Code	Shaker Speed
			T1	T2	T3	T4						
Yes	Test tube	T3 opt	3.119 (0.229)	3.756 (0.259)	4.090 (0.237)	4.121 (0.368)	2.5	0.092	151.2	36.0	0.994	high
Yes	Test tube	T4 opt	2.855 (0.293)	3.409 (0.332)	3.822 (0.303)	4.330 (0.471)	2.5	0.044	26.2	35.5	0.281	low

*Values in parentheses are standard errors

i.If the coded value for shaker speed was between -1 and -0.5 then the shaker speed was defined as “none”. If the coded value for shaker speed was between -0.5 and 0.5 then the shaker speed was defined as “low”. If the coded value for shaker speed was between 0.5 and 1 then the shaker speed was defined as “high”.

Table 49. Predicted Values for Specified Optimum Factor Combinations for Models with Baseline Concentration Removed

hCG Stimu-lation	Vessel Type	Time	Predicted Log(Testosterone Concentration)								Media Volume	hCG Conc	Fragment Size	Temp	Shaker Speed Code	Shaker Speed
			T1		T2		T3		T4							
No	Scintillation vial	Optimal	3.243	(0.228)	3.257	(0.258)	3.687	(0.268)	3.758	(0.280)	5.0		175.0	36.0	0.0	low
No	Scintillation vial	Optimal	3.137	(0.288)	3.012	(0.326)	3.082	(0.339)	3.150	(0.354)	5.0		175.0	36.0	1.0	high
Yes	Scintillation vial	Optimal	4.586	(0.261)	4.888	(0.316)	5.609	(0.390)	5.914	(0.397)	2.5	0.100	175.0	36.0	0.0	low
Yes	Scintillation vial	Optimal	5.178	(0.308)	5.700	(0.374)	6.283	(0.461)	6.468	(0.469)	2.5	0.100	175.0	36.0	1.0	high

*Values in parentheses are standard errors

i. If the coded value for shaker speed was between -1 and -0.5 then the shaker speed was defined as “none”. If the coded value for shaker speed was between -0.5 and 0.5 then the shaker speed was defined as “low”. If the coded value for shaker speed was between 0.5 and 1 then the shaker speed was defined as “high”.

10.0 TESTES PREPARATION EXPERIMENT

10.1 Statistical Analysis of Phase II Testis Preparation Optimization Experiment

10.1.1 Objectives

The objectives of the testis preparation optimization experiment were to determine the effect of various testis preparation methods and aliquot volumes on testosterone production using the sliced testis assay and to identify the factor settings that maximize the amount of testosterone production. These experiments tested the effect(s) of four factors: time delay, organ preparation technique, sample aliquot volume and hCG concentration. Table 50 presents the testis preparation factor settings used in this experiment. Each trial in the experiment was run with and without hCG stimulation and repeated measurements were taken at baseline (time 0) and at 1, 2, 3, and 4 hours after baseline.

10.1.2 Data

A SAS data set was constructed from the raw Excel data files and two fundamental types of dependent variables were used in the analyses: the original testosterone concentrations and the (natural) logarithm of the testosterone concentrations. Each observation includes dependent variable values for 4 time points and a corresponding baseline level. Each observation also includes data identifying the levels of the pertinent factors. The data used in the analysis are displayed in Tables 51 and 52.

10.1.2 Statistical Analysis Methods

In order to assess the effects of each testis preparation factor on the amount of testosterone production, analysis of variance (ANOVA) models were fit to the data separately for the trials with and without hCG stimulation. For each hCG stimulation type, the ANOVA models were fit to each individual time point. All main effects and two-factor interactions (2fi) of the four factors were initially included in the models. Tests for interactions were conducted and where they were not detected as statistically significant (p=0.10), a reduced model was employed that retained the main effects, the baseline covariate and only those 2fi's deemed to have significant effects. Once the final ANOVA model was determined, the data were fit using a response surface regression analysis to determine the maximum predicted value of testosterone and the incubation factor levels associated with the maximum. Additional details are provided in the Results section.

10.1.4 Results

To assess the effects of each testis preparation factor on testosterone production, an ANOVA model was fit separately to the set of data for each hCG stimulation type and each time

point beyond the baseline. The ANOVA models containing all main effects and 2fi's were fit to the original testosterone concentrations and the logarithms of testosterone concentrations using PROC GLM. The logarithm of the baseline testosterone concentration (T0) was also added to the model as a dependent variable. Upon examining the residuals from fitting the full ANOVA models, the residuals for the original testosterone concentrations showed signs of heteroskedasticity. The residuals for the logarithms of testosterone concentrations appear homoskedastic and randomly distributed around 0, thereby satisfying the assumptions necessary for valid ANOVA modeling results. The logarithms of the testosterone concentrations were therefore chosen as the more appropriate dependent variables for the analysis of the incubation experiment data.

For the full ANOVA models using the logarithm of testosterone concentration as the dependent variable, we examined the t-tests for the significance of each factor in the model. In the first round of model evaluation, we examined the t-test results for the 2fi's to see if any of those terms could be removed from the models. Any 2fi's with p-values greater than 0.1 were removed from the models. The reduced models, one model for the data without hCG stimulation and one model for the data with hCG stimulation, were fit to the logarithms of testosterone concentration again and the t-tests of the model terms were re-evaluated for significance. After 2 rounds of removing insignificant 2fi's, we arrived at the final reduced models containing only significant 2fi's and first order factors.

To obtain the optimal combination of factors for each time point, the data were fit using response surface regression models and the SAS procedure RSREG. (The PROC GLM modeling results were used to determine which factors and 2fi's needed to remain in the models. Any factors which did not have any associated 2fi's in the final models were treated as covariates in the response surface regression models.) The ANOVA results for the response surface regressions are summarized in Table 53. The parameter estimates from the response surface regression models for the data without hCG stimulation are presented in Table 54 and the parameter estimates for the data with hCG stimulation are presented in Table 55. Because the organ preparation technique is a categorical variable, we needed to run separate models for each organ preparation technique in PROC RSREG. Differentiation fo the prediction equation is used to determine the location of the stationary point; in most cases the stationary point was not a point of maximal response. Under these circumstances the RIDGE technique in SAS is used to find a point of maximal response within the experimental region. Starting at the center of the experimental region, a hypersphere having a given radius is considered and the point on the hypersphere with the maximal response is determined. The radius is then incremented and the maximum on that hypersphere is determined. This process, when repeated, traces out a "ridge of maximal response." The iterations are terminated when one of the factors reaches the extremity of its range, as defined by the levels used in the experiment. We determined the factor combination that produced the maximum predicted value of logarithm of testosterone concentration for each time point at each combination of with/without hCG stimulation and for

each organ preparation technique. The optimal value factor combinations were plugged back into the response surface regression models to obtain predicted values for the optimal factor combinations at all time points. For example, we used the RIDGE regression results to determine the optimal factor combinations for the first time point (T1) for the scintillation vial without hCG stimulation. We then used the optimal T1 combination to obtain the predicted values for logarithm of testosterone concentration and their standard errors for the scintillation vial without hCG stimulation for that time point and for all the other time points (T2, T3 and T4). The optimal factor results, presented in Table 55, show the effects of the optimal factors for one time point on the other time points in evaluating the best combination of factors to use for further experimentation.

After examining the possible optimal factors for each time point, organ preparation technique and hCG stimulation, the chosen optimal values and their predicted values and standard errors are presented in Table 56.

In order to assess the effects of the baseline testosterone concentrations on the effects of factors, the same analysis steps that were performed above were also repeated on the logarithms of testosterone concentration but with the baseline concentration removed from the models. The ANOVA results for the final reduced model from PROC RSREG are shown in Table 57.

Comparing the results in Table 57 with those in Table 53 (results for models with baseline concentration in the models) indicates that there are many more significant terms for the models without the baseline concentrations.[3] The root mean square errors (RMSEs) in Table 57 are higher than Table 53 for the cases with no hCG stimulation, especially for the cases of warm buffered saline, while the RMSEs for the cases with hCG stimulation vary as to whether the higher values are in Table 53 or Table 57.[4] The response surface regression results from PROC RSREG are presented in Tables 58 and 59. The optimal values for each time point are shown in Table 60. The factor combinations that produced the optimal values for each time point in Table 60 are more stable within the organ preparation techniques than those in Table 55. The standard errors of the predicted values for Table 60 are similar to those in Table 55, except for the cases involving warm buffered saline. For the warm buffered saline cases, the data without hCG stimulation has higher standard errors in Table 57 whereas the data with hCG stimulation has much higher standard errors in Table 55. The predicted values for the chosen optimal combination of factors are presented in Table 61.

[3] This suggests that the baseline levels are affected by (some of) the factors and that their resultant effect on later times is diminished if adjustment for baseline level are made.

[4] This indicates that baseline adjustments for the latter cases were unnecessary, as was also apparent from Table 4 (since baseline effects was not significant).

Table 50. Testis Preparation Factor Settings and Coded Values

Factor Identification	Units	Variable Name	Factor	Experimental Levels			Coded Experimental Levels		
				1	2	3	1	2	3
hCG Concentration	IU/ml	hCG_Conc	X5	0.01	0.1*	1	-1	0	+1
Time Delay	hr	Time_Delay	X7	0.5	1*	2	-1	0	+1
Organ Preparation Technique	NA[a]	Organ_Prep_Tech	X8	Cold buffered saline	Warm buffered saline	Cold media*	-1	0	+1
Sample Aliquot Volume	ml	Sample_vol	X9	0.125	0.25	0.5*	-1	0	+1

*Prototypical value.
[a] NA - not applicable.

Table 51. Data Listing for Incubation Experiment

SET	Organ Prep Technique	hCG Conc	Time Delay	Sample Vol	Weight	Test 0	Test 1	Test 2	Test 3	Test 4
P	Warm Buffered Saline	0.01	0.5	0.125	0.2270	3.35	15.02	14.10	21.23	31.63
PC	Warm Buffered Saline	0.01	0.5	0.125	0.2292	4.01	16.58	36.13	62.83	83.64
Q	Warm Buffered Saline	0.01	0.5	0.500	0.2311	7.53	18.69	18.26	26.66	30.98
QC	Warm Buffered Saline	0.01	0.5	0.500	0.2308	4.81	34.45	121.92	161.61	31.63
AA	Warm Buffered Saline	0.10	0.5	0.250	0.2275	3.69	16.75	22.68	25.36	32.66
AAC	Warm Buffered Saline	0.10	0.5	0.250	0.2255	5.72	20.80	84.12	133.04	166.03
T	Warm Buffered Saline	1.00	0.5	0.125	0.2285	3.28	16.76	24.07	21.23	25.73
TC	Warm Buffered Saline	1.00	0.5	0.125	0.2262	3.05	19.01	29.09	48.10	72.06
U	Warm Buffered Saline	1.00	0.5	0.500	0.2375	1.89	15.20	21.09	24.04	30.57
UC	Warm Buffered Saline	1.00	0.5	0.500	0.2315	4.41	33.05	84.02	123.97	31.63
X	Warm Buffered Saline	0.10	1.0	0.250	0.2311	14.54	55.56	72.70	80.10	89.05
XC	Warm Buffered Saline	0.10	1.0	0.250	0.2294	18.00	92.94			
X*	Warm Buffered Saline	0.10	1.0	0.250	0.2269	20.45	54.96	65.93	73.29	76.51
XC*	Warm Buffered Saline	0.10	1.0	0.250	0.2489	14.42	72.16	141.90	21.23	31.63
CC	Warm Buffered Saline	0.10	1.0	0.125	0.2484	17.31	49.32	67.95	74.40	86.84
CCC	Warm Buffered Saline	0.10	1.0	0.125	0.2262	15.78	81.74	130.11	169.14	190.63
DD	Warm Buffered Saline	0.10	1.0	0.500	0.2302	21.33	63.99	89.88	93.53	100.65
DDC	Warm Buffered Saline	0.10	1.0	0.500	0.2387	21.37	68.50	103.64	124.01	137.91
DD*	Warm Buffered Saline	0.10	1.0	0.500	0.2588	16.38	59.12	74.46	86.32	96.10
DDC*	Warm Buffered Saline	0.10	1.0	0.500	0.2313	19.71	82.45	130.31	172.55	212.02
Z	Warm Buffered Saline	1.00	1.0	0.250	0.2519	2.18	11.47	16.12	28.62	19.02
ZC	Warm Buffered Saline	1.00	1.0	0.250	0.2389	4.48	15.32	33.74	53.24	62.58
Z*	Warm Buffered Saline	1.00	1.0	0.250	0.2385	6.46	21.72	25.49	19.96	36.77
ZC*	Warm Buffered Saline	1.00	1.0	0.250	0.2602	3.54	13.72	35.59	46.85	54.88
R	Warm Buffered Saline	0.01	2.0	0.125	0.2254		12.64	14.15	16.64	12.20
RC	Warm Buffered Saline	0.01	2.0	0.125	0.2262		7.82	7.91	11.72	7.74
S	Warm Buffered Saline	0.01	2.0	0.500	0.2263		22.14	26.78	30.67	7.07
SC	Warm Buffered Saline	0.01	2.0	0.500	0.2265		21.24	38.54	52.32	42.83
BB	Warm Buffered Saline	0.10	2.0	0.250	0.2258	2.21	11.56	18.95	18.87	33.22
BBC	Warm Buffered Saline	0.10	2.0	0.250	0.2269	2.56	45.84	21.82	77.39	95.28
BB*	Warm Buffered Saline	0.10	2.0	0.250	0.2257	2.57	17.63	21.75	54.76	57.60
BBC*	Warm Buffered Saline	0.10	2.0	0.250	0.2251	2.71	24.34	44.96	71.04	68.01
V	Warm Buffered Saline	1.00	2.0	0.125	0.2262	3.01	10.52	15.78	15.92	18.52
VC	Warm Buffered Saline	1.00	2.0	0.125	0.2262	2.08	8.97	15.12	25.95	22.41
W	Warm Buffered Saline	1.00	2.0	0.500	0.2269	3.75	16.22	15.03	18.38	19.00
WC	Warm Buffered Saline	1.00	2.0	0.500	0.2251	2.13	17.95	33.14	49.31	60.46

Table 51. Data Listing for Incubation Experiment (Continued)

SET	Organ Prep Technique	hCG Conc	Time Delay	Sample Vol	Weight	Test 0	Test 1	Test 2	Test 3	Test 4
A	Cold Buffered Saline	0.01	0.5	0.125	0.2284	2.41	40.67	59.81	62.04	68.96
AC	Cold Buffered Saline	0.01	0.5	0.125	0.2272	2.07	50.57	102.55	135.52	173.20
B	Cold Buffered Saline	0.01	0.5	0.500	0.2283		51.82	78.32	90.93	98.82
BC	Cold Buffered Saline	0.01	0.5	0.500	0.2263	15.73	70.35	161.33	246.00	307.07
L	Cold Buffered Saline	0.10	0.5	0.250	0.2280	11.58	75.92	126.84	163.90	189.87
LC	Cold Buffered Saline	0.10	0.5	0.250	0.2369	4.69	53.19	124.61	183.50	190.92
L*	Cold Buffered Saline	0.10	0.5	0.250	0.2335	7.02	59.87	67.45	78.07	89.46
LC*	Cold Buffered Saline	0.10	0.5	0.250	0.2310	3.38	43.07	102.99	142.42	134.94
E	Cold Buffered Saline	1.00	0.5	0.125	0.2268	4.41	42.86	62.70	76.85	91.36
EC	Cold Buffered Saline	1.00	0.5	0.125	0.2268	3.26	63.05	153.13		
F	Cold Buffered Saline	1.00	0.5	0.500	0.2268	4.54	32.10	30.64	36.68	45.06
FC	Cold Buffered Saline	1.00	0.5	0.500	0.2293	5.49	92.63	180.20	302.97	365.94
J	Cold Buffered Saline	0.01	1.0	0.250	0.2553	2.23	14.10	19.94	22.13	24.09
JC	Cold Buffered Saline	0.01	1.0	0.250	0.2400	1.25	13.04	27.50	33.38	39.83
J*	Cold Buffered Saline	0.01	1.0	0.250	0.2605	1.31	19.27	27.91	32.48	34.36
JC*	Cold Buffered Saline	0.01	1.0	0.250	0.2305	1.43	24.60	78.92	125.12	183.51
N	Cold Buffered Saline	0.10	1.0	0.125	0.2277	1.41	14.45	19.85	22.97	23.50
NC	Cold Buffered Saline	0.10	1.0	0.125	0.2357	1.78	15.91		37.89	47.77
N*	Cold Buffered Saline	0.10	1.0	0.125	0.2348	1.92	14.99	16.87	21.76	26.06
NC*	Cold Buffered Saline	0.10	1.0	0.125	0.2605	1.88	19.00	39.50	54.74	65.57
O	Cold Buffered Saline	0.10	1.0	0.500	0.2457	5.90	26.41	30.20	40.37	40.09
OC	Cold Buffered Saline	0.10	1.0	0.500	0.2461	4.51	34.54	76.55	100.16	128.81
K	Cold Buffered Saline	1.00	1.0	0.250	0.2432	4.61	24.59	32.36	32.48	34.91
KC	Cold Buffered Saline	1.00	1.0	0.250	0.2551	6.23	31.75	61.11	95.61	117.72
C	Cold Buffered Saline	0.01	2.0	0.125	0.2334	12.04	59.68	76.22	70.52	92.46
CC	Cold Buffered Saline	0.01	2.0	0.125	0.2303	11.59	59.75	81.37	119.97	149.41
D	Cold Buffered Saline	0.01	2.0	0.500	0.2391	17.69	57.88	69.30	78.59	91.47
DC	Cold Buffered Saline	0.01	2.0	0.500	0.2417	10.76	58.50	88.99	119.57	107.65
I	Cold Buffered Saline	0.10	1.0	0.250	0.2685	1.27	13.52	17.58	20.41	22.16
IC	Cold Buffered Saline	0.10	1.0	0.250	0.2313	1.95	25.25	66.36	96.89	104.37
I*	Cold Buffered Saline	0.10	1.0	0.250	0.2354	3.14	13.85	11.55	20.65	25.45
IC*	Cold Buffered Saline	0.10	1.0	0.250	0.2265	1.72	14.17	33.77	47.90	71.04
M	Cold Buffered Saline	0.10	2.0	0.250	0.2507	13.48	78.30	94.58	96.41	99.88
MC	Cold Buffered Saline	0.10	2.0	0.250	0.2573	8.05	61.52	126.00	129.11	119.20
G	Cold Buffered Saline	1.00	2.0	0.125	0.2555	7.20	60.31	54.68	69.59	48.77
GC	Cold Buffered Saline	1.00	2.0	0.125	0.2594	8.02	67.27	112.80	140.52	151.50
H	Cold Buffered Saline	1.00	2.0	0.500	0.2472	8.58	74.23	94.58	110.32	112.10
HC	Cold Buffered Saline	1.00	2.0	0.500	0.2585	12.42	69.05	100.97	136.25	149.67
EE	Cold Media	0.01	0.5	0.125	0.2247	3.56	22.47	32.80	41.57	43.04
EEC	Cold Media	0.01	0.5	0.125	0.2353	2.72	44.16	73.18	145.13	176.80
FF	Cold Media	0.01	0.5	0.500	0.2311	4.89	58.24	53.05	46.04	59.89
FFC	Cold Media	0.01	0.5	0.500	0.2258	6.91	31.31	19.93	17.98	67.01
PP	Cold Media	0.10	0.5	0.250	0.2264	3.67	29.99	41.21	48.32	52.87
PPC	Cold Media	0.10	0.5	0.250	0.2406	2.91	45.39	105.94	24.02	180.22
PP*	Cold Media	0.10	0.5	0.250	0.2254	4.84	30.43	31.77	43.26	51.77
PPC*	Cold Media	0.10	0.5	0.250	0.2327	6.10	56.51	105.50	110.10	232.14
II	Cold Media	1.00	0.5	0.125	0.2289	5.37	27.13	118.92	48.27	54.74
IIC	Cold Media	1.00	0.5	0.125	0.2321	3.58	40.07	79.15	111.68	35.55
JJ	Cold Media	1.00	0.5	0.500	0.2301	4.48	37.29	49.76	54.89	67.88
JJC	Cold Media	1.00	0.5	0.500	0.2315	3.67	43.97	83.71	126.00	166.95
NN	Cold Media	0.01	1.0	0.250	0.2428	4.32	32.62	41.80	49.30	52.47

Table 51. Data Listing for Incubation Experiment (Continued)

SET	Organ Prep Technique	hCG Conc	Time Delay	Sample Vol	Weight	Test 0	Test 1	Test 2	Test 3	Test 4
NNC	Cold Media	0.01	1.0	0.250	0.2415	7.54	54.99	88.65	117.52	223.81
NN*	Cold Media	0.01	1.0	0.250	0.2272	3.79	47.80	53.13	58.10	62.76
NNC*	Cold Media	0.01	1.0	0.250	0.2554	7.20	46.44	107.44	120.83	155.01
MM	Cold Media	0.10	1.0	0.250	0.2396	9.22	44.45	52.13	61.56	60.60
MMC	Cold Media	0.10	1.0	0.250	0.2466	2.96	58.48	115.98	154.06	238.12
MM*	Cold Media	0.10	1.0	0.250	0.2271	4.89	39.10	54.65	50.07	57.64
MMC*	Cold Media	0.10	1.0	0.250	0.2506	6.58	64.37	107.42	248.92	280.29
RR	Cold Media	0.10	1.0	0.125	0.2599	8.31	43.13	87.69	40.44	48.71
RRC	Cold Media	0.10	1.0	0.125	0.2382	6.76	57.98			
RR*	Cold Media	0.10	1.0	0.125	0.2397	4.84	22.40		40.13	39.88
RRC*	Cold Media	0.10	1.0	0.125	0.2247	3.47	50.96			
SS	Cold Media	0.10	1.0	0.500	0.2347	6.43	34.04	40.90	43.42	49.34
SSC	Cold Media	0.10	1.0	0.500	0.2404	5.57	50.46	105.91	223.00	244.55
OO	Cold Media	1.00	1.0	0.250	0.2470	4.29	48.58	54.94	62.47	63.36
OOC	Cold Media	1.00	1.0	0.250	0.2550	12.63	53.96	123.92	210.00	226.71
KK	Cold Media	1.00	2.0	0.125	0.2386	2.05	11.61	15.17	17.64	20.41
KKC	Cold Media	1.00	2.0	0.125	0.2493		16.69	34.86	43.00	31.33
LL	Cold Media	1.00	2.0	0.500	0.2311	2.64	14.63	17.27	21.12	21.29
LLC	Cold Media	1.00	2.0	0.500	0.2459	3.09	20.46	41.32	96.38	101.38
GG	Cold Media	0.01	2.0	0.125	0.2488	3.26	16.60	23.71	27.17	20.86
GGC	Cold Media	0.01	2.0	0.125	0.2298	2.31	19.41	28.37	43.43	49.56
HH	Cold Media	0.01	2.0	0.500	0.2294	3.62	15.87	19.62	26.07	30.25
HHC	Cold Media	0.01	2.0	0.500	0.2527	2.06	24.93	67.12	135.46	195.84
QQ	Cold Media	0.10	2.0	0.250	0.2252	2.71	16.96	19.94	23.22	32.06
QQC	Cold Media	0.10	2.0	0.250	0.2247	1.78	27.99	80.02	102.67	22.16

Table 52. Data Listing of Coded Factor Values and Logarithm of Testosterone Concentrations

SET	Organ Prep Technique	hCG Stimulation	hCG Conc Code	Time Delay Code	Sample Vol Code	L Test 0	L Test 1	L Test 2	L Test 3	L Test 4
P	Warm Buffered Saline	0	-1	-1	-1	1.21	2.71	2.65	3.06	3.45
PC	Warm Buffered Saline	1	-1	-1	-1	1.39	2.81	3.59	4.14	4.43
Q	Warm Buffered Saline	0	-1	-1	1	2.02	2.93	2.90	3.28	3.43
QC	Warm Buffered Saline	1	-1	-1	1	1.57	3.54	4.80	5.09	3.45
AA	Warm Buffered Saline	0	0	-1	0	1.31	2.82	3.12	3.23	3.49
AAC	Warm Buffered Saline	1	0	-1	0	1.74	3.03	4.43	4.89	5.11
T	Warm Buffered Saline	0	1	-1	-1	1.19	2.82	3.18	3.06	3.25
TC	Warm Buffered Saline	1	1	-1	-1	1.12	2.94	3.37	3.87	4.28
U	Warm Buffered Saline	0	1	-1	1	0.64	2.72	3.05	3.18	3.42
UC	Warm Buffered Saline	1	1	-1	1	1.48	3.50	4.43	4.82	3.45
X	Warm Buffered Saline	0	0	0	0	2.68	4.02	4.29	4.38	4.49
XC	Warm Buffered Saline	1	0	0	0	2.89	4.53			
X*	Warm Buffered Saline	0	0	0	0	3.02	4.01	4.19	4.29	4.34
XC*	Warm Buffered Saline	1	0	0	0	2.67	4.28	4.96	3.06	3.45
CC	Warm Buffered Saline	0	0	0	-1	2.85	3.90	4.22	4.31	4.46
CCC	Warm Buffered Saline	1	0	0	-1	2.76	4.40	4.87	5.13	5.25
DD	Warm Buffered Saline	0	0	0	1	3.06	4.16	4.50	4.54	4.61
DDC	Warm Buffered Saline	1	0	0	1	3.06	4.23	4.64	4.82	4.93
DD*	Warm Buffered Saline	0	0	0	1	2.80	4.08	4.31	4.46	4.57
DDC*	Warm Buffered Saline	1	0	0	1	2.98	4.41	4.87	5.15	5.36
Z	Warm Buffered Saline	0	1	0	0	0.78	2.44	2.78	3.35	2.95
ZC	Warm Buffered Saline	1	1	0	0	1.50	2.73	3.52	3.97	4.14
Z*	Warm Buffered Saline	0	1	0	0	1.87	3.08	3.24	2.99	3.60
ZC*	Warm Buffered Saline	1	1	0	0	1.26	2.62	3.57	3.85	4.01
R	Warm Buffered Saline	0	-1	1	-1		2.54	2.65	2.81	2.50

Table 52. Data Listing of Coded Factor Values and Logarithm of Testosterone Concentrations (Continued)

SET	Organ Prep Technique	hCG Stimulation	hCG Conc Code	Time Delay Code	Sample Vol Code	L Test 0	L Test 1	L Test 2	L Test 3	L Test 4
RC	Warm Buffered Saline	1	-1	1	-1		2.06	2.07	2.46	2.05
S	Warm Buffered Saline	0	-1	1	1		3.10	3.29	3.42	1.96
SC	Warm Buffered Saline	1	-1	1	1		3.06	3.65	3.96	3.76
BB	Warm Buffered Saline	0	0	1	0	0.79	2.45	2.94	2.94	3.50
BBC	Warm Buffered Saline	1	0	1	0	0.94	3.83	3.08	4.35	4.56
BB*	Warm Buffered Saline	0	0	1	0	0.94	2.87	3.08	4.00	4.05
BBC*	Warm Buffered Saline	1	0	1	0	1.00	3.19	3.81	4.26	4.22
V	Warm Buffered Saline	0	1	1	-1	1.10	2.35	2.76	2.77	2.92
VC	Warm Buffered Saline	1	1	1	-1	0.73	2.19	2.72	3.26	3.11
W	Warm Buffered Saline	0	1	1	1	1.32	2.79	2.71	2.91	2.94
WC	Warm Buffered Saline	1	1	1	1	0.76	2.89	3.50	3.90	4.10
A	Cold Buffered Saline	0	-1	-1	-1	0.88	3.71	4.09	4.13	4.23
AC	Cold Buffered Saline	1	-1	-1	-1	0.73	3.92	4.63	4.91	5.15
B	Cold Buffered Saline	0	-1	-1	1		3.95	4.36	4.51	4.59
BC	Cold Buffered Saline	1	-1	-1	1	2.76	4.25	5.08	5.51	5.73
L	Cold Buffered Saline	0	0	-1	0	2.45	4.33	4.84	5.10	5.25
LC	Cold Buffered Saline	1	0	-1	0	1.54	3.97	4.83	5.21	5.25
L*	Cold Buffered Saline	0	0	-1	0	1.95	4.09	4.21	4.36	4.49
LC*	Cold Buffered Saline	1	0	-1	0	1.22	3.76	4.63	4.96	4.90
E	Cold Buffered Saline	0	1	-1	-1	1.48	3.76	4.14	4.34	4.51
EC	Cold Buffered Saline	1	1	-1	-1	1.18	4.14	5.03		
F	Cold Buffered Saline	0	1	-1	1	1.51	3.47	3.42	3.60	3.81
FC	Cold Buffered Saline	1	1	-1	1	1.70	4.53	5.19	5.71	5.90
J	Cold Buffered Saline	0	-1	0	0	0.80	2.65	2.99	3.10	3.18
JC	Cold Buffered Saline	1	-1	0	0	0.22	2.57	3.31	3.51	3.68
J*	Cold Buffered Saline	0	-1	0	0	0.27	2.96	3.33	3.48	3.54
JC*	Cold Buffered Saline	1	-1	0	0	0.36	3.20	4.37	4.83	5.21
N	Cold Buffered Saline	0	0	0	-1	0.34	2.67	2.99	3.13	3.16
NC	Cold Buffered Saline	1	0	0	-1	0.58	2.77		3.63	3.87
N*	Cold Buffered Saline	0	0	0	-1	0.65	2.71	2.83	3.08	3.26
NC*	Cold Buffered Saline	1	0	0	-1	0.63	2.94	3.68	4.00	4.18
O	Cold Buffered Saline	0	0	0	1	1.78	3.27	3.41	3.70	3.69
OC	Cold Buffered Saline	1	0	0	1	1.51	3.54	4.34	4.61	4.86
K	Cold Buffered Saline	0	1	0	0	1.53	3.20	3.48	3.48	3.55
KC	Cold Buffered Saline	1	1	0	0	1.83	3.46	4.11	4.56	4.77
C	Cold Buffered Saline	0	-1	1	-1	2.49	4.09	4.33	4.26	4.53
CC	Cold Buffered Saline	1	-1	1	-1	2.45	4.09	4.40	4.79	5.01
D	Cold Buffered Saline	0	-1	1	1	2.87	4.06	4.24	4.36	4.52
DC	Cold Buffered Saline	1	-1	1	1	2.38	4.07	4.49	4.78	4.68
I	Cold Buffered Saline	0	0	0	0	0.24	2.60	2.87	3.02	3.10
IC	Cold Buffered Saline	1	0	0	0	0.67	3.23	4.20	4.57	4.65
I*	Cold Buffered Saline	0	0	0	0	1.15	2.63	2.45	3.03	3.24
IC*	Cold Buffered Saline	1	0	0	0	0.54	2.65	3.52	3.87	4.26

Table 52. Data Listing of Coded Factor Values and Logarithm of Testosterone Concentrations (Continued)

SET	Organ Prep Technique	hCG Stimulation	hCG Conc Code	Time Delay Code	Sample Vol Code	L Test 0	L Test 1	L Test 2	L Test 3	L Test 4
M	Cold Buffered Saline	0	0	1	0	2.60	4.36	4.55	4.57	4.60
MC	Cold Buffered Saline	1	0	1	0	2.09	4.12	4.84	4.86	4.78
G	Cold Buffered Saline	0	1	1	-1	1.97	4.10	4.00	4.24	3.89
GC	Cold Buffered Saline	1	1	1	-1	2.08	4.21	4.73	4.95	5.02
H	Cold Buffered Saline	0	1	1	1	2.15	4.31	4.55	4.70	4.72
HC	Cold Buffered Saline	1	1	1	1	2.52	4.23	4.61	4.91	5.01
EE	Cold Media	0	-1	-1	-1	1.27	3.11	3.49	3.73	3.76
EEC	Cold Media	1	-1	-1	-1	1.00	3.79	4.29	4.98	5.17
FF	Cold Media	0	-1	-1	1	1.59	4.06	3.97	3.83	4.09
FFC	Cold Media	1	-1	-1	1	1.93	3.44	2.99	2.89	4.20
PP	Cold Media	0	0	-1	0	1.30	3.40	3.72	3.88	3.97
PPC	Cold Media	1	0	-1	0	1.07	3.82	4.66	3.18	5.19
PP*	Cold Media	0	0	-1	0	1.58	3.42	3.46	3.77	3.95
PPC*	Cold Media	1	0	-1	0	1.81	4.03	4.66	4.70	5.45
II	Cold Media	0	1	-1	-1	1.68	3.30	4.78	3.88	4.00
IIC	Cold Media	1	1	-1	-1	1.27	3.69	4.37	4.72	3.57
JJ	Cold Media	0	1	-1	1	1.50	3.62	3.91	4.01	4.22
JJC	Cold Media	1	1	-1	1	1.30	3.78	4.43	4.84	5.12
NN	Cold Media	0	-1	0	0	1.46	3.48	3.73	3.90	3.96
NNC	Cold Media	1	-1	0	0	2.02	4.01	4.48	4.77	5.41
NN*	Cold Media	0	-1	0	0	1.33	3.87	3.97	4.06	4.14
NNC*	Cold Media	1	-1	0	0	1.97	3.84	4.68	4.79	5.04
MM	Cold Media	0	0	0	0	2.22	3.79	3.95	4.12	4.10
MMC	Cold Media	1	0	0	0	1.09	4.07	4.75	5.04	5.47
MM*	Cold Media	0	0	0	0	1.59	3.67	4.00	3.91	4.05
MMC*	Cold Media	1	0	0	0	1.88	4.16	4.68	5.52	5.64
RR	Cold Media	0	0	0	-1	2.12	3.76	4.47	3.70	3.89
RRC	Cold Media	1	0	0	-1	1.91	4.06			
RR*	Cold Media	0	0	0	-1	1.58	3.11		3.69	3.69
RRC*	Cold Media	1	0	0	-1	1.24	3.93			
SS	Cold Media	0	0	0	1	1.86	3.53	3.71	3.77	3.90
SSC	Cold Media	1	0	0	1	1.72	3.92	4.66	5.41	5.50
OO	Cold Media	0	1	0	0	1.46	3.88	4.01	4.13	4.15
OOC	Cold Media	1	1	0	0	2.54	3.99	4.82	5.35	5.42
KK	Cold Media	0	1	1	-1	0.72	2.45	2.72	2.87	3.02
KKC	Cold Media	1	1	1	-1		2.81	3.55	3.76	3.44
LL	Cold Media	0	1	1	1	0.97	2.68	2.85	3.05	3.06
LLC	Cold Media	1	1	1	1	1.13	3.02	3.72	4.57	4.62
GG	Cold Media	0	-1	1	-1	1.18	2.81	3.17	3.30	3.04
GGC	Cold Media	1	-1	1	-1	0.84	2.97	3.35	3.77	3.90
HH	Cold Media	0	-1	1	1	1.29	2.76	2.98	3.26	3.41
HHC	Cold Media	1	-1	1	1	0.72	3.22	4.21	4.91	5.28
QQ	Cold Media	0	0	1	0	1.00	2.83	2.99	3.15	3.47

Table 52. Data Listing of Coded Factor Values and Logarithm of Testosterone Concentrations (Continued)

SET	Organ Prep Technique	hCG Stimulation	hCG Conc Code	Time Delay Code	Sample Vol Code	L Test 0	L Test 1	L Test 2	L Test 3	L Test 4
QQC	Cold Media	1	0	1	0	0.58	3.33	4.38	4.63	3.10

Table 53. ANOVA results for PROC RSREG

hCG Stimulation	Organ Prep.	Error df	Time	R^2	hCG Conc.	Time Delay	Aliquot Volume	Baseline	RMSE
no	Cold Buffered Saline	11	T1	93	NA	***		**	0.220
			T2	85	NA	**			0.342
			T3	87	NA	**		**	0.293
			T4	90	NA	***		**	0.262
	Cold Media	11	T1	89	NA	***	*		0.197
			T2	79	NA	*			0.318
			T3	89	NA	***			0.156
			T4	92	NA	***	*		0.140
	Warm Buffered Saline	9	T1	92	NA			***	0.238
			T2	83	NA			***	0.355
			T3	69	NA			*	0.468
			T4	71	NA			**	0.423
yes	Cold Buffered Saline	6	T1	91		*			0.285
			T2	77					0.404
			T3	76					0.443
			T4	71					0.473
	Cold Media	5	T1	97		***			0.119
			T2	94	*	**	**		0.227
			T3	74					0.649
			T4	81					0.589
	Warm Buffered Saline	4	T1	91					0.412
			T2	87					0.499
			T3	89			*	**	0.407
			T4	90		*	*	**	0.414

*Statistically significant F test at 0.10 level of significance.
**Statistically significant F test at 0.05 level of significance.
***Statistically significant F test at 0.01 level of significance.
Note: R^2 is the percentage of variation accounted for by the model.
RMSE = square root of the residual (error) mean square
NA = not applicable

Table 54. Response Surface Regression Results for No hCG Stimulation

Organ Prep Tech	Model Terms	L Test_1		L Test 2		L Test 3		L Test 4	
		Beta	P Value	Beta	P Value	Beta	P Value	Beta	P Value
Cold Buffered Saline	Time_Delay_Code	0.05	0.536	0.02	0.890	-0.04	0.740	-0.12	0.252
	Sample_vol_code	-0.04	0.625	-0.11	0.422	-0.08	0.471	-0.08	0.428
	Time_Delay_Code*Time_Delay_Code	0.83	0.001	0.84	0.016	0.70	0.018	0.63	0.018
	Sample_vol_code*Time_Delay_Code	0.07	0.434	0.21	0.152	0.20	0.105	0.23	0.047
	Sample_vol_code*Sample_vol_code	-0.12	0.343	-0.19	0.317	-0.16	0.334	-0.16	0.278
	LTest_0	0.33	0.022	0.34	0.107	0.38	0.043	0.43	0.014
Cold Media	Time_Delay_Code	-0.33	0.002	-0.37	0.013	-0.32	0.000	-0.36	<.0001
	Sample_vol_code	0.12	0.090	-0.13	0.215	0.04	0.423	0.09	0.058
	Time_Delay_Code*Time_Delay_Code	-0.46	0.012	-0.39	0.142	-0.34	0.018	-0.30	0.019
	Sample_vol_code*Time_Delay_Code	-0.14	0.065	0.03	0.811	-0.01	0.791	-0.02	0.694
	Sample_vol_code*Sample_vol_code	-0.08	0.504	0.13	0.501	-0.13	0.164	-0.15	0.079
	LTest_0	0.26	0.237	0.49	0.167	0.12	0.462	0.13	0.392
Warm Buffered Saline	Time_Delay_Code	-0.02	0.784	0.01	0.966	0.04	0.828	0.01	0.943
	Sample_vol_code	0.08	0.347	0.01	0.918	0.08	0.660	0.02	0.874
	Time_Delay_Code*Time_Delay_Code	-0.12	0.547	-0.26	0.413	-0.21	0.608	0.06	0.866
	Sample_vol_code*Time_Delay_Code	0.08	0.427	-0.02	0.893	-0.01	0.976	-0.01	0.962
	Sample_vol_code*Sample_vol_code	-0.03	0.817	-0.05	0.814	-0.13	0.657	-0.22	0.398
	LTest_0	0.64	0.000	0.58	0.008	0.50	0.052	0.60	0.015

Note: Ltest_J = log testosterone concentration at time J
Beta = regression coefficient, as applied to coded factors
Pvalue = significance level for t-test of hypothesis that the true Beta = 0

Table 55. Response Surface Regression Results for hCG Stimulation

Organ Prep Tech	Model Terms	L Test 1		L Test 2		L Test 3		L Test 4	
		Beta	P Value	Beta	P Value	Beta	P Value	Beta	P Value
Cold Buffered Saline	Time_Delay_Code	-0.07	0.602	-0.14	0.473	-0.25	0.249	-0.28	0.234
	Sample_vol_code	0.05	0.695	0.14	0.485	0.12	0.574	0.13	0.571
	hCG_conc_code	0.11	0.343	0.06	0.702	0.09	0.624	0.04	0.828
	Time_Delay_Code*Time_Delay_Code	0.69	0.028	0.67	0.098	0.54	0.201	0.32	0.456
	Sample_vol_code*Time_Delay_Code	-0.04	0.797	-0.12	0.569	-0.12	0.596	-0.20	0.417
	Sample_vol_code*Sample_vol_code	0.12	0.499	0.00	0.994	0.01	0.979	0.06	0.853
	hCG_conc_code*Time_Delay_Code	-0.03	0.840	0.05	0.774	-0.01	0.959	0.05	0.811
	hCG_conc_code*Sample_vol_code	0.05	0.706	0.02	0.926	0.03	0.880	0.12	0.585
	hCG_conc_code*hCG_conc_code	0.06	0.722	-0.02	0.940	0.08	0.778	0.14	0.622
	LTest_0	0.23	0.289	0.10	0.720	0.17	0.590	0.17	0.607
Cold Media	Time_Delay_Code	-0.33	0.001	-0.30	0.029	0.04	0.890	-0.47	0.124
	Sample_vol_code	0.04	0.467	0.17	0.157	0.09	0.770	0.66	0.055
	hCG_conc_code	-0.03	0.569	0.01	0.898	0.05	0.863	-0.48	0.102
	Time_Delay_Code*Time_Delay_Code	-0.42	0.013	-0.45	0.085	-0.76	0.256	-0.48	0.415
	Sample_vol_code*Time_Delay_Code	0.13	0.088	0.46	0.011	0.69	0.087	0.70	0.064
	Sample_vol_code*Sample_vol_code	-0.16	0.133	-0.50	0.034	0.25	0.632	-0.18	0.710
	hCG_conc_code*Time_Delay_Code	-0.11	0.119	-0.40	0.018	-0.46	0.219	-0.51	0.147
	hCG_conc_code*Sample_vol_code	0.09	0.205	0.26	0.070	0.43	0.237	0.73	0.054
	hCG_conc_code*hCG_conc_code	-0.17	0.134	-0.22	0.280	-0.36	0.512	-0.38	0.453
	LTest_0	0.07	0.601	-0.03	0.884	0.39	0.580	0.59	0.364
Warm Buffered Saline	Time_Delay_Code	0.49	0.396	-0.24	0.721	-1.71	0.029	-1.64	0.034
	Sample_vol_code	0.13	0.505	0.21	0.387	0.52	0.043	0.27	0.212
	hCG_conc_code	-0.57	0.157	-0.06	0.880	-0.15	0.665	0.26	0.469
	Time_Delay_Code*Time_Delay_Code	0.21	0.861	0.22	0.882	-3.64	0.032	-3.53	0.037
	Sample_vol_code*Time_Delay_Code	0.00	0.999	-0.14	0.626	-0.42	0.113	0.21	0.373
	Sample_vol_code*Sample_vol_code	0.15	0.835	-0.11	0.899	2.46	0.019	2.12	0.032
	hCG_conc_code*Time_Delay_Code	-0.64	0.147	0.04	0.935	0.30	0.441	0.58	0.178
	hCG_conc_code*Sample_vol_code	0.02	0.936	0.05	0.833	0.20	0.357	0.15	0.501
	hCG_conc_code*hCG_conc_code	0.18	0.884	-0.11	0.940	-4.10	0.024	-4.40	0.020
	LTest_0	0.69	0.568	0.70	0.631	-3.70	0.028	-3.36	0.040

Note: Ltest_J = log testosterone concentration at time J
Beta = regression coefficient, as applied to coded factors
Pvalue = significance level for t-test of hypothesis that the true Beta = 0

Table 56. Maximum Values for Each Time Point

hCG Stimu-lation	Organ Prep Technique		Predicted Log(Testosterone Concentration)*								Sample Volume	hCG Conc	Time Delay	log(T0)
			T1		T2		T3		T4					
No	Cold Buffered	T1 opt	3.977	(0.187)	4.181	(0.291)	4.212	(0.249)	4.186	(0.223)	0.253		2.0	1.5058
No	Cold Buffered	T2 opt	3.976	(0.187)	4.182	(0.291)	4.214	(0.249)	4.190	(0.223)	0.258		2.0	1.5058
No	Cold Buffered	T3 opt	3.879	(0.122)	4.183	(0.189)	4.324	(0.162)	4.469	(0.145)	0.224		0.5	1.5058
No	Cold Buffered	T4 opt	3.876	(0.120)	4.182	(0.188)	4.324	(0.161)	4.470	(0.144)	0.221		0.5	1.5058
No	Cold Media	T1 opt	3.823	(0.142)	3.876	(0.230)	3.942	(0.113)	4.092	(0.101)	0.499		0.7	1.4503
No	Cold Media	T2 opt	3.484	(0.145)	4.156	(0.234)	3.842	(0.115)	3.872	(0.103)	0.125		0.8	1.4503
No	Cold Media	T3 opt	3.746	(0.086)	3.876	(0.139)	4.028	(0.068)	4.153	(0.061)	0.283		0.7	1.4503
No	Cold Media	T4 opt	3.753	(0.083)	3.853	(0.134)	4.020	(0.066)	4.162	(0.059)	0.318		0.7	1.4503
No	Warm Buffered	T1 opt	3.273	(0.177)	3.490	(0.264)	3.680	(0.348)	3.615	(0.315)	0.499		1.2	1.7231
No	Warm Buffered	T2 opt	3.226	(0.120)	3.542	(0.178)	3.740	(0.235)	3.804	(0.212)	0.274		1.0	1.7231
No	Warm Buffered	T3 opt	3.237	(0.119)	3.539	(0.177)	3.745	(0.233)	3.794	(0.211)	0.308		1.1	1.7231
No	Warm Buffered	T4 opt	3.072	(0.171)	3.291	(0.254)	3.562	(0.335)	3.877	(0.303)	0.255		2.0	1.7231
Yes	Cold Buffered	T1 opt	3.963	(0.174)	4.829	(0.246)	5.176	(0.270)	5.178	(0.289)	0.264	0.127	0.5	1.4834
Yes	Cold Buffered	T2 opt	3.966	(0.168)	4.858	(0.238)	5.193	(0.261)	5.212	(0.279)	0.282	0.101	0.5	1.4834
Yes	Cold Buffered	T3 opt	3.986	(0.170)	4.868	(0.241)	5.210	(0.264)	5.223	(0.282)	0.284	0.122	0.5	1.4834
Yes	Cold Buffered	T4 opt	4.010	(0.168)	4.916	(0.238)	5.253	(0.261)	5.293	(0.279)	0.326	0.112	0.5	1.4834
Yes	Cold Media	T1 opt	4.133	(0.062)	4.914	(0.118)	4.975	(0.336)	5.273	(0.305)	0.247	0.111	0.8	1.4291
Yes	Cold Media	T2 opt	4.087	(0.068)	4.951	(0.130)	4.902	(0.372)	5.104	(0.338)	0.262	0.375	0.7	1.4291
Yes	Cold Media	T3 opt	3.865	(0.098)	4.572	(0.187)	5.640	(0.535)	5.699	(0.486)	0.497	0.166	1.2	1.4291
Yes	Cold Media	T4 opt	3.927	(0.101)	4.577	(0.193)	5.565	(0.550)	5.706	(0.500)	0.498	0.142	1.1	1.4291
Yes	Warm Buffered	T1 opt	5.429	(2.298)	3.878	(2.781)	-2.568	(2.272)	-3.233	(2.312)	0.266	0.010	2.0	1.6640

Table 56. Maximum Values for Each Time Point, (Continued)

hCG Stimu-lation	Organ Prep Technique		Predicted Log(Testosterone Concentration)*								Sample Volume	hCG Conc	Time Delay	log(T0)
			T1		T2		T3		T4					
Yes	Warm Buffered	3.107	(0.504)	4.555	(0.610)	5.826	(0.498)	5.623	(0.507)	0.321	0.082	0.5	1.6640T	
Yes	Warm Buffered	T3 opt	3.521	(1.594)	4.159	(1.929)	10.264	(1.575)	9.685	(1.603)	0.499	0.100	0.9	1.6640
Yes	Warm Buffered	T4 opt	3.526	(1.580)	4.140	(1.912)	10.193	(1.561)	9.655	(1.589)	0.497	0.106	0.9	1.6640

*Values in parentheses are standard errors

Table 57. Predicted values for Specified Optimum Factor Combinations

hCG Stimu-lation	Organ Prep Technique		Predicted Log(Testosterone Concentration)*								Sample Volume	hCG Concent	Time Delay	log(T0)
			T1		T2		T3		T4					
No	Cold Buffered	Optimal	2.935	(0.151)	3.027	(0.235)	3.312	(0.201)	3.436	(0.180)	0.500		1.0	1.5058
No	Cold Buffered	Optimal	3.643	(0.199)	3.641	(0.310)	3.846	(0.265)	3.959	(0.237)	0.500		0.5	1.5058
Yes	Cold Buffered	Optimal	3.366	(0.213)	4.155	(0.302)	4.499	(0.331)	4.748	(0.354)	0.500	0.100	1.0	1.4834
Yes	Cold Buffered	Optimal	4.168	(0.272)	5.076	(0.385)	5.406	(0.422)	5.545	(0.451)	0.500	0.100	0.5	1.4834

*Values in parentheses are standard errors

Table 58. ANOVA results for PROC RSREG with the Baseline Concentration Removed from Model

hCG Stimulation	Organ Prep.	Error df	Time	R^2	hCG Conc.	Time Delay	Aliquot Volume	RMSE
no	Cold Buffered Saline	13	T1	89	NA	***		0.261
			T2	80	NA	***		0.374
			T3	80	NA	***		0.342
			T4	81	NA	***		0.334
	Cold Media	12	T1	87	NA	***		0.201
			T2	75	NA	***		0.333
			T3	89	NA	***		0.153
			T4	92	NA	***	*	0.139
	Warm Buffered Saline	12	T1	66	NA	***		0.438
			T2	63	NA	***		0.471
			T3	55	NA	**		0.510
			T4	48	NA	*		0.646
yes	Cold Buffered Saline	7	T1	89		***		0.292
			T2	77		**		0.378
			T3	74		*		0.421
			T4	69				0.449
	Cold Media	7	T1	97		***		0.109
			T2	86		**		0.304
			T3	73		*		0.590
			T4	69				0.698
	Warm Buffered Saline	7	T1	84	**			0.450
			T2	87		**		0.455
			T3	67				0.664
			T4	80	*	*		0.578

*Statistically significant F test at 0.10 level of significance.
**Statistically significant F test at 0.05 level of significance.
***Statistically significant F test at 0.01 level of significance.
Note: R^2 is the percentage of variation accounted for by the model.
RMSE = square root of the residual (error) mean square
NA = not applicable

Table 59. Response Surface Regression Results for No hCG Stimulation with Baseline Concentration Removed

Organ Prep Tech	Model Terms	Ltest_1		Ltest_2		Ltest_3		Ltest_4	
		Beta	P value	Beta	P value	Beta	P value	Beta	P value
Cold Buffered Saline	Time_Delay_Code	0.16	0.065	0.09	0.437	0.06	0.604	0.00	0.981
	Sample_vol_code	0.07	0.394	0.05	0.682	0.08	0.474	0.09	0.398
	Time_Delay_Code* Time_Delay_Code	1.25	<.0001	1.28	<.0001	1.19	<.0001	1.19	<.0001
	Sample_vol_code* Time_Delay_Code	0.03	0.766	0.11	0.411	0.12	0.356	0.15	0.238
	Sample_vol_code* Sample_vol_code	-0.16	0.240	-0.20	0.314	-0.18	0.318	-0.20	0.263
Cold Media	Time_Delay_Code	-0.39	<.0001	-0.49	0.000	-0.35	<.0001	-0.39	<.0001
	Sample_vol_code	0.12	0.079	-0.12	0.272	0.04	0.381	0.10	0.047
	Time_Delay_Code* Time_Delay_Code	-0.60	0.000	-0.66	0.003	-0.41	0.000	-0.37	0.000
	Sample_vol_code* Time_Delay_Code	-0.14	0.081	0.04	0.733	-0.01	0.834	-0.02	0.743
	Sample_vol_code* Sample_vol_code	-0.03	0.793	0.22	0.243	-0.11	0.208	-0.13	0.106
Warm Buffered Saline	Time_Delay_Code	-0.04	0.769	-0.02	0.871	0.00	0.982	-0.22	0.280
	Sample_vol_code	0.15	0.284	0.12	0.437	0.16	0.335	0.02	0.901
	Time_Delay_Code* Time_Delay_Code	-0.99	0.001	-1.04	0.001	-0.90	0.005	-0.91	0.018
	Sample_vol_code* Time_Delay_Code	0.11	0.495	0.06	0.735	0.05	0.784	-0.08	0.719
	Sample_vol_code* Sample_vol_code	0.28	0.237	0.21	0.398	0.09	0.736	-0.13	0.710

Note: Ltest_J = log testosterone concentration at time J
Beta = regression coefficient, as applied to coded factors
Pvalue = significance level for t-test of hypothesis that the true Beta = 0

Table 60. Response Surface Regression Results for hCG Stimulation with Baseline Concentration Removed

Organ Prep Tech	Model Terms	Ltest_1		Ltest_2		Ltest_3		Ltest_4	
		Beta	Pvalue	Beta	Pvalue	Beta	Pvalue	Beta	Pvalue
Cold Buffered Saline	Time_Delay_Code	0.02	0.888	-0.10	0.496	-0.19	0.262	-0.21	0.233
	Sample_vol_code	0.14	0.244	0.18	0.254	0.19	0.279	0.20	0.283
	hCG_conc_code	0.13	0.258	0.07	0.625	0.10	0.529	0.06	0.737
	Time_Delay_Code*Time_Delay_Code	0.91	0.001	0.76	0.008	0.70	0.019	0.48	0.091
	Sample_vol_code*Time_Delay_Code	-0.10	0.458	-0.15	0.409	-0.17	0.395	-0.25	0.250
	Sample_vol_code*Sample_vol_code	0.18	0.325	0.02	0.917	0.05	0.846	0.10	0.717
	hCG_conc_code*Time_Delay_Code	-0.01	0.922	0.06	0.730	0.00	0.999	0.06	0.760
	hCG_conc_code*Sample_vol_code	0.03	0.848	0.01	0.971	0.01	0.949	0.10	0.619
	hCG_conc_code*hCG_conc_code	0.07	0.689	-0.01	0.950	0.08	0.745	0.15	0.583
Cold Media	Time_Delay_Code	-0.33	<.0001	-0.16	0.133	0.05	0.790	-0.35	0.145
	Sample_vol_code	0.02	0.584	-0.01	0.949	-0.01	0.962	0.37	0.173
	hCG_conc_code	0.00	0.980	0.14	0.174	0.20	0.309	-0.14	0.534
	Time_Delay_Code*Time_Delay_Code	-0.46	0.000	-0.41	0.066	-1.01	0.028	-0.85	0.090
	Sample_vol_code*Time_Delay_Code	0.09	0.056	0.28	0.033	0.49	0.052	0.25	0.351
	Sample_vol_code*Sample_vol_code	-0.16	0.073	-0.36	0.129	0.26	0.552	-0.06	0.899
	hCG_conc_code*Time_Delay_Code	-0.07	0.096	-0.22	0.076	-0.25	0.262	-0.05	0.835
	hCG_conc_code*Sample_vol_code	0.05	0.246	0.08	0.464	0.23	0.297	0.29	0.279
	hCG_conc_code*hCG_conc_code	-0.13	0.089	-0.22	0.285	-0.17	0.665	-0.07	0.879

Table 60. Response Surface Regression Results for hCG Stimulation with Baseline Concentration Removed (Continued)

Organ Prep Tech	Model Terms	Ltest_1		Ltest_2		Ltest_3		Ltest_4	
		Beta	Pvalue	Beta	Pvalue	Beta	Pvalue	Beta	Pvalue
Warm Buffered Saline	Time_Delay_Code	-0.19	0.209	-0.53	0.007	-0.46	0.058	-0.31	0.118
	Sample_vol_code	0.26	0.099	0.41	0.021	0.38	0.104	0.09	0.624
	hCG_conc_code	-0.02	0.916	-0.04	0.805	0.10	0.668	0.23	0.285
	Time_Delay_Code*Time_Delay_Code	-0.39	0.173	-0.46	0.124	0.13	0.736	-0.14	0.677
	Sample_vol_code*Time_Delay_Code	0.05	0.758	0.01	0.946	0.03	0.899	0.56	0.029
	Sample_vol_code*Sample_vol_code	0.45	0.126	0.24	0.383	0.39	0.346	0.27	0.448
	hCG_conc_code*Time_Delay_Code	-0.02	0.924	0.14	0.427	0.16	0.521	0.19	0.373
	hCG_conc_code*Sample_vol_code	-0.06	0.715	-0.12	0.483	-0.11	0.664	-0.07	0.738
	hCG_conc_code*hCG_conc_code	-1.08	0.005	-0.79	0.024	-0.82	0.079	-1.14	0.013

Note: Ltest_J = log testosterone concentration at time J
Beta = regression coefficient, as applied to coded factors
Pvalue = significance level for t-test of hypothesis that the true Beta = 0

Table 61. Maximum Values for Models with Baseline Concentration Removed

hCG Stimulation	Organ Prep Technique		Predicted Log(Testosterone Concentration)*								Sample Volume	hCG Conc.	Time Delay
			T1		T2		T3		T4				
No	Cold Buffered Saline	T1 opt	4.313	(0.156)	4.495	(0.224)	4.574	(0.205)	4.613	(0.200)	0.256		2.0
No	Cold Buffered Saline	T2 opt	4.313	(0.156)	4.496	(0.224)	4.576	(0.205)	4.616	(0.200)	0.259		2.0
No	Cold Buffered Saline	T3 opt	4.312	(0.156)	4.495	(0.223)	4.576	(0.204)	4.617	(0.199)	0.262		2.0
No	Cold Buffered Saline	T4 opt	4.309	(0.155)	4.494	(0.223)	4.576	(0.204)	4.617	(0.199)	0.265		2.0
No	Cold Media	T1 opt	3.926	(0.120)	4.084	(0.200)	3.991	(0.092)	4.137	(0.083)	0.498		0.8
No	Cold Media	T2 opt	3.595	(0.122)	4.346	(0.202)	3.888	(0.093)	3.917	(0.084)	0.126		0.8
No	Cold Media	T3 opt	3.916	(0.117)	4.052	(0.194)	3.987	(0.089)	4.146	(0.081)	0.497		0.7
No	Cold Media	T4 opt	3.888	(0.117)	4.007	(0.194)	3.969	(0.089)	4.138	(0.081)	0.499		0.6
No	Warm Buffered Saline	T1 opt	3.957	(0.233)	4.151	(0.251)	4.233	(0.271)	4.086	(0.344)	0.500		1.0
No	Warm Buffered Saline	T2 opt	3.957	(0.233)	4.151	(0.251)	4.233	(0.271)	4.091	(0.344)	0.500		1.0
No	Warm Buffered Saline	T3 opt	3.957	(0.233)	4.151	(0.251)	4.233	(0.271)	4.088	(0.344)	0.500		1.0
No	Warm Buffered Saline	T4 opt	3.902	(0.228)	4.103	(0.245)	4.189	(0.265)	4.122	(0.336)	0.497		0.9
Yes	Cold Buffered Saline	T1 opt	3.950	(0.209)	4.611	(0.270)	4.758	(0.301)	4.708	(0.321)	0.255	0.118	2.0
Yes	Cold Buffered Saline	T2 opt	3.971	(0.172)	4.870	(0.223)	5.202	(0.248)	5.224	(0.265)	0.288	0.102	0.5
Yes	Cold Buffered Saline	T3 opt	3.974	(0.171)	4.866	(0.221)	5.206	(0.246)	5.229	(0.262)	0.294	0.118	0.5
Yes	Cold Buffered Saline	T4 opt	4.045	(0.171)	4.937	(0.221)	5.282	(0.246)	5.329	(0.262)	0.334	0.109	0.5
Yes	Cold Media	T1 opt	4.034	(0.075)	4.848	(0.208)	5.070	(0.404)	5.251	(0.478)	0.292	0.978	0.7
Yes	Cold Media	T2 opt	4.006	(0.076)	4.876	(0.214)	4.967	(0.414)	4.952	(0.490)	0.217	0.999	0.6
Yes	Cold Media	T3 opt	3.895	(0.088)	4.558	(0.246)	5.649	(0.478)	5.683	(0.565)	0.498	0.245	1.1
Yes	Cold Media	T4 opt	3.967	(0.090)	4.554	(0.252)	5.556	(0.489)	5.724	(0.578)	0.498	0.214	1.0
Yes	Warm Buffered Saline	T1 opt	4.595	(0.268)	5.182	(0.271)	5.106	(0.395)	5.011	(0.344)	0.499	0.095	1.0
Yes	Warm Buffered Saline	T2 opt	4.591	(0.266)	5.265	(0.269)	5.207	(0.393)	4.933	(0.342)	0.499	0.083	0.8
Yes	Warm Buffered Saline	T3 opt	4.554	(0.270)	5.290	(0.273)	5.317	(0.398)	4.870	(0.347)	0.497	0.094	0.7
Yes	Warm Buffered Saline	T4 opt	4.020	(0.326)	4.463	(0.329)	4.758	(0.480)	5.423	(0.418)	0.126	0.112	0.6

*Values in parentheses are standard errors

Table 62. Predicted values for Specified Optimum Factor Combinations for Models with Baseline Concentration Removed

hCG Stimu-lation	Organ Prep Technique		Predicted Log (Testosterone Concentration)*				Sample Volume	hCG Concent	Time Delay
			T1	T2	T3	T4			
No	Cold Buffered	Optimal	2.815 (0.157)	2.973 (0.225)	3.227 (0.205)	3.317 (0.200)	0.5		1.0
No	Cold Buffered	Optimal	3.872 (0.169)	4.048 (0.243)	4.242 (0.222)	4.359 (0.217)	0.5		0.5
Yes	Cold Buffered	Optimal	3.344 (0.218)	4.145 (0.282)	4.482 (0.314)	4.731 (0.334)	0.5	0.100	1.0
Yes	Cold Buffered	Optimal	4.337 (0.236)	5.154 (0.305)	5.535 (0.339)	5.676 (0.361)	0.5	0.100	0.5

*Values in parentheses are standard errors

11.0 EQUIIBRATION

The optimal values for factors were selected based upon the results of the statistical analyses of the incubation and testes preparation experiments.Further considerations were the practical aspects of the assay and how it will be used as a screen for endocrine disruptors. The temperature chosen was 36▾C which is about 3 degrees lower than the literature value for the rat body temperature. This correlates with the human situation where the testicular temperature is 34▾C which is 3 degrees below the average body temperature. The media chosen is M-199 without phenol red gassed with 95% O_2 :5%CO_2. Five mL of this media is placed in a scintillation vial and a 0175 mg slice of testicular tissue is added and incubated for one hour. At the end of the hour, the media is removed and replaced with fresh media, samples are removed for the determination of LDH and testosterone baselines and the same amount of media is replaced with either plain media or media with hCG. The vials are then incubated for a total of 4 hours with aliquots removed at hourly intervals.

11.1 Statistical Analysis of Phase II Equilibration Period Determination

11.1.1 Objectives

The objective of the equilibration experiment was to determine whether LDH can be used as a marker to determine the period of time needed for parenchymal equilibration (baseline). The assay was performed with and without hCG stimulation at various equilibration times before baseline sampling: 15 minutes, 30 minutes, 45 minutes, 60 minutes, 90 minutes and 120 minutes. Concentrations of LDH and testosterone for each sample were measured at baseline and at 1, 2, 3 and 4 hours after baseline.

11.1.2 Data

A SAS data set was constructed from the raw Excel data file. Each observation includes concentration measurements for LDH and testosterone and the corresponding sampling time and repeated measurement times. The data used in the analysis are displayed in Tables 63 and 64.

11.1.3 Statistical Analysis Methods and Results

In order to examine the effects of the sampling times on the concentrations of LDH and testosterone, summary statistics were calculated for the combinations of sampling times and repeated measurements. Results of the means, standard deviations, sample sizes and coefficients of variation for LDH concentrations are shown in Tables 65 through 72 and the results for testosterone concentrations are shown in Tables 73 through 80. Graphs of the means and coefficients of variation for LDH and testosterone concentrations are presented at the end of the section report. The summary of these data is shown in Tables 81 through 84.

Table 63. Equilibration Data for No hCG Stimulation

Sample ID	Timepoint	Equilibration time	LDH (mU/mg)	Testosterone (ng/mg)
A	Baseline	15 min.	233.2	0.0029
A	T-1 (1 hr)	15 min.	1355.7	0.0300
A	T-2 (2 hr)	15 min.	1420.8	0.0350
A	T-3 (3 hr)	15 min.	1480.5	0.0322
A	T-4 (4 hr)	15 min.	1556.4	0.0306
B	Baseline	15 min.	331.8	0.0030
B	T-1 (1 hr)	15 min	1117.6	0.0314
B	T-2 (2 hr)	15 min.	1146.7	0.0132
B	T-3 (3 hr)	15 min.	1152.5	0.0291
B	T-4 (4 hr)	15 min.	1146.7	0.0295
C	Baseline	15 min.	394.1	0.0038
C	T-1 (1 hr)	15 min.	1092.3	0.0303
C	T-2 (2 hr)	15 min.	1064.2	0.0304
C	T-3 (3 hr)	15 min.	1013.5	0.0276
C	T-4 (4 hr)	15 min.	929.1	0.0271
D	Baseline	30 min	218.1	0.0041
D	T-1 (1 hr)	30 min	839.7	0.0261
D	T-2 (2 hr)	30 min	932.4	0.0274
D	T-3 (3 hr)	30 min	970.6	0.0275
D	T-4 (4 hr)	30 min	970.6	0.0260
E	Baseline	30 min	135.6	0.0043
E	T-1 (1 hr)	30 min	672.3	0.0289
E	T-2 (2 hr)	30 min	785.3	0.0302
E	T-3 (3 hr)	30 min	853.1	0.0299
E	T-4 (4 hr)	30 min	870.1	0.0295
F	Baseline	30 min	110.6	0.0023
F	T-1 (1 hr)	30 min	504.0	0.0293
F	T-2 (2 hr)	30 min	522.4	0.0314
F	T-3 (3 hr)	30 min	565.5	0.0301
F	T-4 (4 hr)	30 min	596.2	0.0315
G	Baseline	45 min	262.6	0.0031
G	T-1 (1 hr)	45 min	1061.3	0.0176
G	T-2 (2 hr)	45 min	1198.0	0.0211

Table 63. Equilibration Data for No hCG Stimulation (Continued)

Sample ID	Timepoint	Equilibration time	LDH (mU/mg)	Testosterone (ng/mg)
G	T-3 (3 hr)	45 min	1247.3	0.0187
G	T-4 (4 hr)	45 min	1187.1	0.0183
H	Baseline	45 min	300.6	0.0039
H	T-1 (1 hr)	45 min	1043.7	0.0252
H	T-2 (2 hr)	45 min	1111.7	0.0288
H	T-3 (3 hr)	45 min	1179.8	0.0251
H	T-4 (4 hr)	45 min	1174.1	0.0252
I	Baseline	45 min	1055.4	0.0070
I	T-1 (1 hr)	45 min	1032.1	0.0351
I	T-2 (2 hr)	45 min	1061.2	0.0334
I	T-3 (3 hr)	45 min	1008.7	0.0308
I	T-4 (4 hr)	45 min	262.4	0.0287
J	Baseline	60 min	159.4	0.0080
J	T-1 (1 hr)	60 min	615.7	0.0521
J	T-2 (2 hr)	60 min	698.2	0.0476
J	T-3 (3 hr)	60 min	830.1	0.0437
J	T-4 (4 hr)	60 min	874.1	0.0398
K	Baseline	60 min	300.9	0.0096
K	T-1 (1 hr)	60 min	886.2	0.0463
K	T-2 (2 hr)	60 min	809.6	0.0408
K	T-3 (3 hr)	60 min	820.6	0.0359
K	T-4 (4 hr)	60 min	820.6	0.0327
L	Baseline	60 min	124.9	0.0057
L	T-1 (1 hr)	60 min	772.9	0.0326
L	T-2 (2 hr)	60 min	796.7	0.0368
L	T-3 (3 hr)	60 min	909.6	0.0346
L	T-4 (4 hr)	60 min	1076.1	0.0348
M	Baseline	90 min	179.8	0.0104
M	T-1 (1 hr)	90 min	859.6	0.0408
M	T-2 (2 hr)	90 min	955.1	0.0402
M	T-3 (3 hr)	90 min	1005.6	0.0349
M	T-4 (4 hr)	90 min	988.8	0.0392
N	Baseline	90 min	140.1	0.0111
N	T-1 (1 hr)	90 min	786.6	0.0530
N	T-2 (2 hr)	90 min	856.7	0.0466
N	T-3 (3 hr)	90 min	878.2	0.0418
N	T-4 (4 hr)	90 min	862.1	0.0330

Table 63. Equilibration Data for No hCG Stimulation (Continued)

Sample ID	Timepoint	Equilibration time	LDH (mU/mg)	Testosterone (ng/mg)
O	Baseline	90 min	139.6	0.0054
O	T-1 (1 hr)	90 min	722.5	0.0198
O	T-2 (2 hr)	90 min	777.2	0.0251
O	T-3 (3 hr)	90 min	710.4	0.0208
O	T-4 (4 hr)	90 min	1135.4	0.0214
P	Baseline	120 min	192.8	0.0089
P	T-1 (1 hr)	120 min	1000.0	0.0396
P	T-2 (2 hr)	120 min	1174.7	0.0403
P	T-3 (3 hr)	120 min	626.5	0.0211
P	T-4 (4 hr)	120 min	1741.0	0.0404
Q	Baseline	120 min	943.1	0.0135
Q	T-1 (1 hr)	120 min	1406.3	0.0454
Q	T-2 (2 hr)	120 min	1484.4	0.0493
Q	T-3 (3 hr)	120 min	1523.4	0.0411
Q	T-4 (4 hr)	120 min	948.7	0.0332
R	Baseline	120 min	318.6	0.0073
R	T-1 (1 hr)	120 min	950.6	0.0314
R	T-2 (2 hr)	120 min	918.7	0.0297
R	T-3 (3 hr)	120 min	892.2	0.0297
R	T-4 (4 hr)	120 min	897.5	0.0258

Table 64. Equilibration Data for hCG Stimulation

Sample ID	Timepoint	Equilibrationtime	LDH (mU/mg)	Testosterone (ng/mg)
AC	Baseline	15 min.	195.4	0.0026
AC	T-1 (1 hr)	15 min.	770.9	0.0545
AC	T-2 (2 hr)	15 min.	830.6	0.1024
AC	T-3 (3 hr)	15 min.	879.5	0.1979
AC	T-4 (4 hr)	15 min.	928.3	0.2350
BC	Baseline	15 min.	535.8	0.0044
BC	T-1 (1 hr)	15 min.	1147.4	0.0674
BC	T-2 (2 hr)	15 min.	1170.6	0.2153
BC	T-3 (3 hr)	15 min.	1176.5	0.1449
BC	T-4 (4 hr)	15 min.	1071.6	0.2566
CC	Baseline	15 min.	332.0	0.0041
CC	T-1 (1 hr)	15 min.	797.9	0.0620
CC	T-2 (2 hr)	15 min.	862.0	0.1501
CC	T-3 (3 hr)	15 min.	885.3	0.2394
CC	T-4 (4 hr)	15 min.	850.3	0.2543
DC	Baseline	30 min	178.9	0.0030
DC	T-1 (1 hr)	30 min	773.3	0.0428
DC	T-2 (2 hr)	30 min	804.8	0.0854
DC	T-3 (3 hr)	30 min	804.8	0.1385
DC	T-4 (4 hr)	30 min	799.6	0.2101
EC	Baseline	30 min	353.7	0.0034
EC	T-1 (1 hr)	30 min	1024.4	0.0486
EC	T-2 (2 hr)	30 min	1085.4	0.1080
EC	T-3 (3 hr)	30 min	1274.4	0.1755
EC	T-4 (4 hr)	30 min	1146.3	0.2261
FC	Baseline	30 min	133.8	0.0018
FC	T-1 (1 hr)	30 min	711.6	0.0512
FC	T-2 (2 hr)	30 min	775.8	0.1221
FC	T-3 (3 hr)	30 min	802.6	0.1801
FC	T-4 (4 hr)	30 min	936.3	0.2385

Table 64. Equilibration Data for hCG Stimulation (Continued)

Sample ID	Timepoint	Equilibrationtime	LDH (mU/mg)	Testosterone (ng/mg)
GC	Baseline	45 min	341.0	0.0043
GC	T-1 (1 hr)	45 min	1221.1	0.0448
GC	T-2 (2 hr)	45 min	1281.6	0.0811
GC	T-3 (3 hr)	45 min	1199.1	0.1266
GC	T-4 (4 hr)	45 min	1276.1	0.1431
HC	Baseline	45 min	383.4	0.0053
HC	T-1 (1 hr)	45 min	1053.0	0.0620
HC	T-2 (2 hr)	45 min	1156.4	0.1326
HC	T-3 (3 hr)	45 min	1241.6	0.2413
HC	T-4 (4 hr)	45 min	1284.2	0.2879
IC	Baseline	45 min	1056.3	0.0085
IC	T-1 (1 hr)	45 min	1098.7	0.0630
IC	T-2 (2 hr)	45 min	1098.7	0.1382
IC	T-3 (3 hr)	45 min	1045.6	0.1700
IC	T-4 (4 hr)	45 min	376.9	0.2271
JC	Baseline	60 min	204.3	0.0085
JC	T-1 (1 hr)	60 min	859.1	0.1175
JC	T-2 (2 hr)	60 min	1000.5	0.1923
JC	T-3 (3 hr)	60 min	1052.9	0.2481
JC	T-4 (4 hr)	60 min	1094.8	0.2962
KC	Baseline	60 min	274.4	0.0096
KC	T-1 (1 hr)	60 min	533.0	0.0952
KC	T-2 (2 hr)	60 min	723.0	0.1664
KC	T-3 (3 hr)	60 min	781.0	0.2142
KC	T-4 (4 hr)	60 min	854.9	0.2057
LC	Baseline	60 min	148.4	0.0072
LC	T-1 (1 hr)	60 min	679.2	0.1074
LC	T-2 (2 hr)	60 min	724.9	0.2139
LC	T-3 (3 hr)	60 min	724.9	0.2438
LC	T-4 (4 hr)	60 min	707.8	0.3026
MC	Baseline	90 min	201.7	0.0093
MC	T-1 (1 hr)	90 min	690.0	0.0731
MC	T-2 (2 hr)	90 min	843.9	0.1478

Table 64. Equilibration Data for hCG Stimulation (Continued)

Sample ID	Timepoint	Equilibrationtime	LDH (mU/mg)	Testosterone (ng/mg)
MC	T-3 (3 hr)	90 min	966.0	0.1636
MC	T-4 (4 hr)	90 min	1008.5	0.1847
NC	Baseline	90 min	229.2	0.0106
NC	T-1 (1 hr)	90 min	648.4	0.1025
NC	T-2 (2 hr)	90 min	760.2	0.1874
NC	T-3 (3 hr)	90 min	877.6	0.2360
NC	T-4 (4 hr)	90 min	944.7	0.2666
OC	Baseline	90 min	208.0	0.0053
OC	T-1 (1 hr)	90 min	582.3	0.2442
OC	T-2 (2 hr)	90 min	635.8	0.4070
OC	T-3 (3 hr)	90 min	760.5	0.4769
OC	T-4 (4 hr)	90 min	837.8	0.4797
PC	Baseline	120 min	1188.5	0.0089
PC	T-1 (1 hr)	120 min	1842.7	0.0711
PC	T-2 (2 hr)	120 min	2046.8	0.1131
PC	T-3 (3 hr)	120 min	2028.8	0.1545
PC	T-4 (4 hr)	120 min	1200.5	0.1628
QC	Baseline	120 min	789.9	0.0125
QC	T-1 (1 hr)	120 min	1190.9	0.2552
QC	T-2 (2 hr)	120 min	1268.7	0.4781
QC	T-3 (3 hr)	120 min	1238.8	0.4377
QC	T-4 (4 hr)	120 min	784.0	0.4604
RC	Baseline	120 min	403.1	0.0082
RC	T-1 (1 hr)	120 min	1019.0	0.1031
RC	T-2 (2 hr)	120 min	985.4	0.1619
RC	T-3 (3 hr)	120 min	946.2	0.1889
RC	T-4 (4 hr)	120 min	1192.6	0.2415

Table 65. Means of LDH Concentration (mU/mg) by Equilibration Time (minutes) hCG Stimulation

Time point	15 minutes	30 minutes	45 minutes	60 minutes	90 minutes	120 minutes
Baseline	319.7	154.8	539.5	195.1	153.2	484.8
T-1 (1 hr)	1,188.6	672.0	1,045.7	758.3	789.6	1,119.0
T-2 (2 hr)	1,210.6	746.7	1,123.7	768.2	863.0	1,192.6
T-3 (3 hr)	1,215.5	796.4	1,145.3	853.4	864.7	1,014.0
T-4 (4 hr)	1,210.7	812.3	874.5	923.6	995.4	1,195.7

Table 66. Standard Deviations of LDH concentration (mU/mg) by Equilibration Time (minutes). No hCG Stimulation

Time point	15 minutes	30 minutes	45 minutes	60 minutes	90 minutes	120 minutes
Baseline	81.2	56.2	447.2	93.3	23.0	401.8
T-1 (1 hr)	145.3	167.9	14.7	135.8	68.6	250.0
T-2 (2 hr)	186.7	207.7	69.2	60.9	89.1	283.2
T-3 (3 hr)	239.8	208.4	123.0	48.9	148.1	460.7
T-4 (4 hr)	318.5	193.8	530.2	134.8	136.8	472.9

Table 67. Sample sizes of LDH concentration (mU/mg) by Equilibration Time (minutes) No hCG Stimulation

Time point	15 minutes	30 minutes	45 minutes	60 minutes	90 minutes	120 minutes
Baseline	3	3	3	3	3	3
T-1 (1 hr)	3	3	3	3	3	3
T-2 (2 hr)	3	3	3	3	3	3
T-3 (3 hr)	3	3	3	3	3	3
T-4 (4 hr)	3	3	3	3	3	3

Table 68. Coeff of Variation of LDH concentration (mU/mg) by Equilibration Time (minutes)No hCG Stimulation

Time point	15 minutes	30 minutes	45 minutes	60 minutes	90 minutes	120 minutes
Baseline	25.4	36.3	82.9	47.8	15.0	82.9
T-1 (1 hr)	12.2	25.0	1.4	17.9	8.7	22.3
T-2 (2 hr)	15.4	27.8	6.2	7.9	10.3	23.8
T-3 (3 hr)	19.7	26.2	10.7	5.7	17.1	45.4
T-4 (4 hr)	26.3	23.9	60.6	14.6	13.7	39.5

Table 69. Means of LDH concentration (mU/mg) by Equilibration Time (minutes) with hCG Stimulation

Time point	15 minutes	30 minutes	45 minutes	60 minutes	90 minutes	120 minutes
Baseline	354.4	222.1	593.6	209.0	213.0	793.9
T-1 (1 hr)	905.4	836.4	1,124.3	690.4	640.2	1,350.9
T-2 (2 hr)	954.4	888.7	1,178.9	816.1	746.6	1,433.7
T-3 (3 hr)	980.4	960.6	1,162.1	852.9	868.1	1,404.6
T-4 (4 hr)	950.1	960.8	979.1	885.8	930.3	1,059.0

Table 70. Standard Deviations of LDH concentration (mU/mg) by Equilibration Time (minutes) with hCG Stimulation

Time point	15 minutes	30 minutes	45 minutes	60 minutes	90 minutes	120 minutes
Baseline	171.3	116.2	401.3	63.1	14.4	392.7
T-1 (1 hr)	210.0	165.7	86.9	163.3	54.3	434.5
T-2 (2 hr)	187.9	171.0	93.5	159.7	104.8	549.6
T-3 (3 hr)	169.8	271.8	103.1	175.4	103.1	560.0
T-4 (4 hr)	112.3	174.7	521.6	195.4	86.3	238.2

Table 71. Sample sizes of LDH concentration (mU/mg) by Equilibration Time (minutes) with hCG Stimulation

Time point	15 minutes	30 minutes	45 minutes	60 minutes	90 minutes	120 minutes
Baseline	3	3	3	3	3	3
T-1 (1 hr)	3	3	3	3	3	3
T-2 (2 hr)	3	3	3	3	3	3
T-3 (3 hr)	3	3	3	3	3	3
T-4 (4 hr)	3	3	3	3	3	3

Table 72. Coeff of Variation of LDH concentration (mU/mg) by Equilibration Time (minutes) with hCG Stimulation

Timepoint	15 minutes	30 minutes	45 minutes	60 minutes	90 minutes	120 minutes
Baseline	**48.3**	**52.3**	**67.6**	**30.2**	**6.8**	**49.5**
T-1 (1 hr)	**23.2**	**19.8**	**7.7**	**23.7**	**8.5**	**32.2**
T-2 (2 hr)	**19.7**	**19.2**	**7.9**	**19.6**	**14.0**	**38.3**
T-3 (3 hr)	**17.3**	**28.3**	**8.9**	**20.6**	**11.9**	**39.9**
T-4 (4 hr)	**11.8**	**18.2**	**53.3**	**22.1**	**9.3**	**22.5**

Table 73. Means of Testosterone concentration (ng/mg) by Equilibration Time (minutes) No hCG Stimulation

Timepoint	15 minutes	30 minutes	45 minutes	60 minutes	90 minutes	120 minutes
Baseline	0.0032	0.0036	0.0047	0.0078	0.0090	0.0099
T-1 (1 hr)	0.0306	0.0281	0.0259	0.0437	0.0379	0.0388
T-2 (2 hr)	0.0262	0.0297	0.0277	0.0417	0.0373	0.0398
T-3 (3 hr)	0.0297	0.0292	0.0249	0.0381	0.0325	0.0307
T-4 (4 hr)	0.0291	0.0290	0.0241	0.0358	0.0312	0.0331

Table 74. Standard Deviations of Testosterone concentration (ng/mg) by Equilibration Time (minutes) No hCG Stimulation

Time point	15 minutes	30 minutes	45 minutes	60 minutes	90 minutes	120 minutes
Baseline	0.0005	0.0011	0.0021	0.0019	0.0031	0.0032
T-1 (1 hr)	0.0007	0.0017	0.0088	0.0100	0.0168	0.0070
T-2 (2 hr)	0.0115	0.0021	0.0062	0.0054	0.0110	0.0098
T-3 (3 hr)	0.0023	0.0014	0.0061	0.0049	0.0107	0.0100
T-4 (4 hr)	0.0018	0.0028	0.0053	0.0036	0.0091	0.0073

Table 75. Sample sizes of Testosterone concentration (ng/mg) by Equilibration Time (minutes) No hCG Stimulation

Timepoint	15 minutes	30 minutes	45 minutes	60 minutes	90 minutes	120 minutes
Baseline	3	3	3	3	3	3
T-1 (1 hr)	3	3	3	3	3	3
T-2 (2 hr)	3	3	3	3	3	3
T-3 (3 hr)	3	3	3	3	3	3
T-4 (4 hr)	3	3	3	3	3	3

Table 76. Coeff of Variation of Testosterone concentration (ng/mg) by Equilibration Time (minutes) No hCG Stimulation

Timepoint	15 minutes	30 minutes	45 minutes	60 minutes	90 minutes	120 minutes
Baseline	15.7	31.3	44.4	25.0	34.7	32.8
T-1 (1 hr)	2.4	6.2	33.9	22.8	44.4	18.1
T-2 (2 hr)	43.8	7.0	22.3	13.0	29.5	24.6
T-3 (3 hr)	7.9	4.9	24.4	12.8	33.0	32.7
T-4 (4 hr)	6.1	9.5	22.0	10.2	29.0	22.1

Table 77. Means of Testosterone concentration (ng/mg) by Equilibration Time (minutes) with hCG Stimulation

Timepoint	15 minutes	30 minutes	45 minutes	60 minutes	90 minutes	120 minutes
Baseline	0.0037	0.0027	0.0061	0.0084	0.0084	0.0099
T-1 (1 hr)	0.0613	0.0475	0.0566	0.1067	0.1400	0.0871
T-2 (2 hr)	0.1559	0.1052	0.1173	0.1909	0.1676	0.1375
T-3 (3 hr)	0.1941	0.1647	0.1793	0.2354	0.1998	0.2604
T-4 (4 hr)	0.2486	0.2385	0.2271	0.2994	0.2256	0.3510

Table 78. Standard Deviations of Testosterone concentration (ng/mg) by Equilibration Time (minutes) with hCG Stimulation

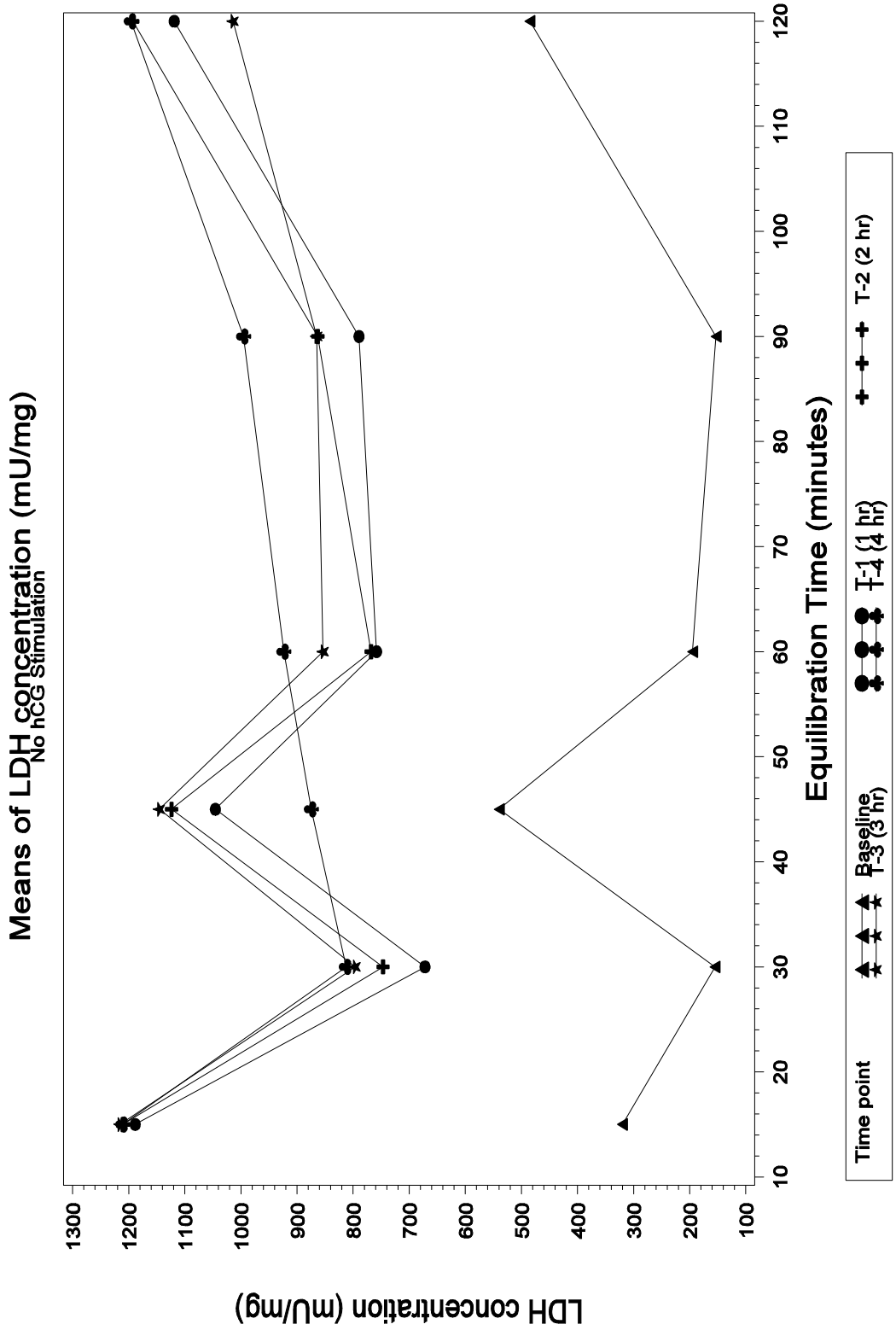
Timepoint	15 minutes	30 minutes	45 minutes	60 minutes	90 minutes	120 minutes
Baseline	0.0010	0.0008	0.0022	0.0012	0.0028	0.0023
T-1 (1 hr)	0.0065	0.0043	0.0102	0.0112	0.0915	0.0226
T-2 (2 hr)	0.0566	0.0185	0.0315	0.0238	0.0280	0.0345
T-3 (3 hr)	0.0474	0.0228	0.0579	0.0184	0.0512	0.1545
T-4 (4 hr)	0.0118	.	.	0.0045	0.0579	0.1548

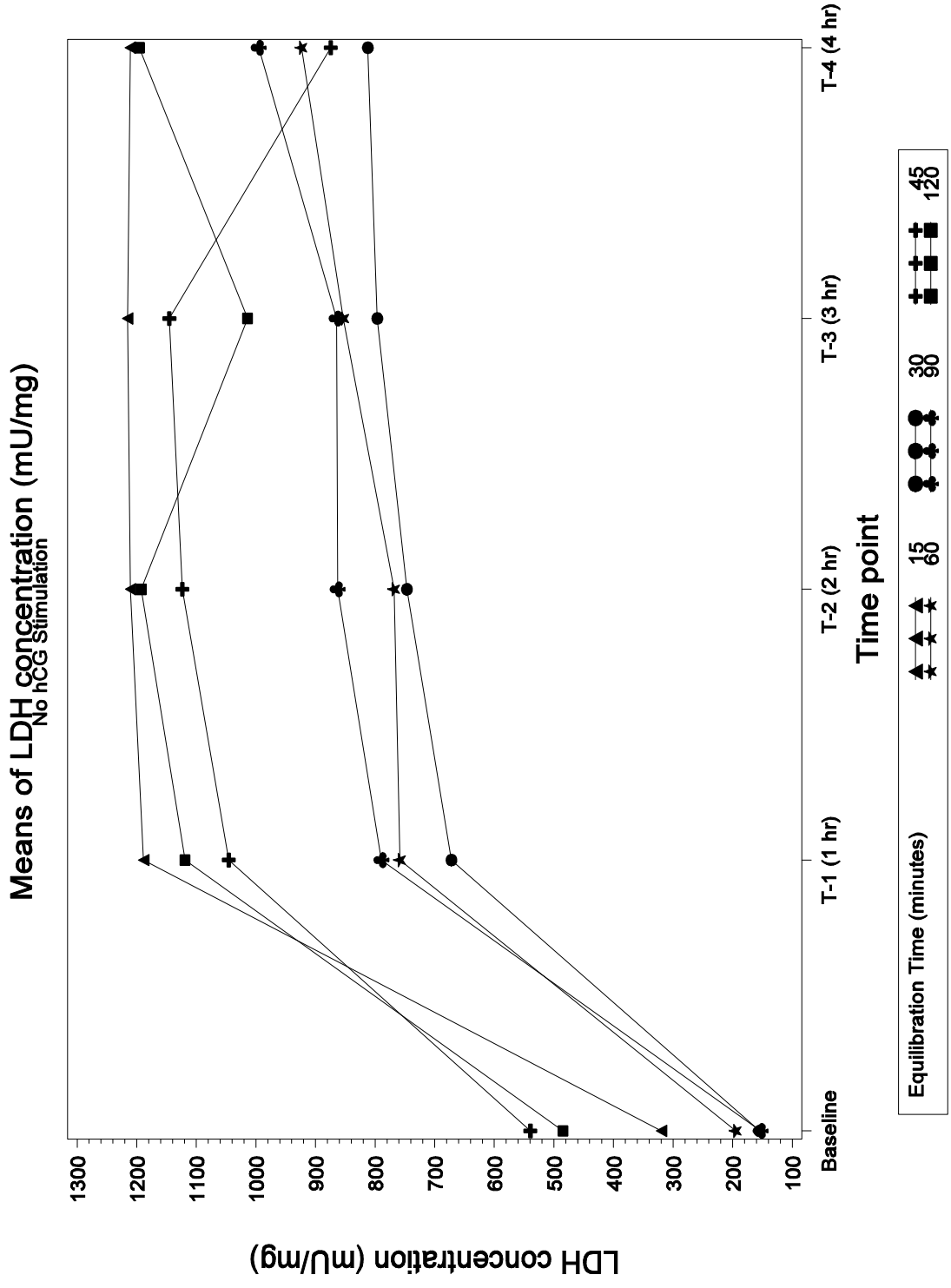
Table 79. Sample sizes of Testosterone concentration (ng/mg) by Equilibration Time (minutes) with hCG Stimulation

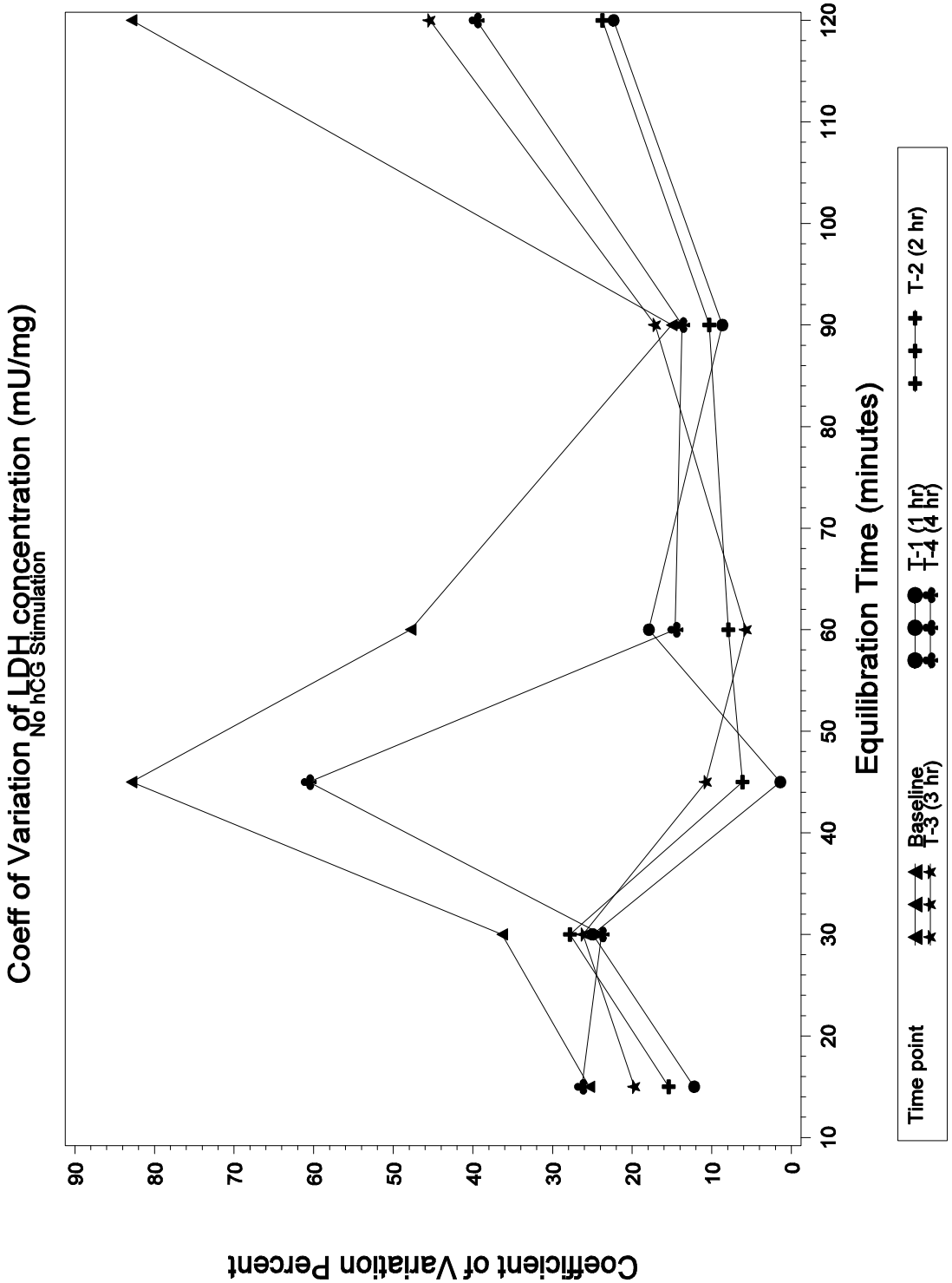
Time point	15 minutes	30 minutes	45 minutes	60 minutes	90 minutes	120 minutes
Baseline	3	3	3	3	3	3
T-1 (1 hr)	3	3	3	3	3	2
T-2 (2 hr)	3	3	3	3	2	2
T-3 (3 hr)	3	3	3	3	2	3
T-4 (4 hr)	3	1	1	2	2	2

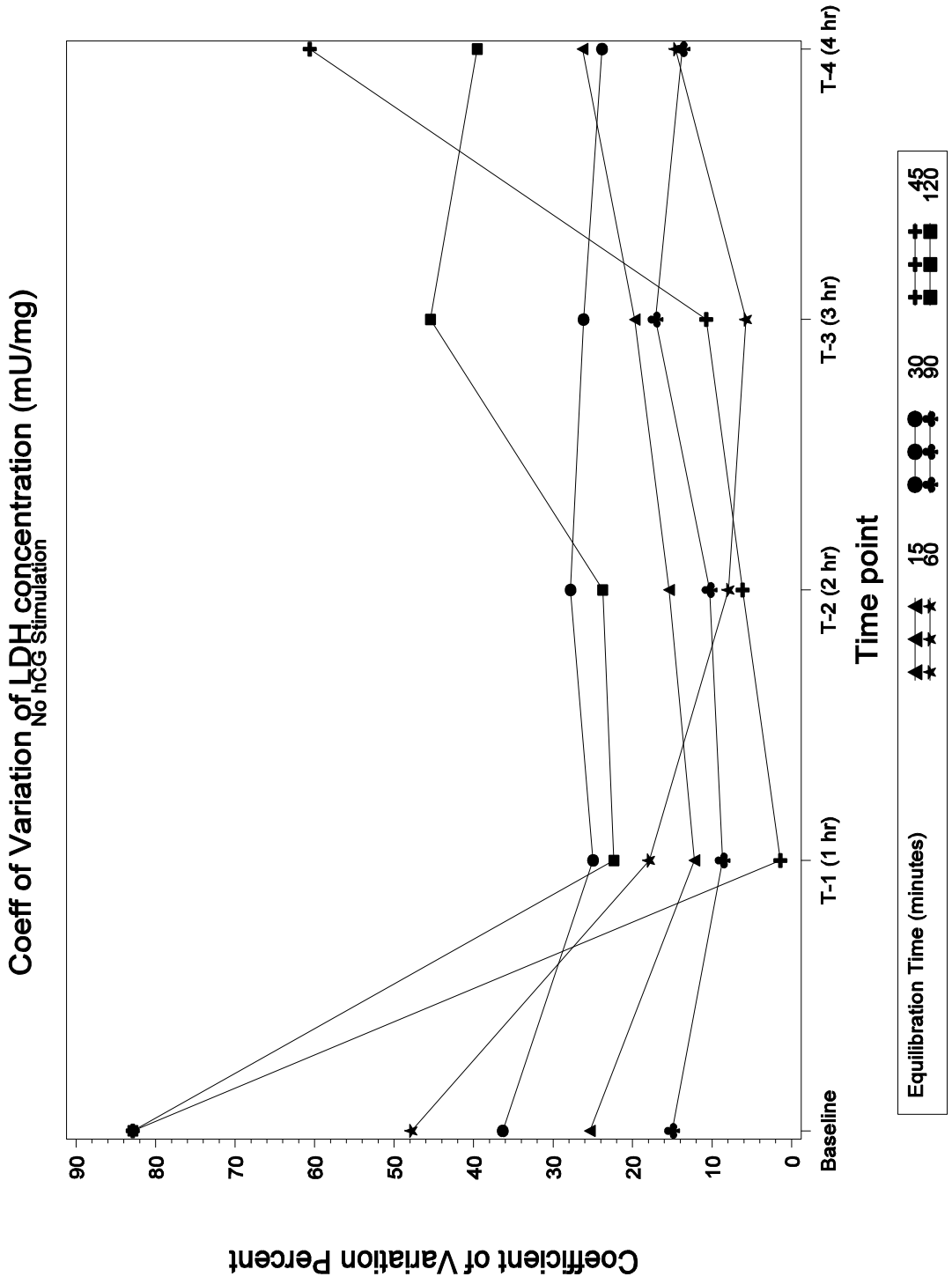
Table 80. Coeff of Variation of Testosterone concentration (ng/mg) by Equilibration Time (minutes) with hCG Stimulation

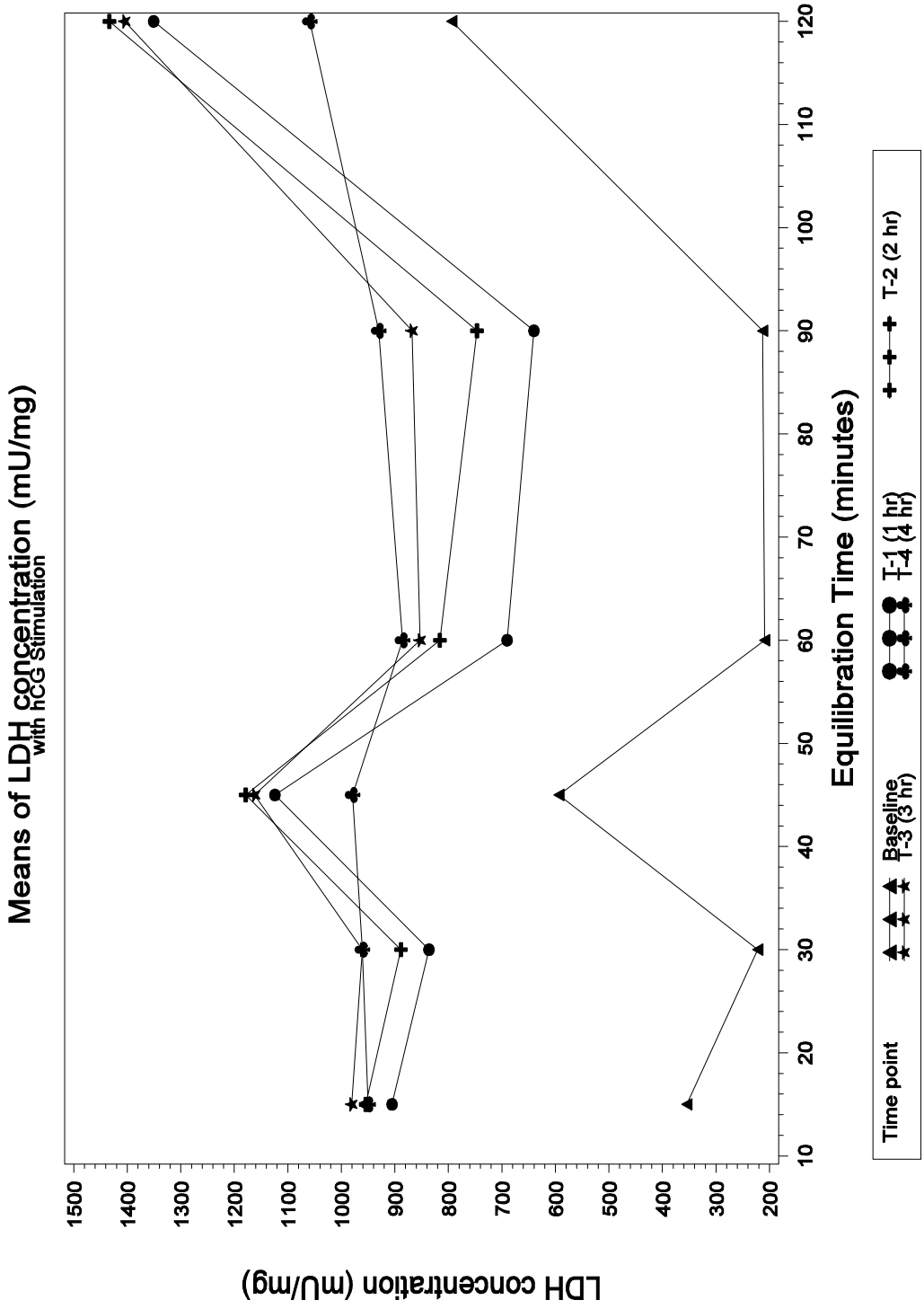
Timepoint	15 minutes	30 minutes	45 minutes	60 minutes	90 minutes	120 minutes
Baseline	25.9	29.5	36.3	14.3	33.0	23.3
T-1 (1 hr)	10.6	9.0	18.0	10.5	65.4	26.0
T-2 (2 hr)	36.3	17.6	26.8	12.5	16.7	25.1
T-3 (3 hr)	24.4	13.9	32.3	7.8	25.6	59.4
T-4 (4 hr)	4.8	.	.	1.5	25.7	44.1

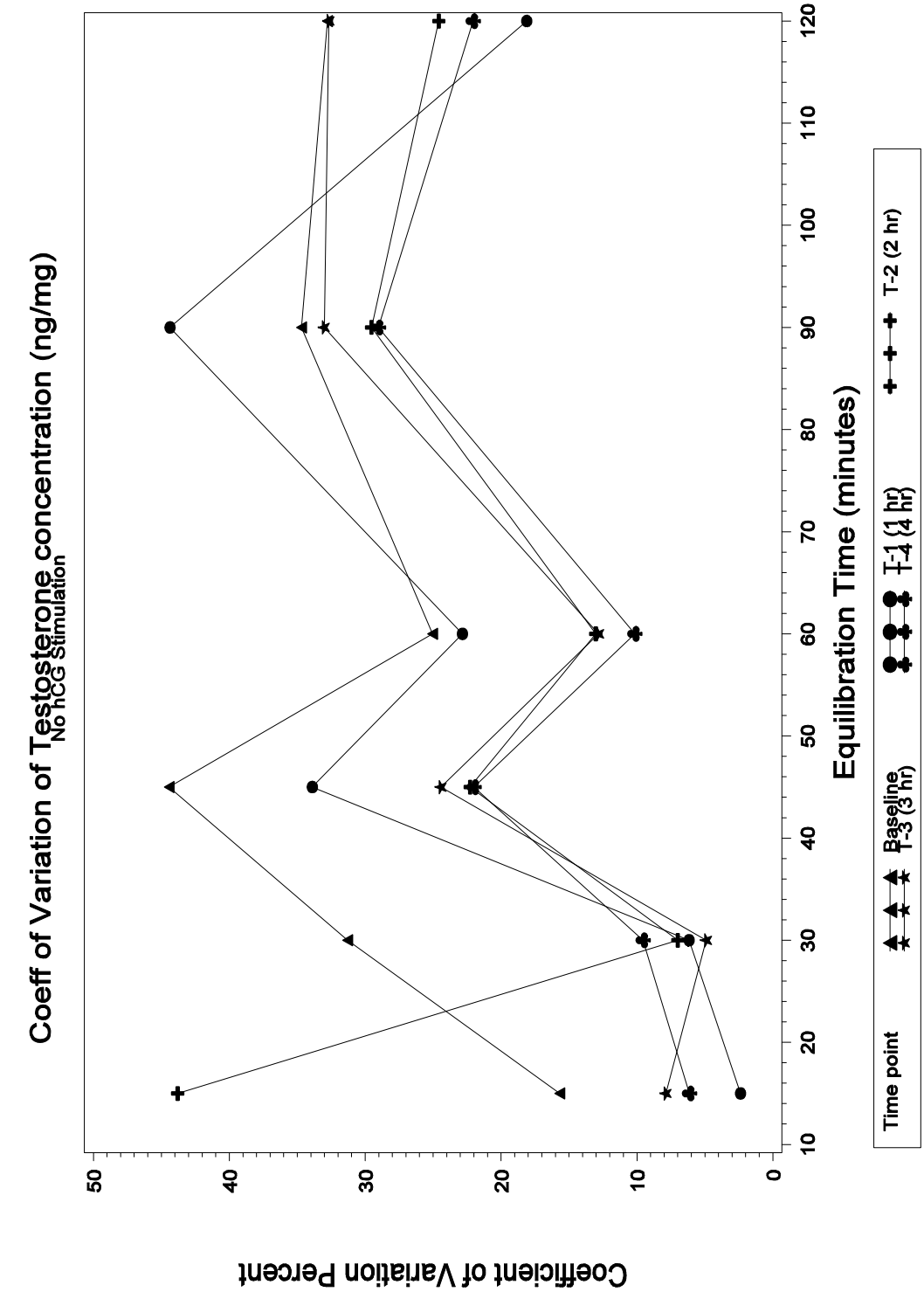

Means of LDH concentration (mU/mg)
No hCG Stimulation
LDH concentration (mU/mg)
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
10
20
30
40
50
60
70
80
90
100
110
120
Equilibration Time (minutes)
Time point
Baseline
T-3 (3 hr)
T-1 (1 hr)
T-4 (4 hr)
T-2 (2 hr)

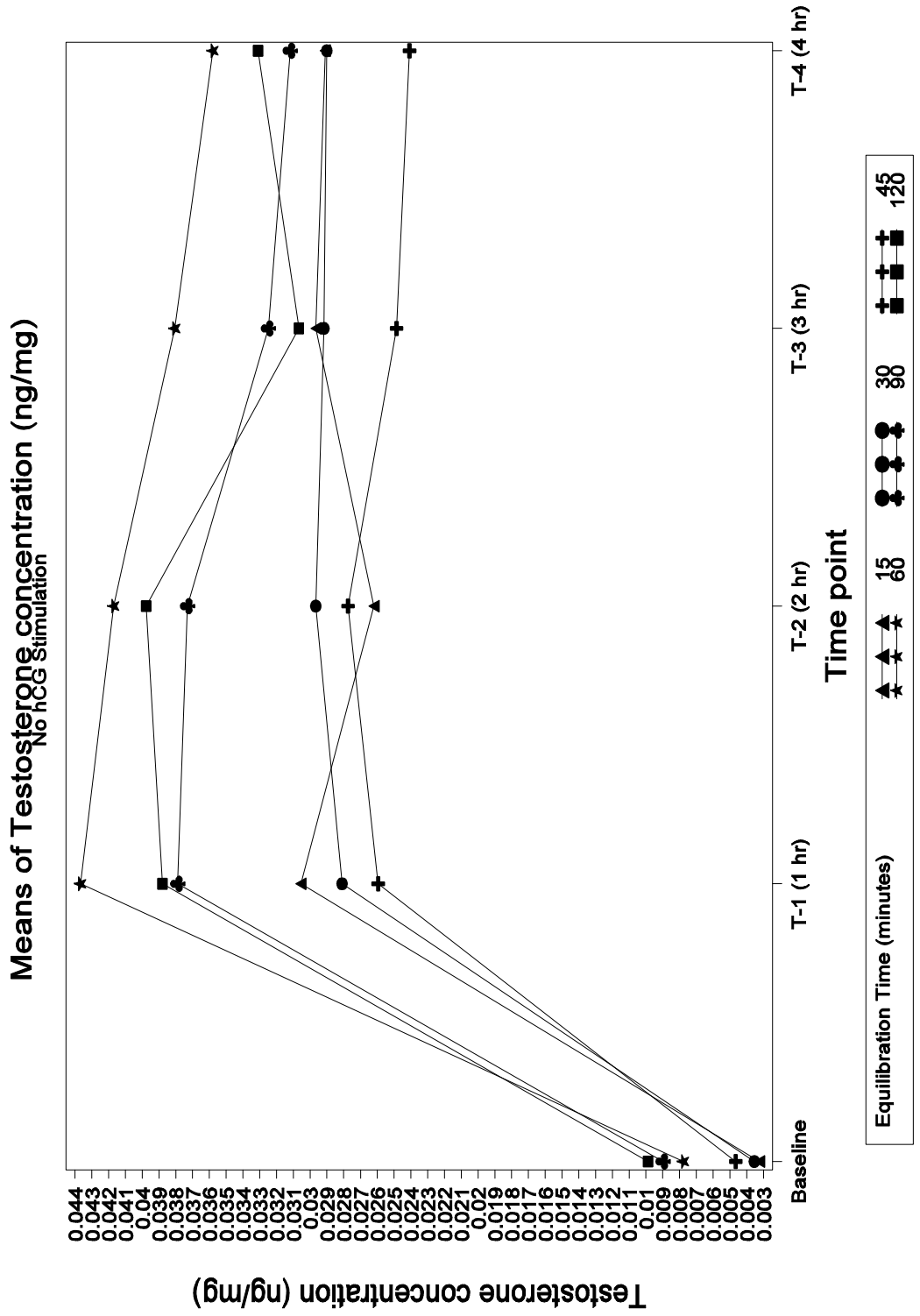

Means of LDH concentration (mU/mg)
No hCG Stimulation
LDH concentration (mU/mg)
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)
Time point
Equilibration Time (minutes)
15
60
30
90
45
120


Coeff of Variation of LDH concentration (mU/mg)
No hCG Stimulation
Coefficient of Variation Percent
Equilibration Time (minutes)
Time point
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)


Coeff of Variation of LDH concentration (mU/mg)
No hCG Stimulation
Coefficient of Variation Percent
0
10
20
30
40
50
60
70
80
90
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)
Time point
Equilibration Time (minutes)
15
30
45
60
90
120


Means of LDH concentration (mU/mg)
with hCG Stimulation
LDH concentration (mU/mg)
Equilibration Time (minutes)
Time point
Baseline
T-3 (3 hr)
T-1 (1 hr)
T-4 (4 hr)
T-2 (2 hr)


Coeff of Variation of Testosterone concentration (ng/mg)
No hCG Stimulation
Coefficient of Variation Percent
0
10
20
30
40
50
10
20
30
40
50
60
70
80
90
100
110
120
Equilibration Time (minutes)
Time point
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)


Means of Testosterone concentration (ng/mg)
No hCG Stimulation
Testosterone concentration (ng/mg)
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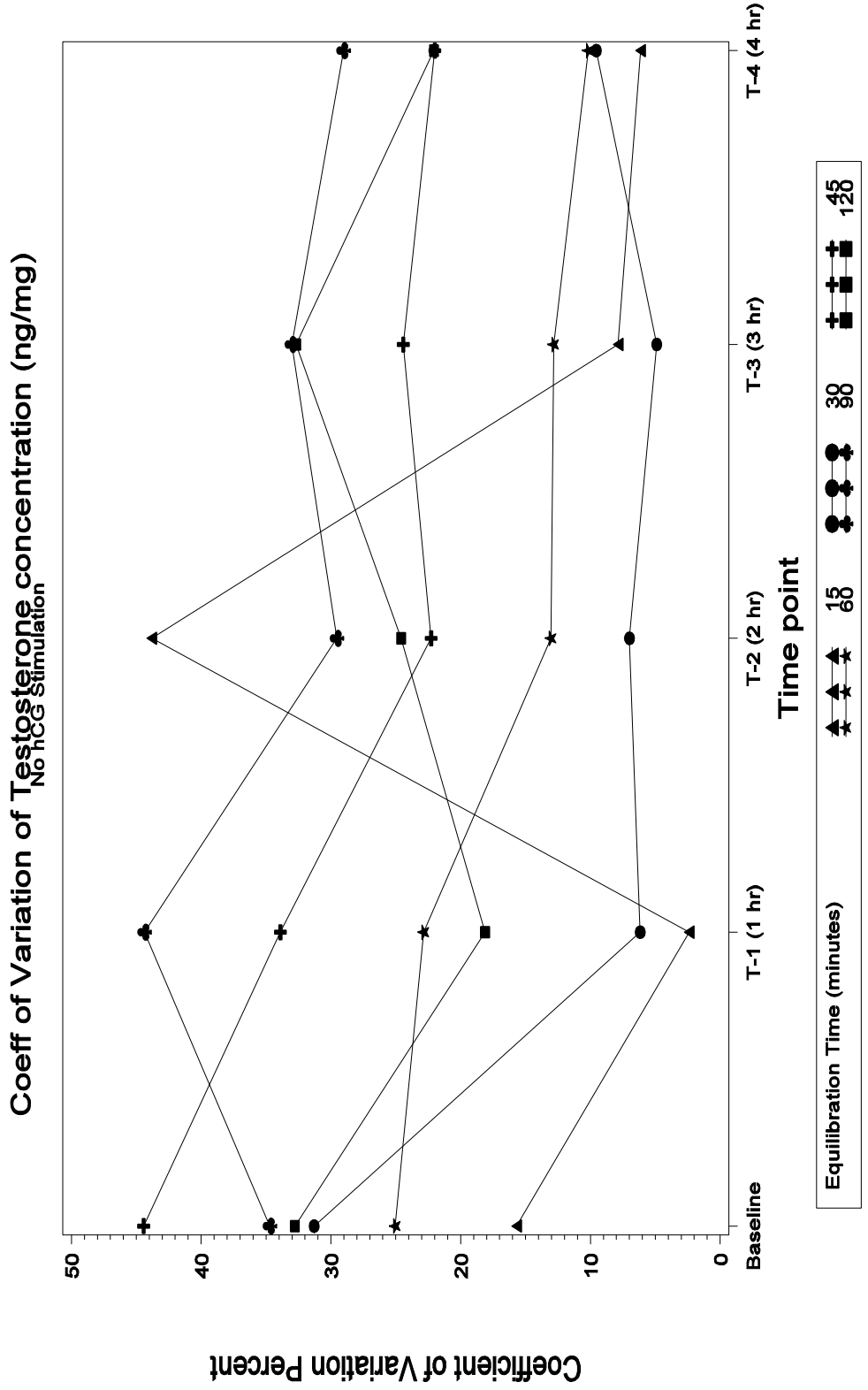
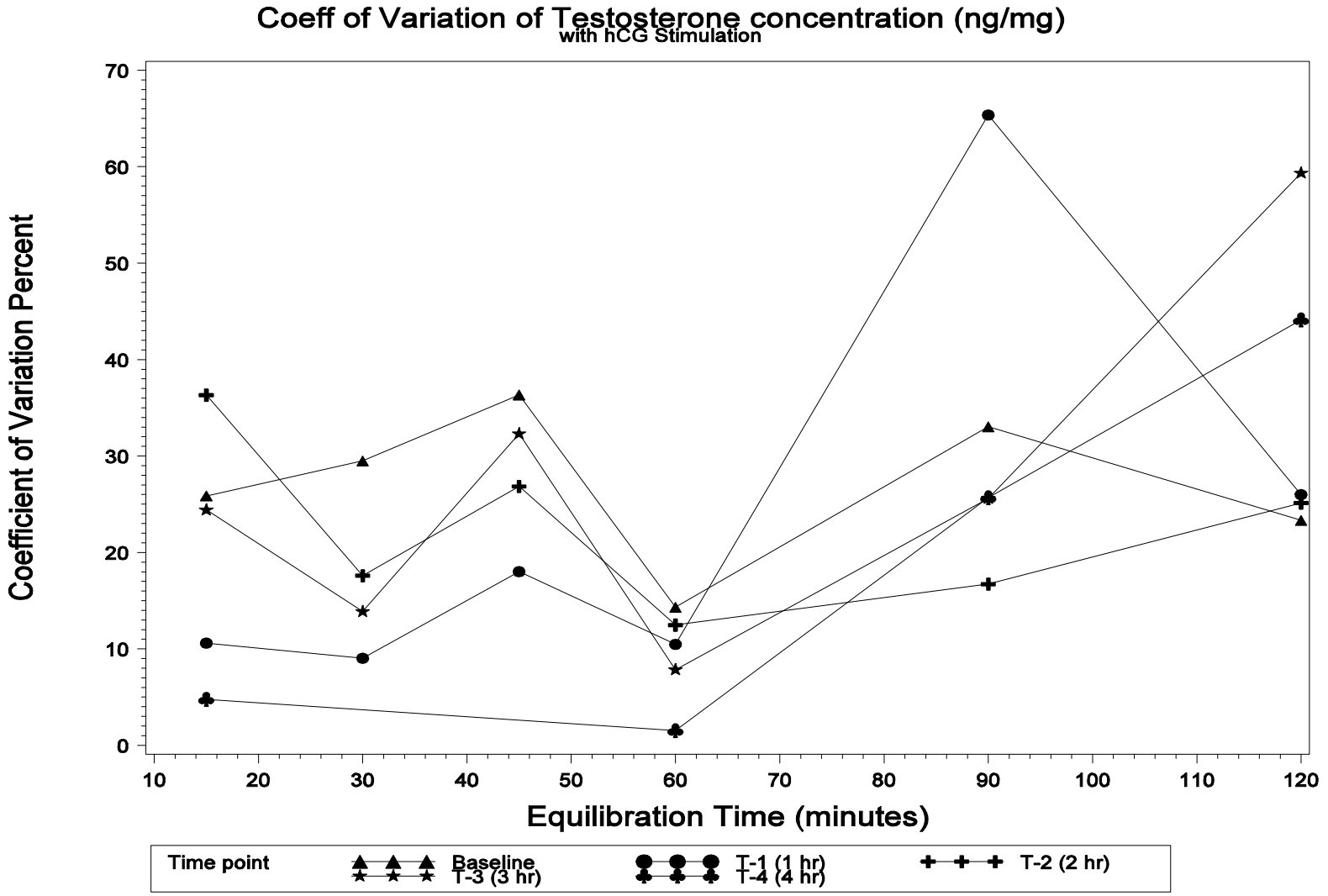
0.043
0.042
0.041
0.04
0.039
0.038
0.037
0.036
0.035
0.034
0.033
0.032
0.031
0.03
0.029
0.028
0.027
0.026
0.025
0.024
0.023
0.022
0.021
0.02
0.019
0.018
0.017
0.016
0.015
0.014
0.013
0.012
0.011
0.01
0.009
0.008
0.007
0.006
0.005
0.004
0.003
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)
Time point
Equilibration Time (minutes)
15
60
30
90
45
120


Coeff of Variation of Testosterone concentration (ng/mg)
No hCG Stimulation
Coefficient of Variation Percent
50
40
30
20
10
0
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)
Time point
Equilibration Time (minutes)
15
60
30
90
45
120


Coeff of Variation of Testosterone concentration (ng/mg)
with hCG Stimulation
Coefficient of Variation Percent
70
60
50
40
30
20
10
0
10
20
30
40
50
60
70
80
90
100
110
120
Equilibration Time (minutes)
Time point
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)

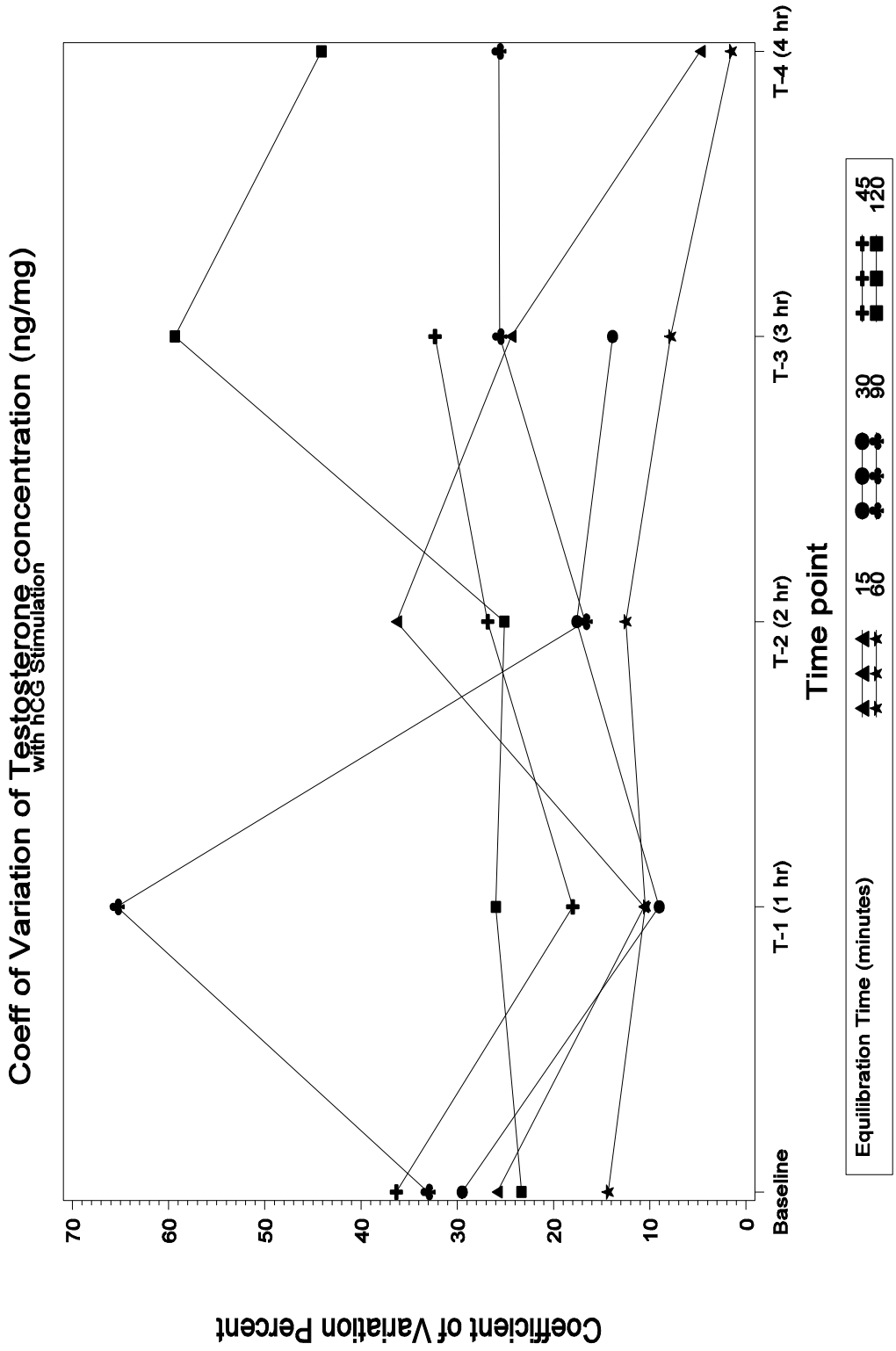

Coeff of Variation of Testosterone concentration (ng/mg)
with hCG Stimulation
Coefficient of Variation Percent
70
60
50
40
30
20
10
0
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)
Time point
Equilibration Time (minutes)
15
60
30
90
45
120

Table 81. Summary of Equilibration Experiment: Testosterone Concentration*(ng/mg) by Time Point With No hCG Stimulation

Equilibration Time (minutes)	Baseline	One Hour	Two Hours	Three Hours	Four Hours
15	0.0032 ± 0.0005	0.0306 ± 0.0007	0.0262 ± 0.0115	0.0297 ± 0.0023	0.0291 ± 0.0018
30	0.0036 ± 0.0011	0.0281 ± 0.0017	0.0297 ± 0.0021	0.0292 ± 0.0014	0.0290 ± 0.0028
45	0.0047 ± 0.0021	0.0259 ± 0.0088	0.0277 ± 0.0062	0.0249 ± 0.0061	0.0241 ± 0.0053
60	0.0078 ± 0.0019	0.0437 ± 0.0100	0.0417 ± 0.0054	0.0381 ± 0.0049	0.0358 ± 0.0036
90	0.0090 ± 0.0031	0.0379 ± 0.0168	0.0373 ± 0.0110	0.0325 ± 0.0107	0.0312 ± 0.0091
120	0.0099 ± 0.0032	0.0388 ± 0.0070	0.0398 ± 0.0098	0.0307 ± 0.0100	0.0331 ± 0.0073

*Mean ± Standard Deviation

Table 82. Summary of Equilibration Experiment: Testosterone Concentration*(ng/mg) by Time Point With hCG Stimulation

Equilibration Time (minutes)	Baseline	One Hour	Two Hours	Three Hours	Four Hours
15	0.0037 ± 0.0010	0.0613 ± 0.0065	0.1559 ± 0.0566	0.1941 ± 0.0474	0.2486 ± 0.0118
30	0.0027 ± 0.0008	0.0475 ± 0.0043	0.1052 ± 0.0185	0.1647 ± 0.0228	0.2385
45	0.0061 ± 0.0022	0.0566 ± 0.0102	0.1173 ± 0.0315	0.1793 ± 0.0579	0.2271
60	0.0084 ± 0.0012	0.1067 ± 0.0112	0.1909 ± 0.0238	0.2354 ± 0.0184	0.2994 ± 0.0045
90	0.0084 ± 0.0028	0.1400 ± 0.0915	0.1676 ± 0.0280	0.1998 ± 0.0512	0.2256 ± 0.0579
120	0.0099 ± 0.0023	0.0871 ± 0.0226	0.1375 ± 0.0345	0.2604 ± 0.1545	0.3510 ± 0.1548

* Mean ± Standard Deviation

Table 83. Summary of Equilibration Experiment: LDH Concentration*(mU/mg) by Time Point With hCG Stimulation

Equilibration Time (minutes)	Baseline	One Hour	Two Hours	Three Hours	Four Hours
15	354.41 ± 171.29	905.38 ± 209.98	954.41 ± 187.92	980.40 ± 169.82	950.10 ± 112.25
30	222.09 ± 116.15	836.43 ± 165.68	888.67 ± 170.96	960.60 ± 271.75	960.75 ± 174.67
45	593.58 ± 401.26	1124.30 ± 86.95	1178.90 ± 93.51	1162.10 ± 103.09	979.07 ± 521.55
60	209.03 ± 63.14	690.43 ± 163.34	816.12 ± 159.70	852.93 ± 175.44	885.82 ± 195.37
90	212.95 ± 14.40	640.24 ± 54.33	746.64 ± 104.75	868.05 ± 103.07	930.31 ± 86.25
120	793.85 ± 392.68	1350.90 ± 434.53	1433.7 ± 549.58	1404.60 ± 560.01	1059.00 ± 238.24

*Mean ± Standard Deviation

Table 84. Summary of Equilibration Experiment:: LDH Concentration*(mU/mg) by Time Point Without hCG Stimulation

Equilibration Time (minutes)	Baseline	One Hour	Two Hours	Three Hours	Four Hours
15	319.70 ± 81.15	1188.60 ± 145.34	1210.60 ± 186.70	1215.50 ± 239.77	1210.70 ± 318.54
30	154.78 ± 56.24	672.00 ± 167.85	746.71 ± 207.69	796.37 ± 208.42	812.27 ± 193.76
45	539.53 ± 447.15	1045.70 ± 14.70	1123.7 ± 69.18	1145.30 ± 122.95	874.54 ± 530.18
60	195.05 ± 93.26	758.28 ± 135.84	768.16 ± 60.95	853.44 ± 48.90	923.59 ± 134.76
90	153.17 ± 23.04	789.57 ± 68.56	862.97 ± 89.11	864.74 ± 148.08	995.41 ± 136.79
120	484.83 ± 401.82	1119.00 ± 250.03	1192.60 ± 283.24	1014.00 ± 460.71	1195.70 ± 472.9

*Mean ±Standard Deviation

12.0 SAMPLING TIMES

12.1 Statistical Analysis of Phase II Sampling Time Factors Determination

12.1.1 Objectives

The objective of the sampling time factors experiment were to determine the effect of sampling times on testosterone production using the sliced testis assay. Concentrations of LDH and testosterone were measured at the following time points: baseline and 0.5, 1, 2, 3, 4, 8, 12 and 24 hours. The sampling points of interest in this experiment are those at which the average concentration remains linear and at which the coefficient of variation settles down to an approximately constant value.

12.1.2 Data

A SAS data set was constructed from the raw Excel data file. Each observation includes concentration measurements for LDH and testosterone and the corresponding repeated measurement times. The data used in the analysis are displayed in Tables 85 and 86.

12.1.3 Statistical Analysis Methods and Results

In order to examine the effects of the sampling times on the concentrations of LDH and testosterone, summary statistics were calculated for each sampling time. Results of the means, standard deviations, sample sizes and coefficients of variation for LDH concentrations are shown in Tables 87 through 90 and the results for testosterone concentrations are shown in Tables 91 through 94. Graphs of the means and coefficients of variation for LDH and testosterone concentrations are presented at the end of the report.

Table 85. Sampling Time Factors Data for No hCG Stimulation

Sample_ID	Timepoint	LDH (mU/mg)	Testosterone (ng/mg)
A	Baseline	147.9	0.0033
A	T-1 (0.5 hr)	834.3	0.0318
A	T-2 (1 hr)	822.5	0.0338
A	T-3 (2 hr)	964.5	0.0420
A	T-4 (3 hr)	1142.0	0.0404
A	T-5 (4 hr)	1355.0	0.0402
A	T-6 (8 hr)	2763.3	0.0376
A	T-7 (12 hr)	3171.6	0.0373
A	T-8 (24 hr)	4053.3	0.0270
B	Baseline	84.9	0.0027
B	T-1 (0.5 hr)	928.7	0.0270
B	T-2 (1 hr)	860.7	0.0300
B	T-3 (2 hr)	1002.3	0.0370
B	T-4 (3 hr)	1126.8	0.0366
B	T-5 (4 hr)	1228.8	0.0307
B	T-6 (8 hr)	2463.2	0.0341
B	T-7 (12 hr)	2769.0	0.0260
B	T-8 (24 hr)	3023.8	0.0191
C	Baseline	135.3	0.0041
C	T-1 (0.5 hr)	641.2	0.0361
C	T-2 (1 hr)	770.6	0.0348
C	T-3 (2 hr)	852.9	0.0462
C	T-4 (3 hr)	1023.5	0.0474
C	T-5 (4 hr)	1370.6	0.0448
C	T-6 (8 hr)	3441.2	0.0448
C	T-7 (12 hr)	3811.8	0.0358
C	T-8 (24 hr)	3747.1	0.0314
D	Baseline	198.0	0.0043
D	T-1 (0.5 hr)	741.0	0.0295
D	T-2 (1 hr)	763.6	0.0314
D	T-3 (2 hr)	893.7	0.0592
D	T-4 (3 hr)	1295.2	0.0413
D	T-5 (4 hr)	1583.7	0.0390

Table 85. Sampling Time Factors Data for No hCG Stimulation, (Continued)

Sample_ID	Timepoint	LDH (mU/mg)	Testosterone (ng/mg)
D	T-6 (8 hr)	2856.3	0.0371
D	T-7 (12 hr)	3535.1	0.0314
D	T-8 (24 hr)	2867.6	0.0243
E	Baseline	140.5	0.0041
E	T-1 (0.5 hr)	696.9	0.0305
E	T-2 (1 hr)	659.1	0.0317
E	T-3 (2 hr)	756.3	0.0347
E	T-4 (3 hr)	956.2	0.0348
E	T-5 (4 hr)	1053.5	0.0332
E	T-6 (8 hr)	2582.4	0.0365
E	T-7 (12 hr)	3446.8	0.0280
E	T-8 (24 hr)	3463.0	0.0216

Table 86. Sampling Time Factors Data for hCG Stimulation

Sample ID	Timepoint	LDH (mU/mg)	Testosterone (ng/mg)
AC	Baseline	104.0	0.0029
AC	T-1 (0.5 hr)	630.4	0.0278
AC	T-2 (1 hr)	636.5	0.0348
AC	T-3 (2 hr)	795.6	0.0636
AC	T-4 (3 hr)	1003.7	0.0867
AC	T-5 (4 hr)	1211.8	0.1035
AC	T-6 (8 hr)	2894.7	0.1228
AC	T-7 (12 hr)	3237.5	0.1045
AC	T-8 (24 hr)	3445.5	0.0797
BC	Baseline	160.6	0.0047
BC	T-1 (0.5 hr)	725.4	0.0352
BC	T-2 (1 hr)	664.5	0.0352
BC	T-3 (2 hr)	858.3	0.0815
BC	T-4 (3 hr)	952.4	0.0406
BC	T-5 (4 hr)	1096.3	0.1039
BC	T-6 (8 hr)	2530.5	0.1297
BC	T-7 (12 hr)	3028.8	0.0782
BC	T-8 (24 hr)	3693.2	0.0683
CC	Baseline	234.2	0.0047
CC	T-1 (0.5 hr)	626.4	0.0294
CC	T-2 (1 hr)	697.2	0.0401
CC	T-3 (2 hr)	866.0	0.0566
CC	T-4 (3 hr)	1029.4	0.1016
CC	T-5 (4 hr)	1345.3	0.1039
CC	T-6 (8 hr)	2963.0	0.1166
CC	T-7 (12 hr)	3507.6	0.0731
CC	T-8 (24 hr)	3104.6	0.0650
DC	Baseline	187.4	0.0050
DC	T-1 (0.5 hr)	871.0	0.0382
DC	T-2 (1 hr)	815.9	0.0476
DC	T-3 (2 hr)	970.2	0.0828
DC	T-4 (3 hr)	1102.5	0.1125
DC	T-5 (4 hr)	1185.2	0.1355

Table 86. Sampling Time Factors Data for hCG Stimulation, (Continued)

Sample ID	Timepoint	LDH (mU/mg)	Testosterone (ng/mg)
DC	T-6 (8 hr)	2817.0	0.2103
DC	T-7 (12 hr)	3847.9	0.1407
DC	T-8 (24 hr)	2932.7	0.1113
EC	Baseline	112.9	0.0041
EC	T-1 (0.5 hr)	807.0	0.0270
EC	T-2 (1 hr)	807.0	0.0318
EC	T-3 (2 hr)	953.7	0.0501
EC	T-4 (3 hr)	1038.4	0.0562
EC	T-5 (4 hr)	1179.5	0.0884
EC	T-6 (8 hr)	2590.3	0.0980
EC	T-7 (12 hr)	3820.5	0.0870
EC	T-8 (24 hr)	2844.2	0.0678

Table 87. Means of LDH Concentration (mU/mg) by hCG Stimulation

Time point	No hCG Stimulation	With hCG Stimulation
Baseline	141.3	159.8
T-1 (0.5 hr)	768.4	732.0
T-2 (1 hr)	775.3	724.2
T-3 (2 hr)	893.9	888.8
T-4 (3 hr)	1,108.8	1,025.3
T-5 (4 hr)	1,318.3	1,203.6
T-6 (8 hr)	2,821.3	2,759.1
T-7 (12 hr)	3,346.8	3,488.5
T-8 (24 hr)	3,430.9	3,204.1

Table 88. Standard Deviations of LDH Concentration (mU/mg) by hCG Stimulation

Time point	No hCG Stimulation	With hCG Stimulation
Baseline	40.2	53.9
T-1 (0.5 hr)	114.1	107.8
T-2 (1 hr)	76.1	82.5
T-3 (2 hr)	96.6	72.4
T-4 (3 hr)	129.2	54.6
T-5 (4 hr)	195.4	90.2
T-6 (8 hr)	378.9	189.8
T-7 (12 hr)	395.7	358.5
T-8 (24 hr)	492.8	357.3

Table 89. Sample sizes of LDH concentration (mU/mg) by hCG stimulation

Time point	No hCG Stimulation	With hCG Stimulation
Baseline	5	5
T-1 (0.5 hr)	5	5
T-2 (1 hr)	5	5
T-3 (2 hr)	5	5
T-4 (3 hr)	5	5
T-5 (4 hr)	5	5
T-6 (8 hr)	5	5
T-7 (12 hr)	5	5

Time point	No hCG Stimulation	With hCG Stimulation
T-8 (24 hr)	5	5

Table 90. Coeff of Variation of LDH Concentration (mU/mg) by hCG Stimulation

Time point	No hCG Stimulation	With hCG Stimulation
Baseline	28.5	33.7
T-1 (0.5 hr)	14.8	14.7
T-2 (1 hr)	9.8	11.4
T-3 (2 hr)	10.8	8.2
T-4 (3 hr)	11.7	5.3
T-5 (4 hr)	14.8	7.5
T-6 (8 hr)	13.4	6.9
T-7 (12 hr)	11.8	10.3
T-8 (24 hr)	14.4	11.2

Table 91. Means of Testosterone concentration (ng/mg) by hCG stimulation

Time point	No hCG Stimulation	With hCG Stimulation
Baseline	0.0037	0.0043
T-1 (0.5 hr)	0.0310	0.0315
T-2 (1 hr)	0.0323	0.0379
T-3 (2 hr)	0.0438	0.0669
T-4 (3 hr)	0.0401	0.0795
T-5 (4 hr)	0.0376	0.1071
T-6 (8 hr)	0.0380	0.1369
T-7 (12 hr)	0.0317	0.1013
T-8 (24 hr)	0.0247	0.0784

Table 92. Standard Deviations of Testosterone concentration (ng/mg) by hCG Stimulation

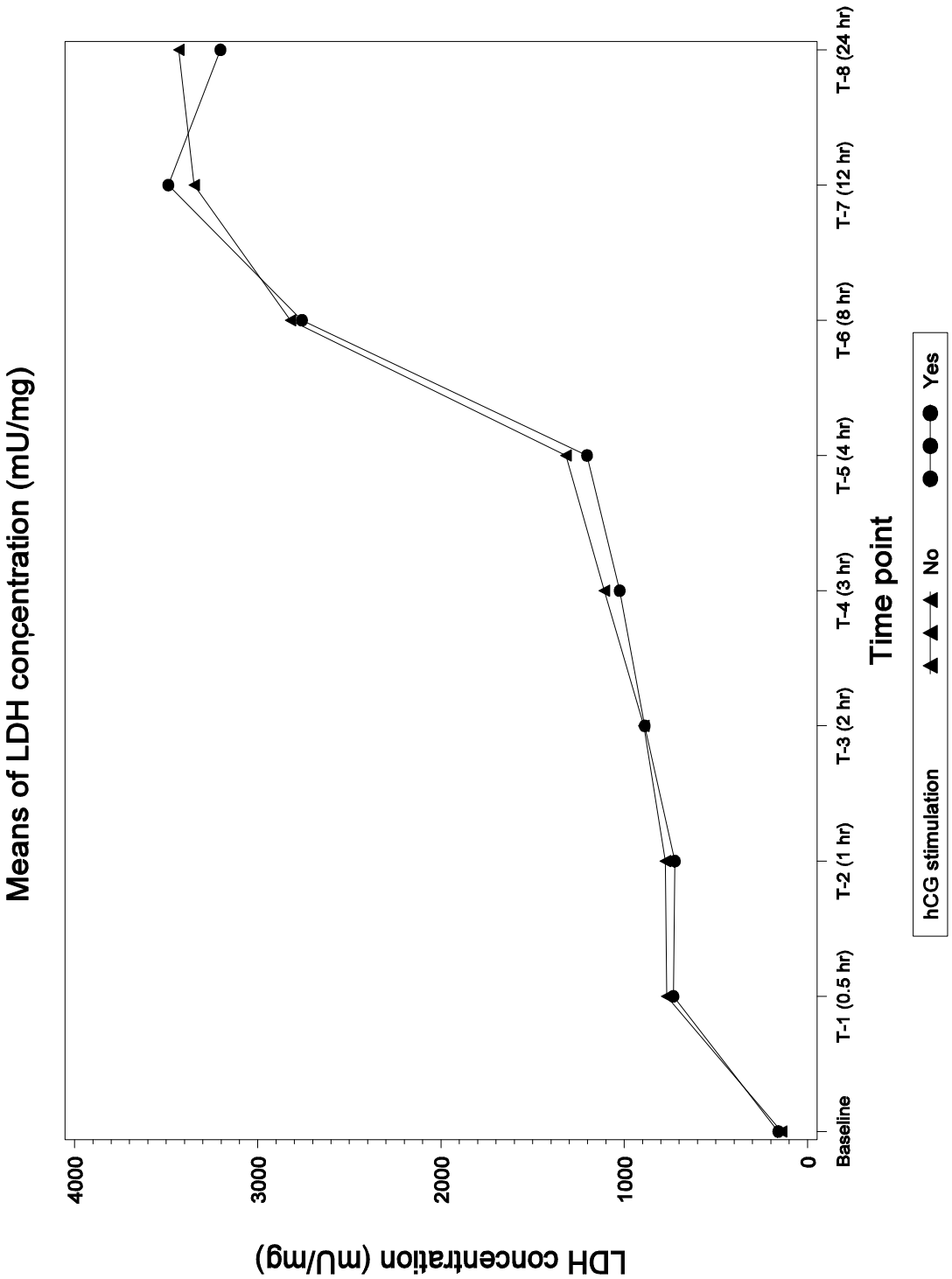
Time point	No hCG Stimulation	With hCG Stimulation
Baseline	0.0007	0.0008
T-1 (0.5 hr)	0.0034	0.0049
T-2 (1 hr)	0.0020	0.0062
T-3 (2 hr)	0.0097	0.0147
T-4 (3 hr)	0.0049	0.0303
T-5 (4 hr)	0.0056	0.0172
T-6 (8 hr)	0.0040	0.0501
T-7 (12 hr)	0.0049	0.0292
T-8 (24 hr)	0.0048	0.0192

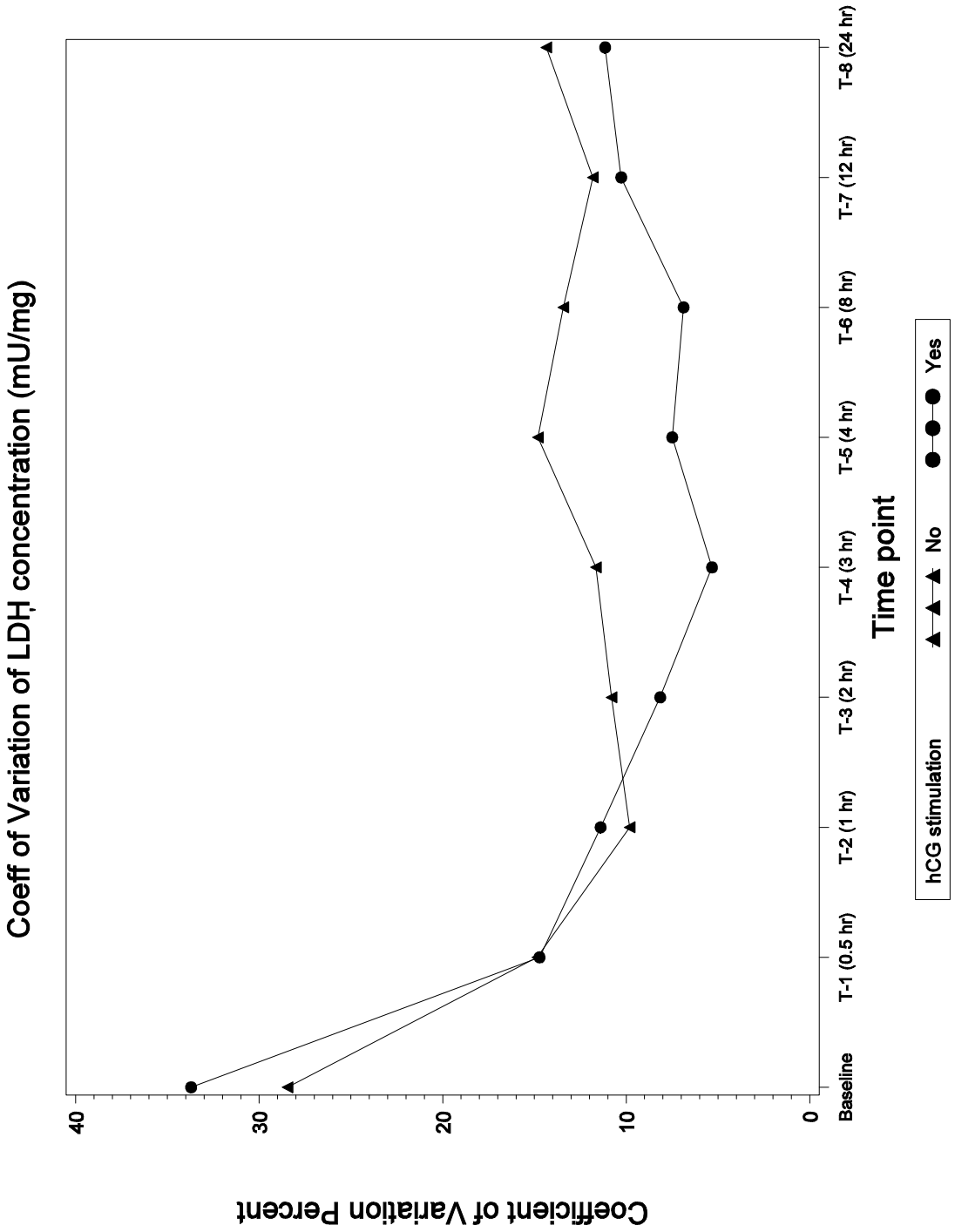
Table 93. Sample sizes of Testosterone concentration (ng/mg) by hCG Stimulation

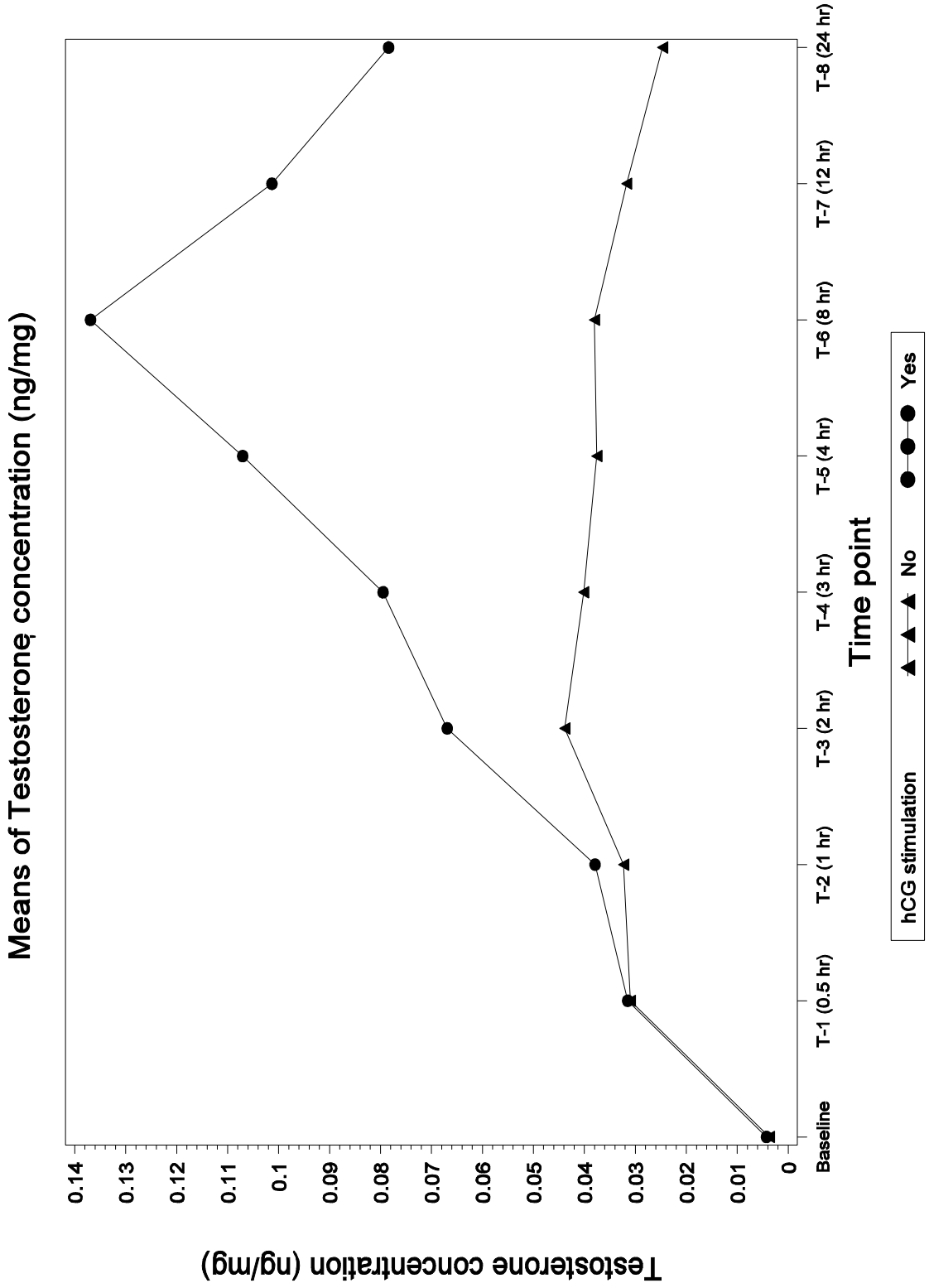
Timepoint	No hCG Stimulation	With hCG Stimulation
Baseline	5	5
T-1 (0.5 hr)	5	5
T-2 (1 hr)	5	5
T-3 (2 hr)	5	5
T-4 (3 hr)	5	5
T-5 (4 hr)	5	5
T-6 (8 hr)	5	4
T-7 (12 hr)	5	4
T-8 (24 hr)	5	5

Table 94. Coeff of Variation of Testosterone concentration (ng/mg) by hCG stimulation

Timepoint	No hCG Stimulation	With hCG Stimulation
Baseline	18.6	19.6
T-1 (0.5 hr)	10.8	15.6
T-2 (1 hr)	6.1	16.3
T-3 (2 hr)	22.1	22.0
T-4 (3 hr)	12.1	38.1
T-5 (4 hr)	14.9	16.1
T-6 (8 hr)	10.5	36.6
T-7 (12 hr)	15.3	28.8
T-8 (24 hr)	19.4	24.5


Means of LDH concentration (mU/mg)
LDH concentration (mU/mg)
4000
3000
2000
1000
0
Baseline
T-1 (0.5 hr)
T-2 (1 hr)
T-3 (2 hr)
T-4 (3 hr)
T-5 (4 hr)
T-6 (8 hr)
T-7 (12 hr)
T-8 (24 hr)
Time point
hCG stimulation
No
Yes


Coeff of Variation of LDH concentration (mU/mg)
Coefficient of Variation Percent
40
30
20
10
0
Baseline
T-1 (0.5 hr)
T-2 (1 hr)
T-3 (2 hr)
T-4 (3 hr)
T-5 (4 hr)
T-6 (8 hr)
T-7 (12 hr)
T-8 (24 hr)
Time point
hCG stimulation
No
Yes


Means of Testosterone concentration (ng/mg)
Testosterone concentration (ng/mg)
0.14
0.13
0.12
0.11
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Baseline
T-1 (0.5 hr)
T-2 (1 hr)
T-3 (2 hr)
T-4 (3 hr)
T-5 (4 hr)
T-6 (8 hr)
T-7 (12 hr)
T-8 (24 hr)
Time point
hCG stimulation
No
Yes

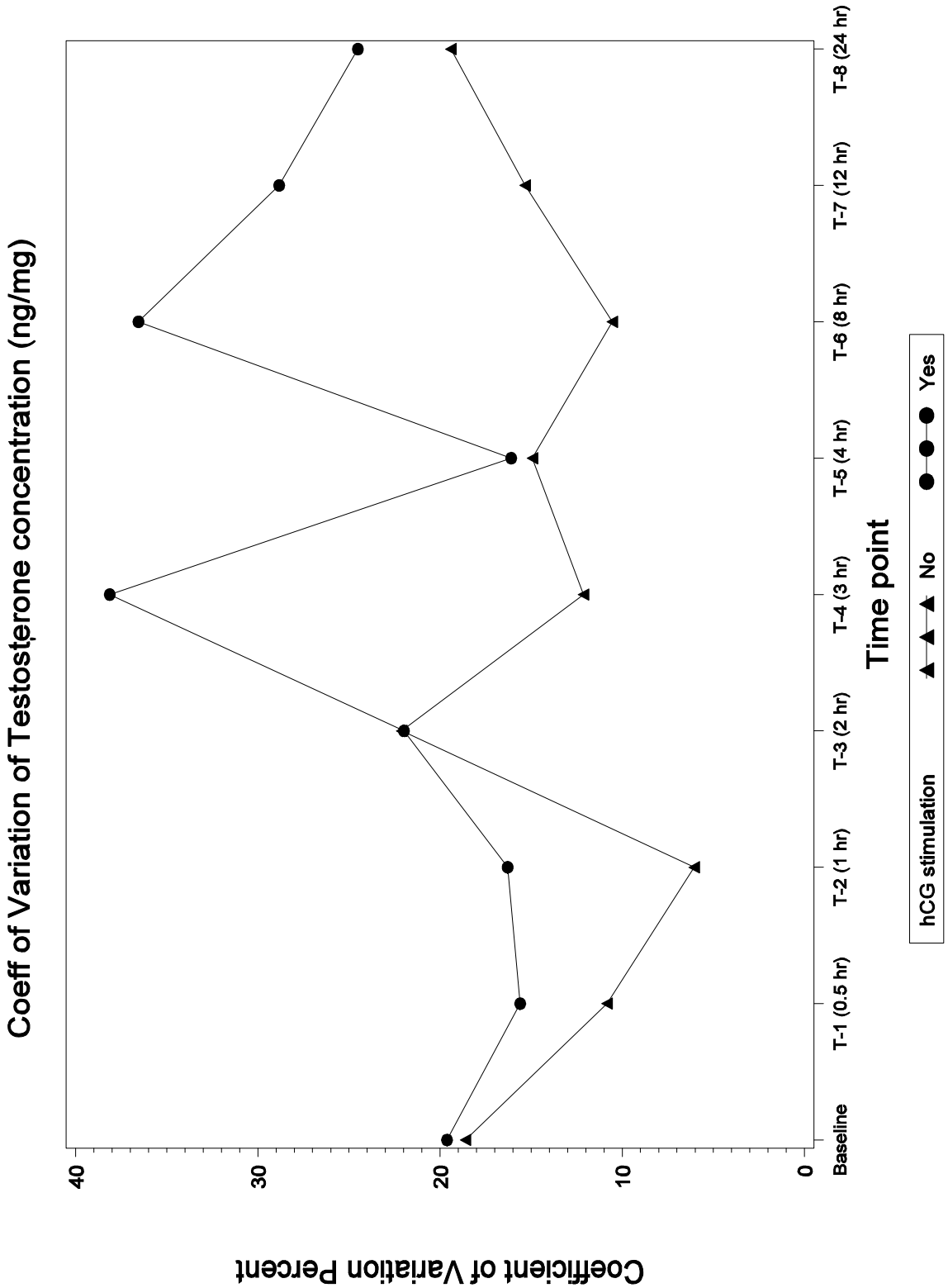

Coeff of Variation of Testosterone concentration (ng/mg)
Coefficient of Variation Percent
0
10
20
30
40
Baseline
T-1 (0.5 hr)
T-2 (1 hr)
T-3 (2 hr)
T-4 (3 hr)
T-5 (4 hr)
T-6 (8 hr)
T-7 (12 hr)
T-8 (24 hr)
Time point
hCG stimulation
No
Yes

Table 95. Summary of Sampling Times Experiment: Testosterone Concentration* (ng/mg) by Sampling Time Points over 24 Hours

hCG Stimulation	Baseline	0.5 hr	1 hr	2 hr	3 hr	4 hr	8 hr	12 hr	24 hr
No	0.0037 ± 0.0007	0.0310 ±0.0034	0.0323 ±0.0020	0.0438 ±0.0097	0.0401 ±0.0049	0.0376 ±0.0056	0.0380 ±0.0040	0.0317 ±0.0049	0.0247 ±0.0048
Yes	0.0043 ±0.0008	0.0315 ±0.0049	0.0379 ±0.0062	0.0669 ±0.0147	0.0795 ±0.0303	0.1071 ±0.0172	0.1369 ±0.0501	0.1013 ±0.0292	0.0784 ±0.0192

*Means ± Standard Deviation

Table 96 . Summary of Sampling Times Experiment: LDH Concentration* (mU/mg) by Sampling Time Points over 24 Hours

hCG Stimulation	Baseline	0.5 hr	1 hr	2 hr	3 hr	4 hr	8 hr	12 hr	24 hr
No	141.32 ±40.21	768.40 ±114.10	775.29 ±76.13	893.94 ±96.60	1108.8 ±129.17	1318.3 ±195.36	2821.3 ±378.87	3346.8 ±395.67	3430.9 ±492.78
Yes	159.82 ±53.88	732.02 ±107.8	724.19 ±82.55	888.76 ±72.43	1025.3 ±54.63	1203.6 ±90.24	2759.1 ±189.79	3488.5 ±358.51	3204.1 ±357.28

*Means ± Standard Deviation

13.0 INDUCED PARENCHYMAL FRAGMENT INJURY

13.1. Statistical Analysis of Phase II LDH Determination—Induced Parenchymal Fragment Injury

13.1.1 Objectives

The objective of the induced parenchymal fragment injury experiment is to determine the effect of various types of injuries on LDH concentration. The assay was performed with and without hCG stimulation under three different inflicted injury types: chemical, heat and trauma. Concentrations of LDH and testosterone for each sample were measured at baseline and at 1, 2, 3 and 4 hours after baseline.

13.1.2 Data

A SAS data set was constructed from the raw Excel data file. Each observation includes concentration measurements for LDH and testosterone, the type of injury inflicted on the sample and the corresponding repeated measurement times. The data used in the analysis are displayed in Tables 97 and 98.

13.1.3 Statistical Analysis Methods and Results

In order to examine the effects of the injury types on the concentrations of LDH and testosterone, summary statistics were calculated for each injury type. Results of the means, standard deviations, sample sizes and coefficients of variation for LDH concentrations are shown in Tables 99 through 106 and the results for testosterone concentrations are shown in Tables 107 through 114. Graphs of the means and coefficients of variation for LDH and testosterone concentrations are presented at the end of the section report.

Table 97. Induced Parenchymal Fragment Injury Data for No hCG Stimulation

Sample ID	Timepoint	Injury	LDH (mU/mg)	Testosterone (ng/mg)
A	Baseline	Heat	390.9	0.0012
A	T-1 (1 hr)	Heat	1455.3	0.0081
A	T-2 (2 hr)	Heat	1742.2	0.0095
A	T-3 (3 hr)	Heat	1950.1	0.0087
A	T-4 (4 hr)	Heat	2241.2	0.0083
B	Baseline	Heat	132.2	0.0011
B	T-1 (1 hr)	Heat	941.5	0.0078
B	T-2 (2 hr)	Heat	1121.8	0.0078
B	T-3 (3 hr)	Heat	1370.2	0.0081
B	T-4 (4 hr)	Heat	1718.8	0.0075
C	Baseline	Heat	310.9	0.0012
C	T-1 (1 hr)	Heat	779.3	0.0053
C	T-2 (2 hr)	Heat	1028.8	0.0061
C	T-3 (3 hr)	Heat	1220.7	0.0056
C	T-4 (4 hr)	Heat	1493.3	0.0054
D	Baseline	Heat	788.2	0.0012
D	T-1 (1 hr)	Heat	1216.4	0.0060
D	T-2 (2 hr)	Heat	1356.6	0.0069
D	T-3 (3 hr)	Heat	1583.9	0.0059
D	T-4 (4 hr)	Heat	1879.5	0.0058
E	Baseline	Heat	541.4	0.0017
E	T-1 (1 hr)	Heat	1094.2	0.0068
E	T-2 (2 hr)	Heat	1361.0	0.0070
E	T-3 (3 hr)	Heat	1608.8	0.0072
E	T-4 (4 hr)	Heat	1849.0	0.0061
F	Baseline	Heat	255.9	0.0015
F	T-1 (1 hr)	Heat	1012.4	0.0066
F	T-2 (2 hr)	Heat	1309.7	0.0070
F	T-3 (3 hr)	Heat	1614.6	0.0070
F	T-4 (4 hr)	Heat	1825.4	0.0063

Table 97. Induced Parenchymal Fragment Injury Data for No hCG Stimulation, (Continued)

Sample ID	Timepoint	Injury	LDH (mU/mg)	Testosterone (ng/mg)
G	Baseline	Trauma	243.9	0.0213
G	T-1 (1 hr)	Trauma	815.8	0.0196
G	T-2 (2 hr)	Trauma	811.6	0.0339
G	T-3 (3 hr)	Trauma	832.6	0.0327
G	T-4 (4 hr)	Trauma	878.9	0.0325
H	Baseline	Trauma	493.2	0.0030
H	T-1 (1 hr)	Trauma	978.0	0.0309
H	T-2 (2 hr)	Trauma	949.0	0.0330
H	T-3 (3 hr)	Trauma	944.9	0.0322
H	T-4 (4 hr)	Trauma	1036.1	0.0274
I	Baseline	Trauma	432.5	0.0040
I	T-1 (1 hr)	Trauma	774.2	0.0381
I	T-2 (2 hr)	Trauma	778.5	0.0456
I	T-3 (3 hr)	Trauma	787.2	0.0407
I	T-4 (4 hr)	Trauma	847.8	0.0378
J	Baseline	Trauma	242.8	0.0016
J	T-1 (1 hr)	Trauma	595.1	0.0410
J	T-2 (2 hr)	Trauma	665.6	0.0484
J	T-3 (3 hr)	Trauma	743.9	0.0491
J	T-4 (4 hr)	Trauma	947.5	0.0498
K	Baseline	Trauma	514.2	0.0034
K	T-1 (1 hr)	Trauma	913.8	0.0325
K	T-2 (2 hr)	Trauma	933.5	0.0330
K	T-3 (3 hr)	Trauma	925.6	0.0344
K	T-4 (4 hr)	Trauma	969.1	0.0299
L	Baseline	Trauma	460.7	0.0019
L	T-1 (1 hr)	Trauma	790.3	0.0364
L	T-2 (2 hr)	Trauma	802.2	0.0419
L	T-3 (3 hr)	Trauma	849.9	0.0415
L	T-4 (4 hr)	Trauma	961.1	0.0386
M	Baseline	Chemical	919.7	High
M	T-1 (1 hr)	Chemical	1554.2	0.1108
M	T-2 (2 hr)	Chemical	1421.7	0.1279
M	T-3 (3 hr)	Chemical	1160.6	0.0972

Table 97. Induced Parenchymal Fragment Injury Data for No hCG Stimulation, (Continued)

Sample ID	Timepoint	Injury	LDH (mU/mg)	Testosterone (ng/mg)
M	T-4 (4 hr)	Chemical	947.8	0.1133
N	Baseline	Chemical	1256.3	0.0854
N	T-1 (1 hr)	Chemical	2103.7	0.1341
N	T-2 (2 hr)	Chemical	1943.5	0.1162
N	T-3 (3 hr)	Chemical	1648.4	0.1165
N	T-4 (4 hr)	Chemical	1340.6	0.1006
O	Baseline	Chemical	884.0	0.1111
O	T-1 (1 hr)	Chemical	1629.7	0.1258
O	T-2 (2 hr)	Chemical	1223.3	0.1149
O	T-3 (3 hr)	Chemical	1206.5	0.1142
O	T-4 (4 hr)	Chemical	988.7	0.1055
P	Baseline	Chemical	863.9	High
P	T-1 (1 hr)	Chemical	1500.2	0.1366
P	T-2 (2 hr)	Chemical	1234.7	0.1540
P	T-3 (3 hr)	Chemical	1015.6	0.1290
P	T-4 (4 hr)	Chemical	813.3	0.1075
Q	Baseline	Chemical	929.9	0.1242
Q	T-1 (1 hr)	Chemical	1483.0	0.1092
Q	T-2 (2 hr)	Chemical	1210.4	0.1108
Q	T-3 (3 hr)	Chemical	973.9	0.1016
Q	T-4 (4 hr)	Chemical	793.6	0.0966
R	Baseline	Chemical	855.8	0.0997
R	T-1 (1 hr)	Chemical	1539.7	0.1058
R	T-2 (2 hr)	Chemical	1363.8	0.1232
R	T-3 (3 hr)	Chemical	1133.3	0.1277
R	T-4 (4 hr)	Chemical	937.9	0.0825

Table 98. Induced Parenchymal Fragment Injury Data for hCG Stimulation

Sample_ID	Timepoint	Injury	LDH (mU/mg)	Testosterone (ng/mg)
AC	Baseline	Heat	195.5	0.0014
AC	T-1 (1 hr)	Heat	1280.2	0.0079
AC	T-2 (2 hr)	Heat	1767.0	0.0084
AC	T-3 (3 hr)	Heat	2027.6	0.0084
AC	T-4 (4 hr)	Heat	2453.0	0.0079
BC	Baseline	Heat	121.0	0.0010
BC	T-1 (1 hr)	Heat	1147.7	0.0063
BC	T-2 (2 hr)	Heat	1644.4	0.0068
BC	T-3 (3 hr)	Heat	1944.9	0.0074
BC	T-4 (4 hr)	Heat	2220.4	0.0065
CC	Baseline	Heat	312.1	0.0016
CC	T-1 (1 hr)	Heat	1526.3	0.0081
CC	T-2 (2 hr)	Heat	1898.2	0.0094
CC	T-3 (3 hr)	Heat	2227.4	0.0087
CC	T-4 (4 hr)	Heat	2548.1	0.0086
DC	Baseline	Heat	272.3	0.0013
DC	T-1 (1 hr)	Heat	1214.1	0.0074
DC	T-2 (2 hr)	Heat	1633.9	0.0077
DC	T-3 (3 hr)	Heat	2015.9	0.0070
DC	T-4 (4 hr)	Heat	2341.1	0.0078
EC	Baseline	Heat	231.6	0.0013
EC	T-1 (1 hr)	Heat	1330.5	0.0070
EC	T-2 (2 hr)	Heat	1650.5	0.0082
EC	T-3 (3 hr)	Heat	1898.9	0.0083
EC	T-4 (4 hr)	Heat	2143.2	0.0099
FC	Baseline	Heat	281.5	0.0014
FC	T-1 (1 hr)	Heat	1441.2	0.0087
FC	T-2 (2 hr)	Heat	1705.9	0.0078
FC	T-3 (3 hr)	Heat	2016.8	0.0085
FC	T-4 (4 hr)	Heat	2357.1	0.0108
GC	Baseline	Trauma	845.5	0.0037
GC	T-1 (1 hr)	Trauma	778.8	0.0341
GC	T-2 (2 hr)	Trauma	853.8	0.0537
GC	T-3 (3 hr)	Trauma	903.8	0.0733
GC	T-4 (4 hr)	Trauma	1082.9	0.0947

Table 98. Induced Parenchymal Fragment Injury Data for hCG Stimulation (Continued)

Sample_ID	Timepoint	Injury	LDH (mU/mg)	Testosterone (ng/mg)
HC	Baseline	Trauma	646.6	0.0025
HC	T-1 (1 hr)	Trauma	814.7	0.0589
HC	T-2 (2 hr)	Trauma	866.4	0.1228
HC	T-3 (3 hr)	Trauma	931.0	0.1460
HC	T-4 (4 hr)	Trauma	1060.3	0.2081
IC	Baseline	Trauma	342.0	0.0027
IC	T-1 (1 hr)	Trauma	731.1	0.0438
IC	T-2 (2 hr)	Trauma	786.7	0.0703
IC	T-3 (3 hr)	Trauma	795.2	0.0956
IC	T-4 (4 hr)	Trauma	961.9	0.1105
JC	Baseline	Trauma	388.7	0.0022
JC	T-1 (1 hr)	Trauma	837.2	0.0358
JC	T-2 (2 hr)	Trauma	965.4	0.0679
JC	T-3 (3 hr)	Trauma	1008.1	0.0891
JC	T-4 (4 hr)	Trauma	1213.2	0.1214
KC	Baseline	Trauma	454.7	0.0031
KC	T-1 (1 hr)	Trauma	770.7	0.0296
KC	T-2 (2 hr)	Trauma	820.8	0.0492
KC	T-3 (3 hr)	Trauma	828.5	0.0625
KC	T-4 (4 hr)	Trauma	1009.6	0.0874
LC	Baseline	Trauma	349.8	0.0023
LC	T-1 (1 hr)	Trauma	718.3	0.0262
LC	T-2 (2 hr)	Trauma	815.0	0.0447
LC	T-3 (3 hr)	Trauma	841.1	0.0576
LC	T-4 (4 hr)	Trauma	908.1	0.0771
MC	Baseline	Chemical	968.4	0.1205
MC	T-1 (1 hr)	Chemical	1616.6	0.1083
MC	T-2 (2 hr)	Chemical	1347.1	High
MC	T-3 (3 hr)	Chemical	1089.4	0.2762
MC	T-4 (4 hr)	Chemical	870.8	0.2551
NC	Baseline	Chemical	927.3	0.0891
NC	T-1 (1 hr)	Chemical	2142.9	0.1298
NC	T-2 (2 hr)	Chemical	1783.6	0.1312
NC	T-3 (3 hr)	Chemical	1453.6	0.1772
NC	T-4 (4 hr)	Chemical	1173.8	0.2370
OC	Baseline	Chemical	1142.4	0.1318
OC	T-1 (1 hr)	Chemical	2003.1	0.1368
OC	T-2 (2 hr)	Chemical	1811.4	0.1433
OC	T-3 (3 hr)	Chemical	1561.0	High

Table 98. Induced Parenchymal Fragment Injury Data for hCG Stimulation (Continued)

Sample_ID	Timepoint	Injury	LDH (mU/mg)	Testosterone (ng/mg)
OC	T-4 (4 hr)	Chemical	1291.1	0.2996
PC	Baseline	Chemical	1038.3	0.1282
PC	T-1 (1 hr)	Chemical	1223.8	0.0971
PC	T-2 (2 hr)	Chemical	1046.2	0.1090
PC	T-3 (3 hr)	Chemical	884.3	0.2131
PC	T-4 (4 hr)	Chemical	730.4	0.1683
QC	Baseline	Chemical	1005.4	0.1091
QC	T-1 (1 hr)	Chemical	1416.7	0.1160
QC	T-2 (2 hr)	Chemical	1117.6	0.1032
QC	T-3 (3 hr)	Chemical	951.4	0.2807
QC	T-4 (4 hr)	Chemical	756.1	0.3867
RC	Baseline	Chemical	885.7	0.1446
RC	T-1 (1 hr)	Chemical	2396.5	0.1189
RC	T-2 (2 hr)	Chemical	2223.9	0.1296
RC	T-3 (3 hr)	Chemical	1894.2	High
RC	T-4 (4 hr)	Chemical	1549.1	0.1813

Table 99. Means of LDH concentration (mU/mg) by Type of Injury Induced No hCG Stimulation

Timepoint	Chemical	Heat	Trauma
Baseline	951.6	403.3	397.9
T-1 (1 hr)	1,635.1	1,083.2	811.2
T-2 (2 hr)	1,399.6	1,320.0	823.4
T-3 (3 hr)	1,189.7	1,558.1	847.4
T-4 (4 hr)	970.3	1,834.5	940.1

Table 100. Standard Deviations of LDH concentration (mU/mg) by Type of Injury Induced No hCG Stimulation

Timepoint	Chemical	Heat	Trauma
Baseline	152.2	232.9	122.9
T-1 (1 hr)	235.2	234.0	131.8
T-2 (2 hr)	279.9	247.2	105.3
T-3 (3 hr)	241.5	248.7	77.7
T-4 (4 hr)	197.4	243.9	67.6

Table 101. Sample sizes of LDH concentration (mU/mg) by Type of Injury Induced No hCG Stimulation

Timepoint	Chemical	Heat	Trauma
Baseline	6	6	6
T-1 (1 hr)	6	6	6
T-2 (2 hr)	6	6	6
T-3 (3 hr)	6	6	6
T-4 (4 hr)	6	6	6

Table 102. Coeff of Variation of LDH concentration (mU/mg) by Type of Injury Induced No hCG Stimulation

Timepoint	Chemical	Heat	Trauma
Baseline	16.0	57.7	30.9
T-1 (1 hr)	14.4	21.6	16.2
T-2 (2 hr)	20.0	18.7	12.8
T-3 (3 hr)	20.3	16.0	9.2
T-4 (4 hr)	20.3	13.3	7.2

Table 103. Means of LDH concentration (mU/mg) by Type of Injury Induced with hCG Stimulation

Timepoint	Chemical	Heat	Trauma
Baseline	994.6	235.7	504.6
T-1 (1 hr)	1,799.9	1,323.3	775.1
T-2 (2 hr)	1,555.0	1,716.7	851.4
T-3 (3 hr)	1,305.7	2,021.9	884.6
T-4 (4 hr)	1,061.9	2,343.8	1,039.3

Table 104. Standard Deviations of LDH concentration (mU/mg) by Type of Injury Induced with hCG Stimulation

Timepoint	Chemical	Heat	Trauma
Baseline	90.5	69.4	201.3
T-1 (1 hr)	453.2	141.3	46.1
T-2 (2 hr)	460.2	102.0	62.7
T-3 (3 hr)	395.8	112.6	78.5
T-4 (4 hr)	329.3	147.9	106.4

Table 105. Sample sizes of LDH concentration (mU/mg) by Type of Injury Induced with hCG Stimulation

Timepoint	Chemical	Heat	Trauma
Baseline	6	6	6
T-1 (1 hr)	6	6	6
T-2 (2 hr)	6	6	6
T-3 (3 hr)	6	6	6
T-4 (4 hr)	6	6	6

Table 106. Coeff of Variation of LDH concentration (mU/mg) by Type of Injury Induced with hCG Stimulation

Timepoint	Chemical	Heat	Trauma
Baseline	9.1	29.4	39.9
T-1 (1 hr)	25.2	10.7	5.9
T-2 (2 hr)	29.6	5.9	7.4
T-3 (3 hr)	30.3	5.6	8.9
T-4 (4 hr)	31.0	6.3	10.2

Table 107. Means of Testosterone concentration (ng/mg) by Type of Injury Induced No hCG Stimulation

Timepoint	Chemical	Heat	Trauma
Baseline	0.1051	0.0013	0.0059
T-1 (1 hr)	0.1204	0.0068	0.0331
T-2 (2 hr)	0.1245	0.0074	0.0393
T-3 (3 hr)	0.1144	0.0071	0.0384
T-4 (4 hr)	0.1010	0.0066	0.0360

Table 108. Standard Deviations of Testosterone concentration (ng/mg) by Type of Injury Induced No hCG Stimulation

Timepoint	Chemical	Heat	Trauma
Baseline	0.0165	0.0002	0.0076
T-1 (1 hr)	0.0135	0.0010	0.0076
T-2 (2 hr)	0.0157	0.0012	0.0069
T-3 (3 hr)	0.0130	0.0012	0.0066
T-4 (4 hr)	0.0107	0.0011	0.0081

Table 109. Sample sizes of Testosterone concentration (ng/mg) by Type of Injury Induced No hCG Stimulation

Timepoint	Chemical	Heat	Trauma
Baseline	4	6	6
T-1 (1 hr)	6	6	6
T-2 (2 hr)	6	6	6
T-3 (3 hr)	6	6	6
T-4 (4 hr)	6	6	6

Table 110. Coeff of Variation of Testosterone concentration (ng/mg) by Type of Injury Induced No hCG Stimulation

Timepoint	Chemical	Heat	Trauma
Baseline	15.7	16.4	129.6
T-1 (1 hr)	11.2	15.5	22.9
T-2 (2 hr)	12.6	15.8	17.6
T-3 (3 hr)	11.4	17.1	17.1
T-4 (4 hr)	10.6	16.7	22.4

Table 111. Means of Testosterone concentration (ng/mg) by Type of Injury Induced with hCG Stimulation

Timepoint	Chemical	Heat	Trauma
Baseline	0.1206	0.0013	0.0027
T-1 (1 hr)	0.1178	0.0076	0.0381
T-2 (2 hr)	0.1233	0.0081	0.0681
T-3 (3 hr)	0.2368	0.0081	0.0873
T-4 (4 hr)	0.2547	0.0086	0.1165

Table 112. Standard Deviations of Testosterone concentration (ng/mg) by Type of Injury Induced with hCG Stimulation

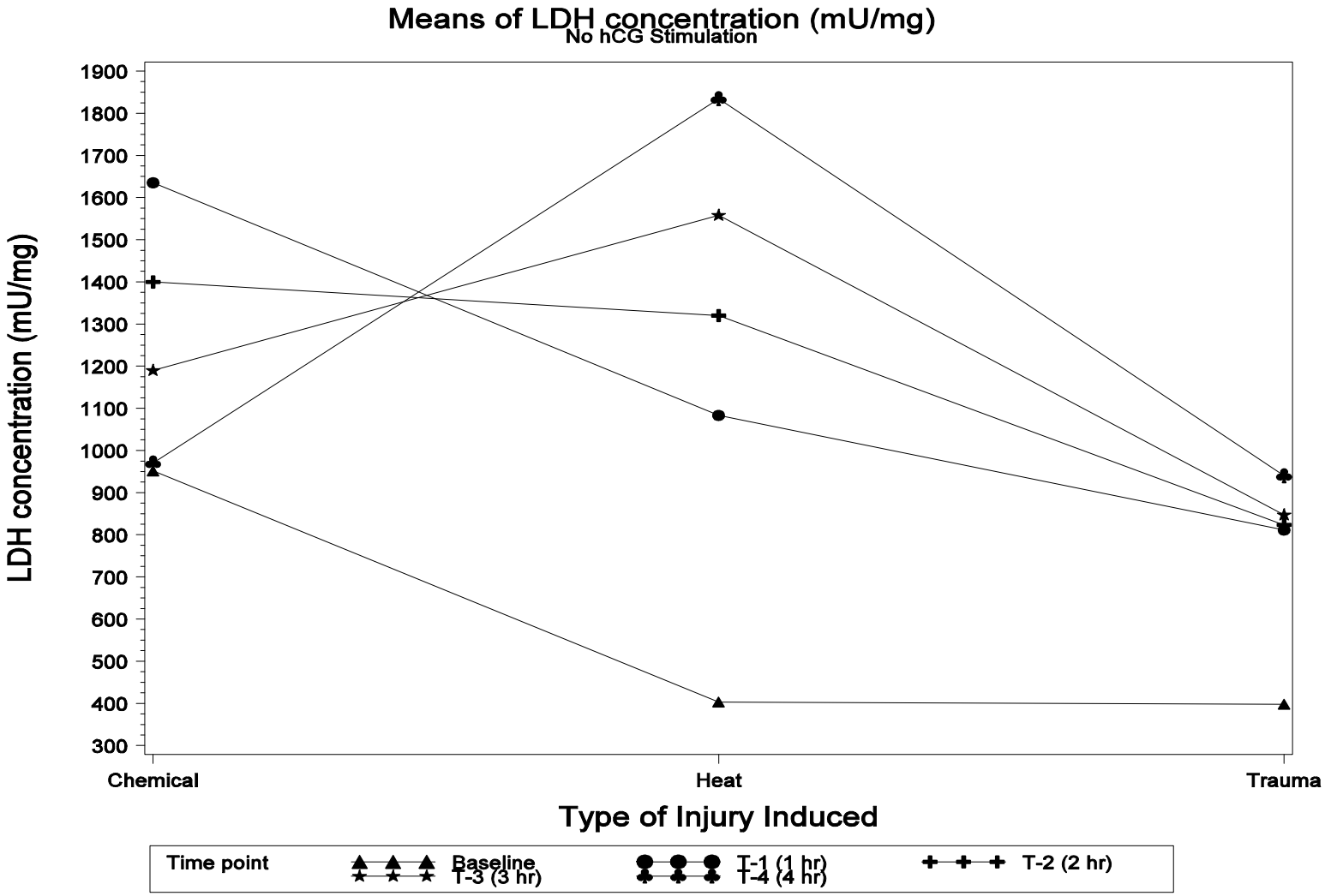
Timepoint	Chemical	Heat	Trauma
Baseline	0.0194	0.0002	0.0006
T-1 (1 hr)	0.0143	0.0008	0.0119
T-2 (2 hr)	0.0167	0.0009	0.0287
T-3 (3 hr)	0.0503	0.0007	0.0323
T-4 (4 hr)	0.0808	0.0016	0.0476

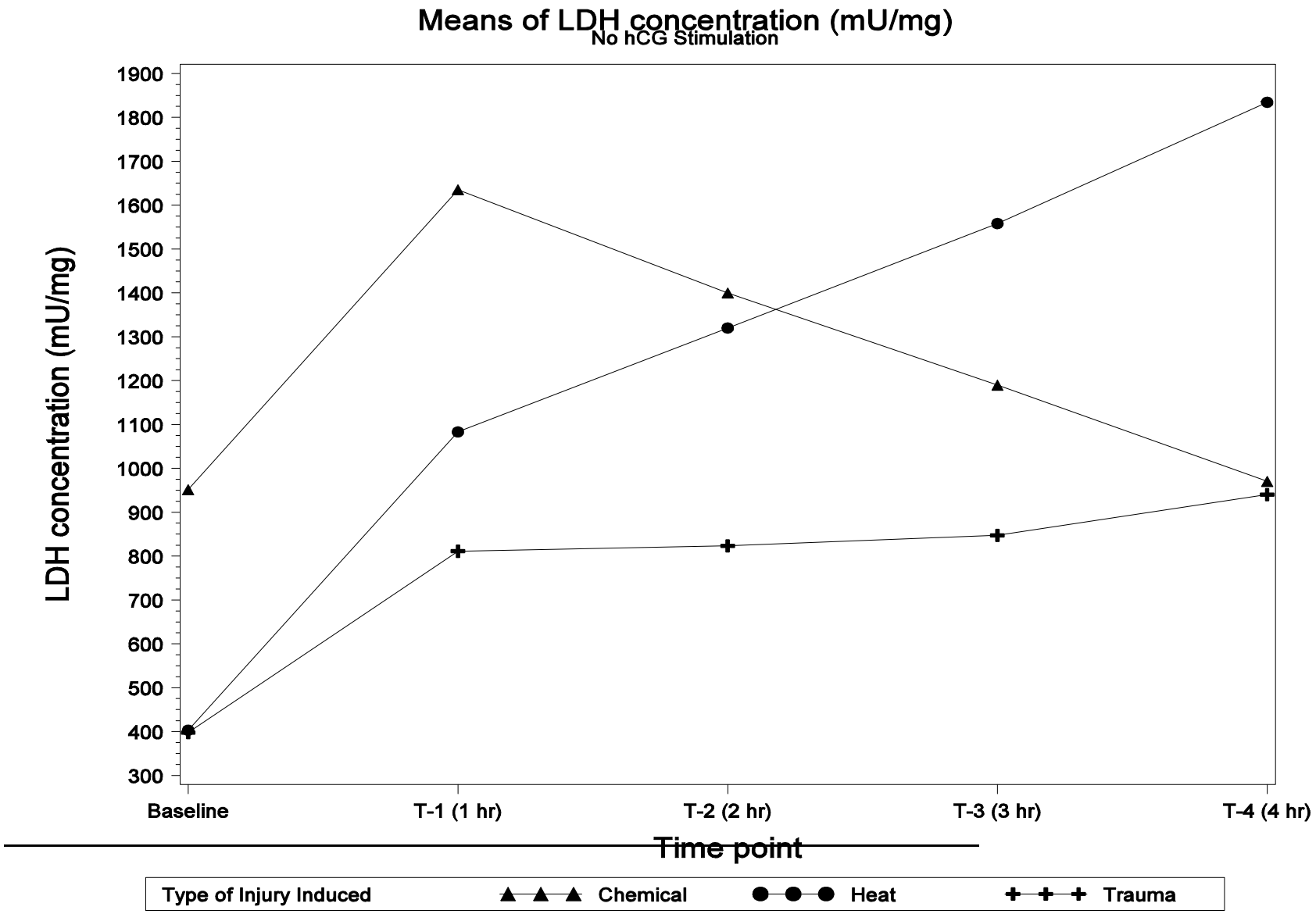
Table 113. Sample sizes of Testosterone concentration (ng/mg) by Type of Injury Induced with hCG Stimulation

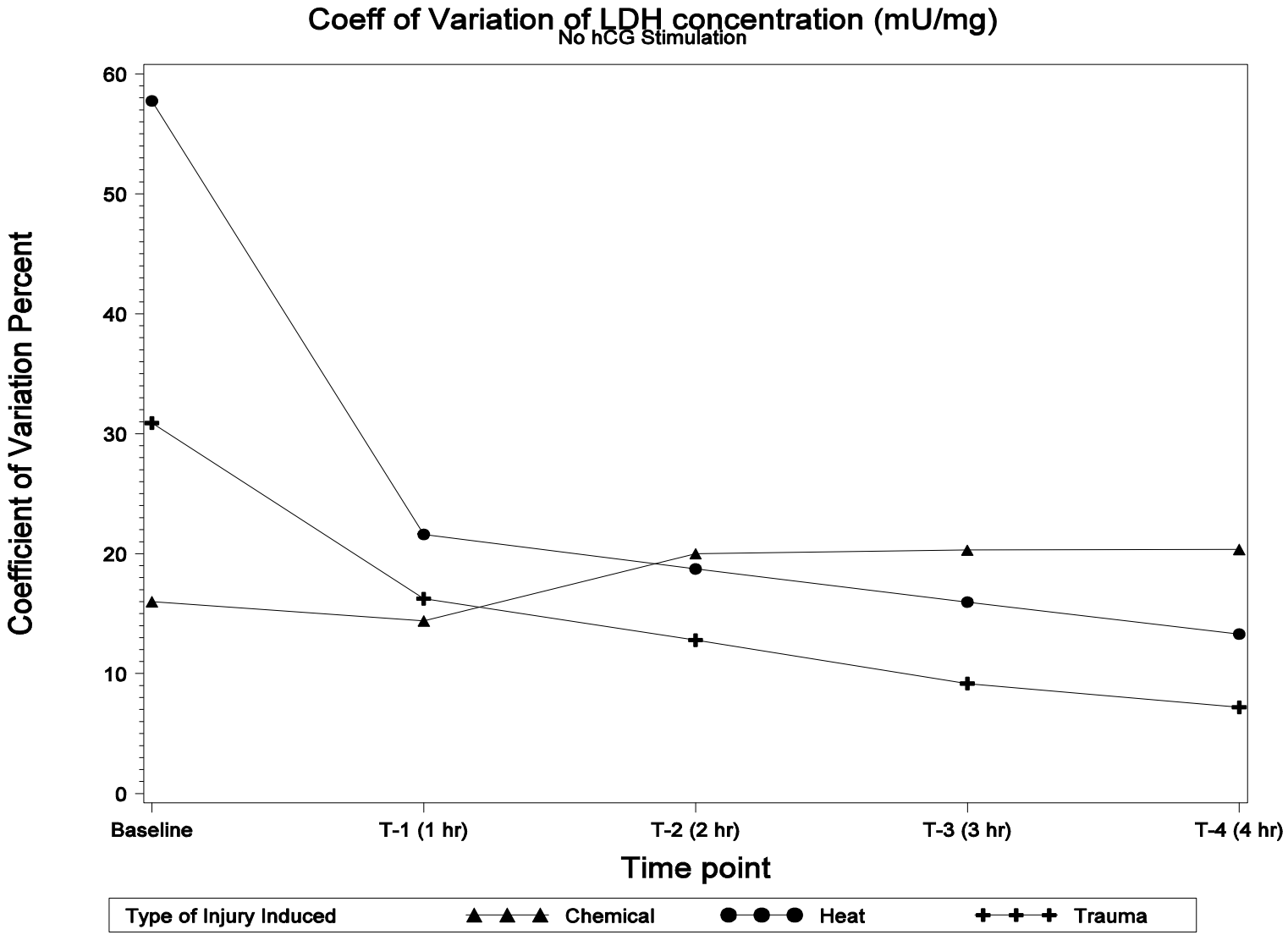
Timepoint	Chemical	Heat	Trauma
Baseline	6	6	6
T-1 (1 hr)	6	6	6
T-2 (2 hr)	5	6	6
T-3 (3 hr)	4	6	6
T-4 (4 hr)	6	6	6

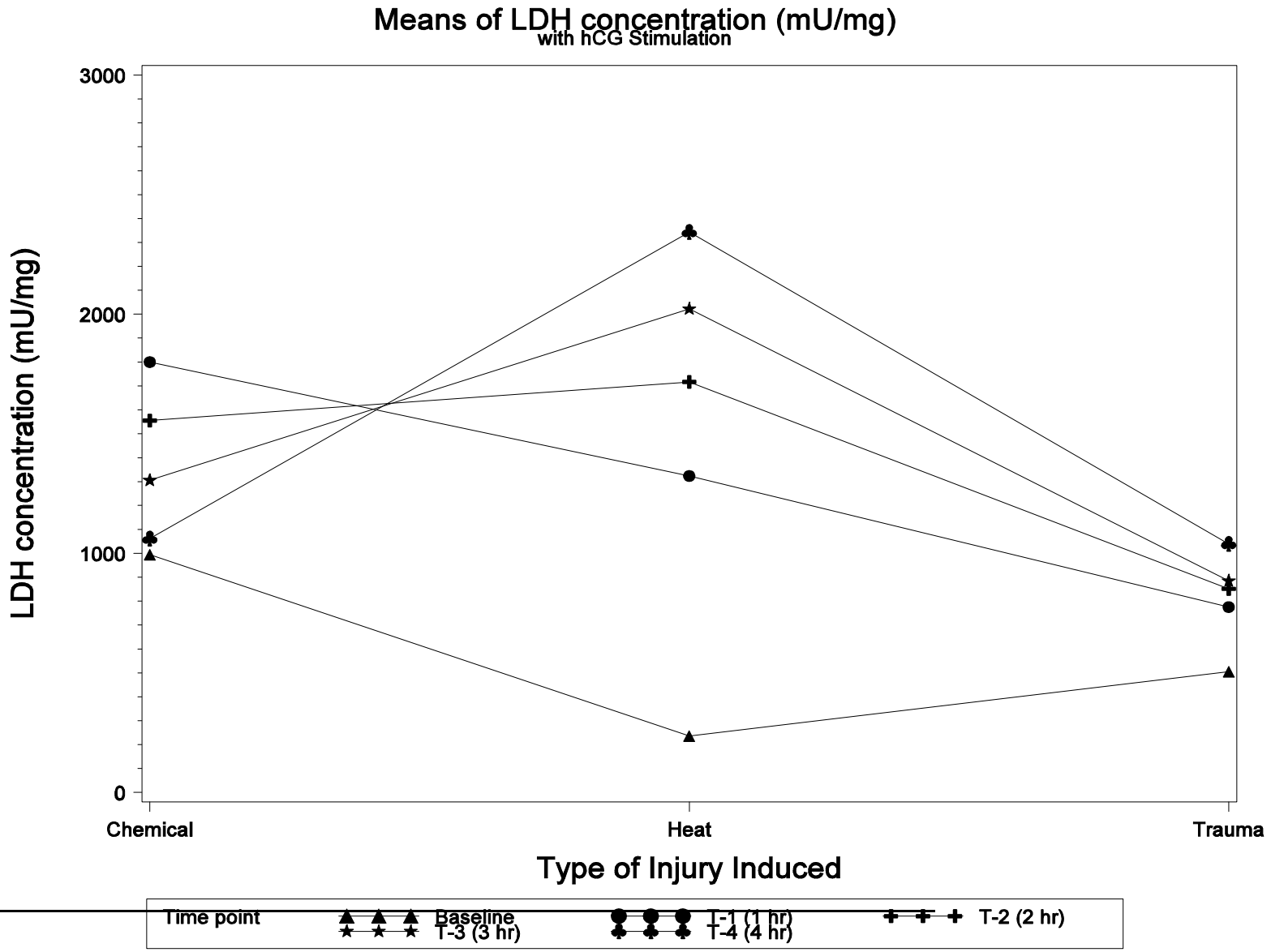
Table 114. Coeff of Variation of Testosterone concentration (ng/mg) by Type of Injury Induced with hCG Stimulation

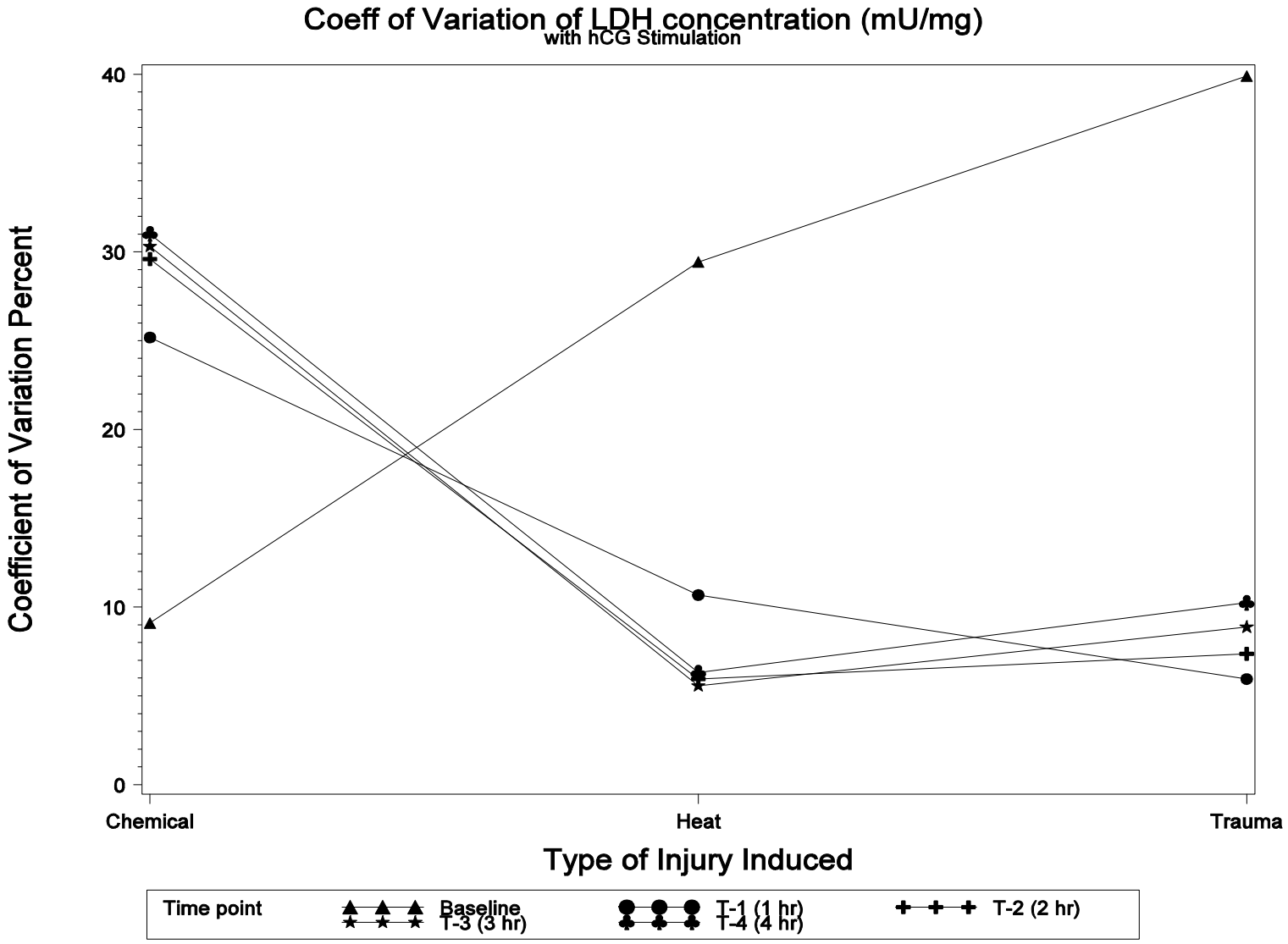
Timepoint	Chemical	Heat	Trauma
Baseline	16.1	15.5	20.2
T-1 (1 hr)	12.2	10.9	31.1
T-2 (2 hr)	13.5	10.8	42.1
T-3 (3 hr)	21.2	8.5	37.0
T-4 (4 hr)	31.7	18.3	40.8

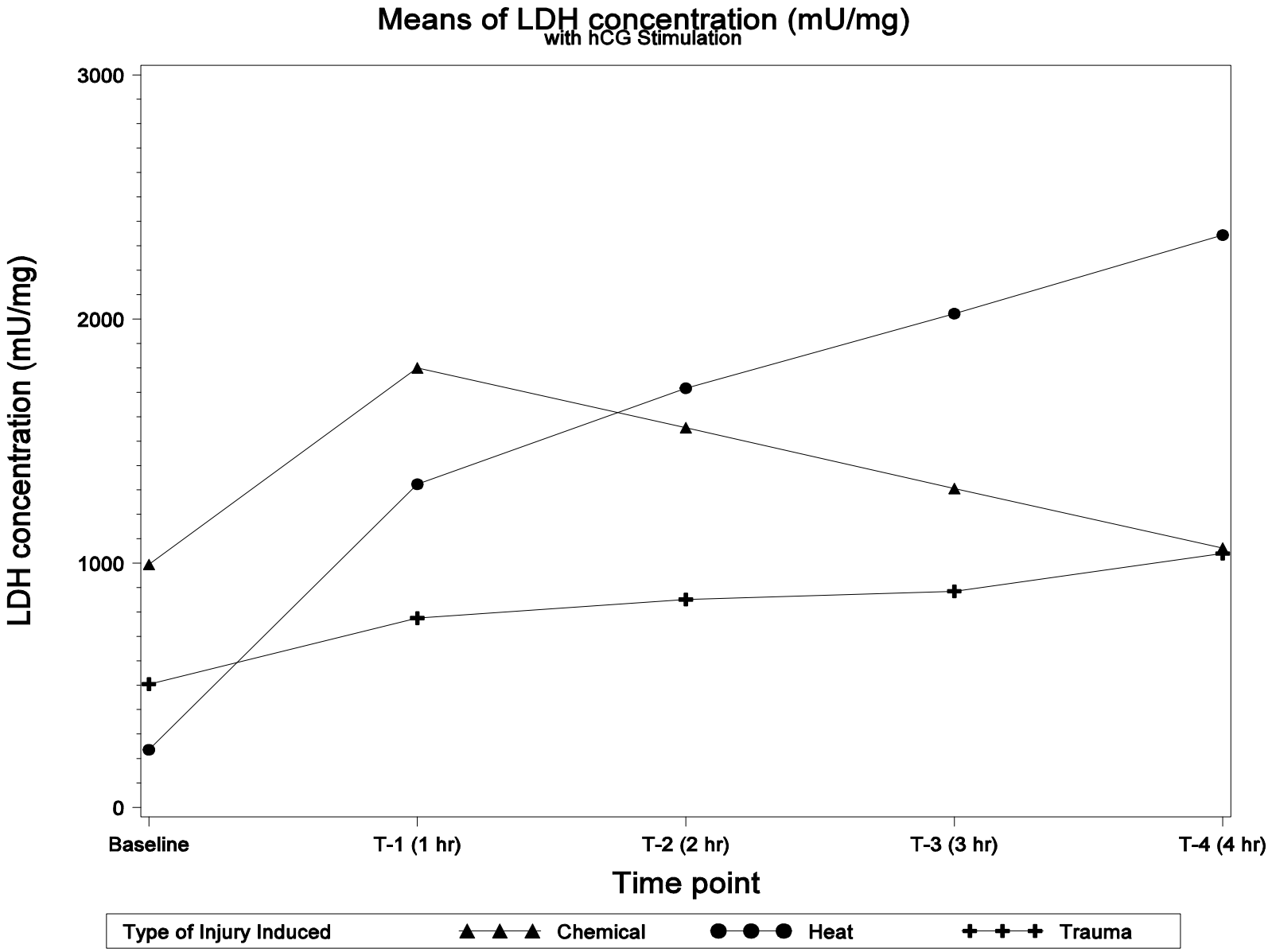

Means of LDH concentration (mU/mg)
No hCG Stimulation
LDH concentration (mU/mg)
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
Chemical
Heat
Trauma
Type of Injury Induced
Time point
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)

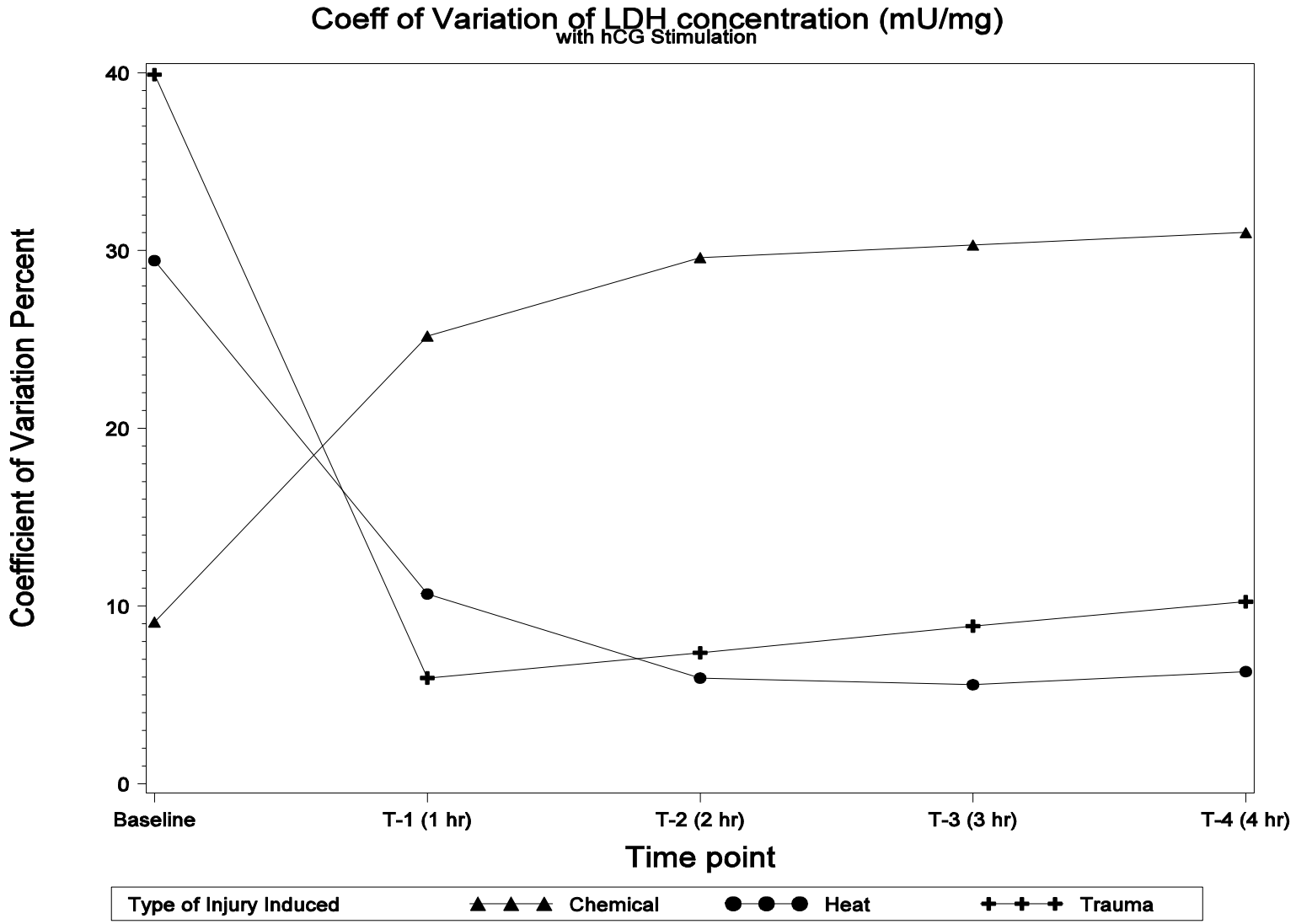

Means of LDH concentration (mU/mg)
No hCG Stimulation
LDH concentration (mU/mg)
1900
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)
Time point
Type of Injury Induced
Chemical
Heat
Trauma

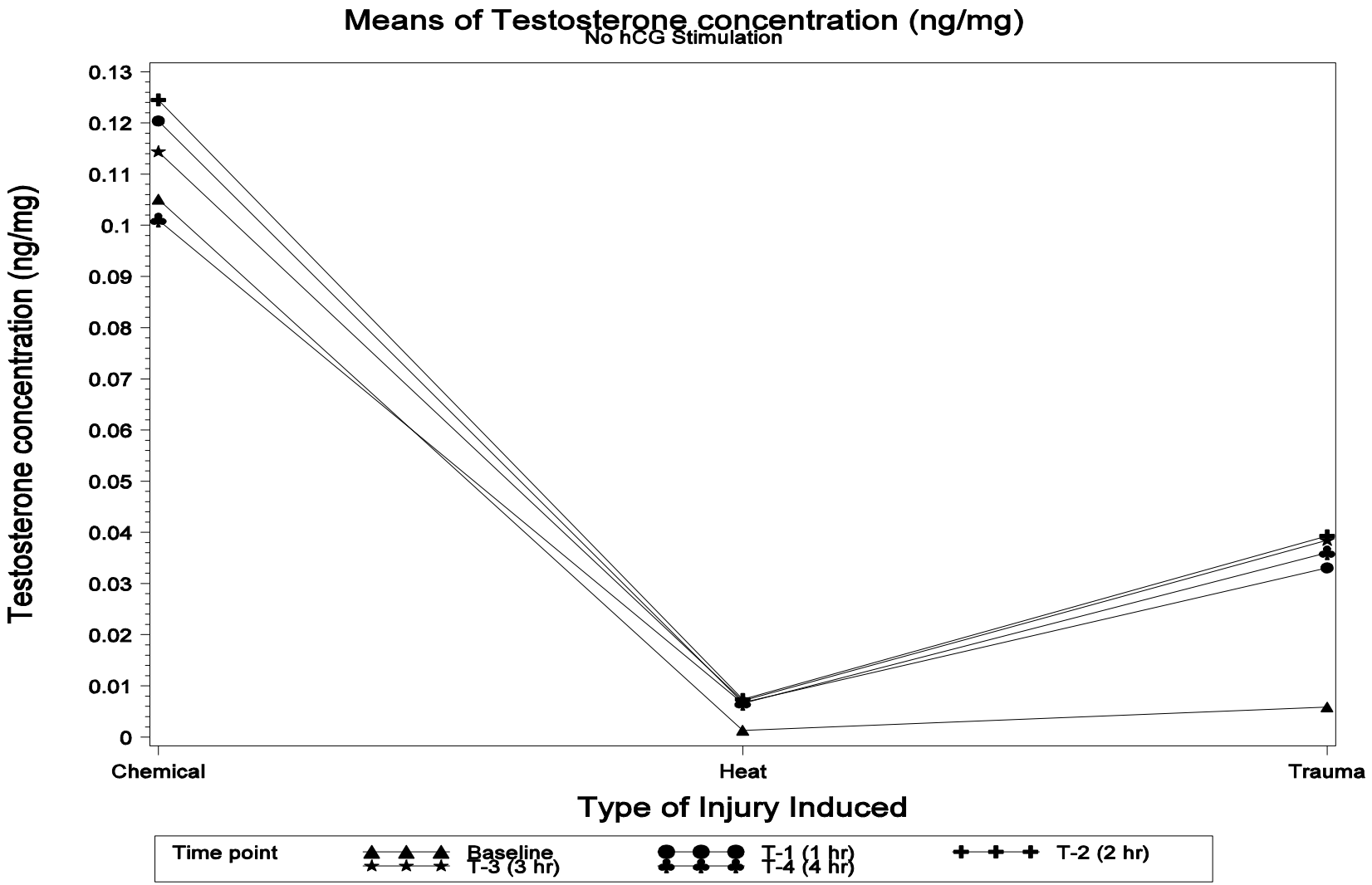

Coeff of Variation of LDH concentration (mU/mg)
No hCG Stimulation
Coefficient of Variation Percent
60
50
40
30
20
10
0
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)
Time point
Type of Injury Induced
Chemical
Heat
Trauma

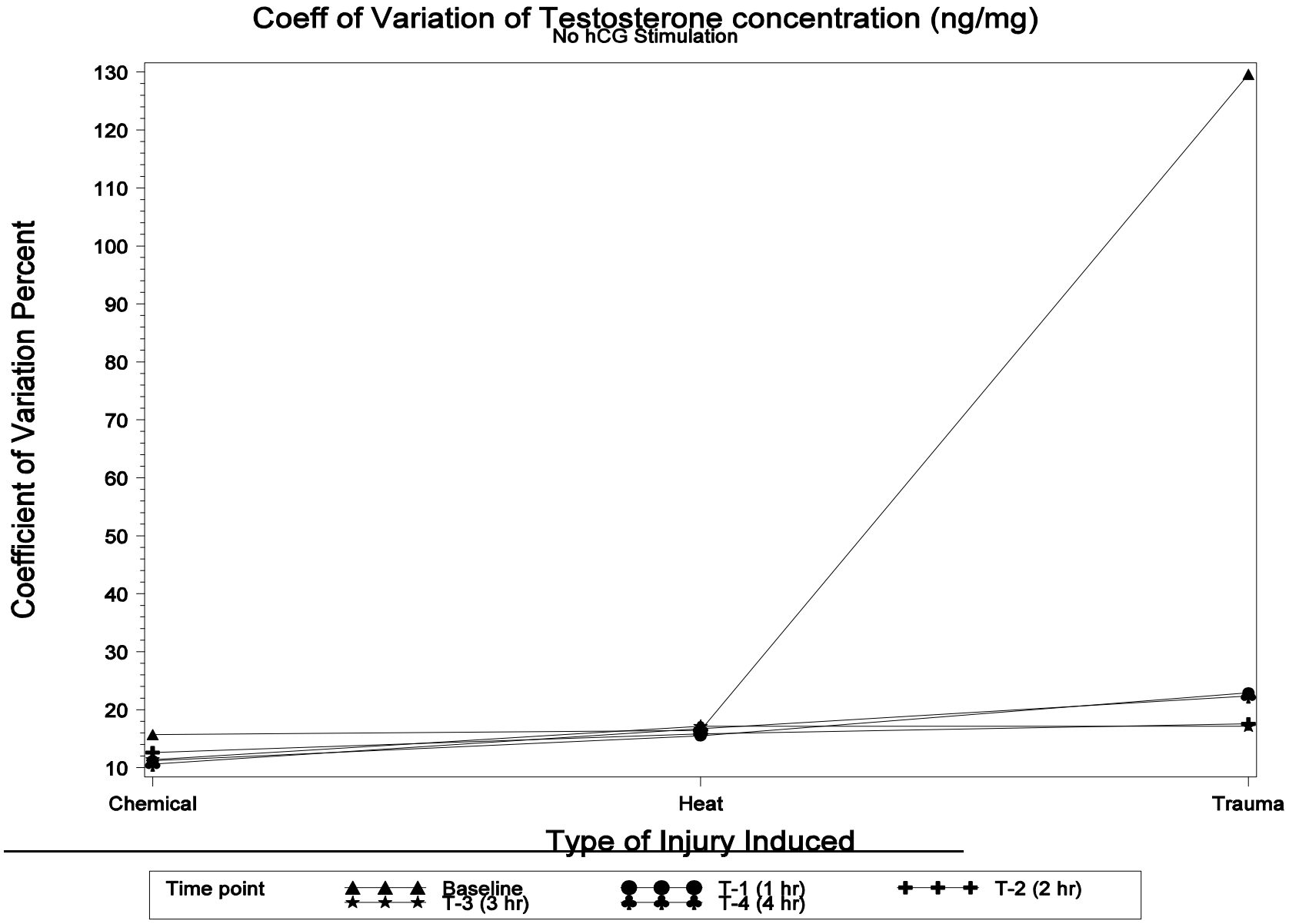

Means of LDH concentration (mU/mg)
with hCG Stimulation
LDH concentration (mU/mg)
3000
2000
1000
0
Chemical
Heat
Trauma
Type of Injury Induced
Time point
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)

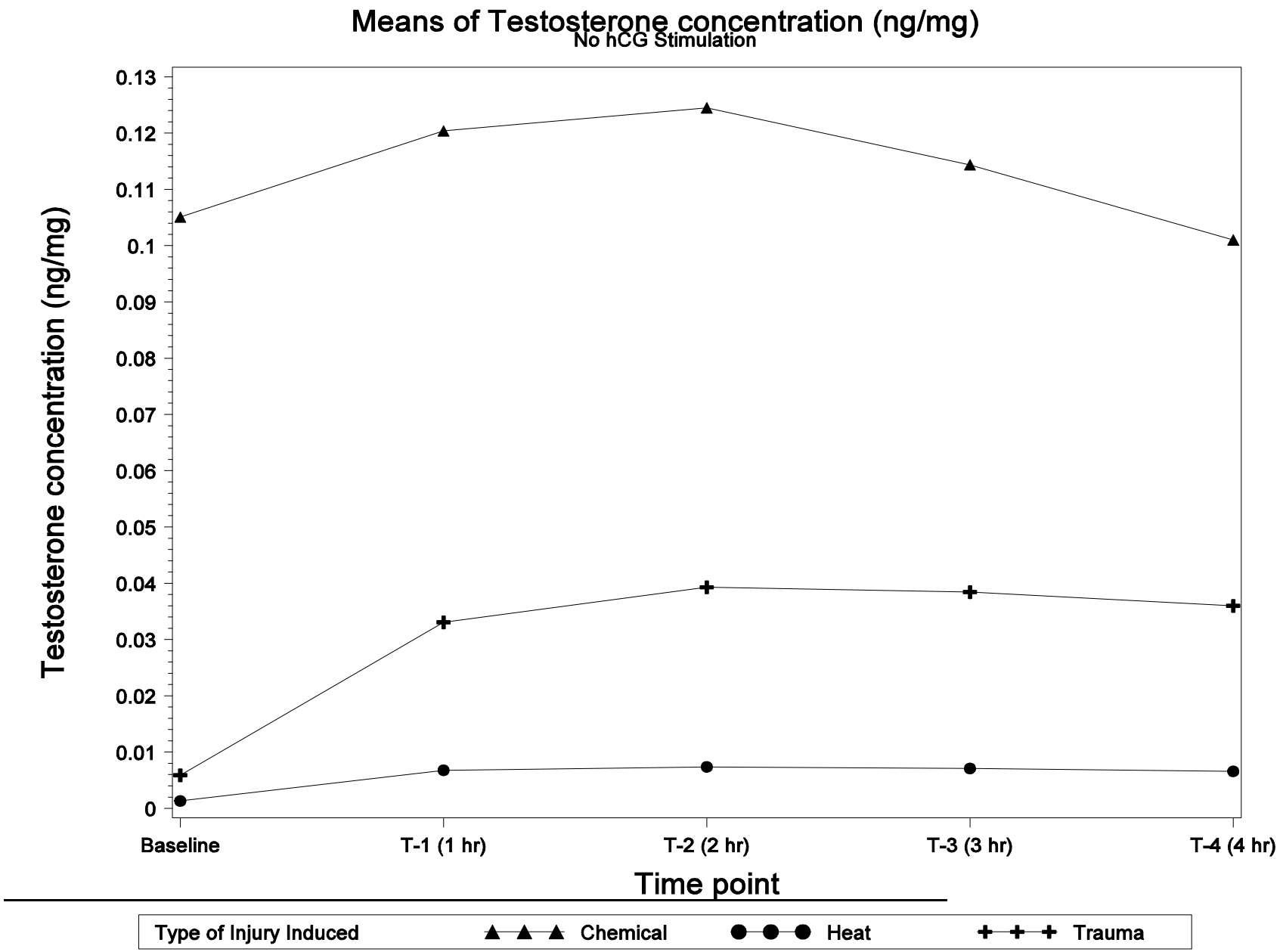

Coeff of Variation of LDH concentration (mU/mg)
with hCG Stimulation
Coefficient of Variation Percent
40
30
20
10
0
Chemical
Heat
Trauma
Type of Injury Induced
Time point
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)

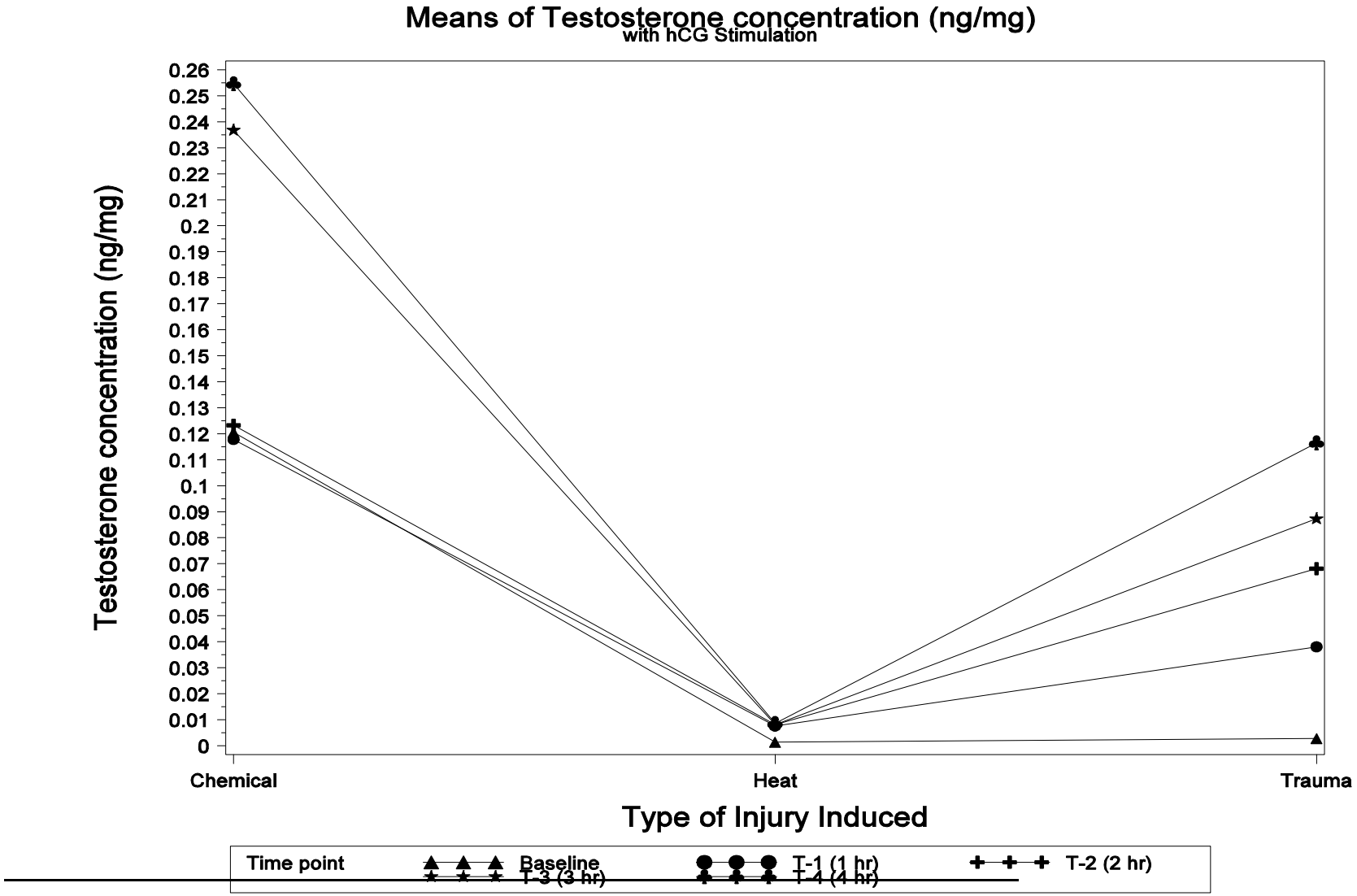

Means of LDH concentration (mU/mg)
with hCG Stimulation
LDH concentration (mU/mg)
3000
2000
1000
0
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)
Time point
Type of Injury Induced
Chemical
Heat
Trauma

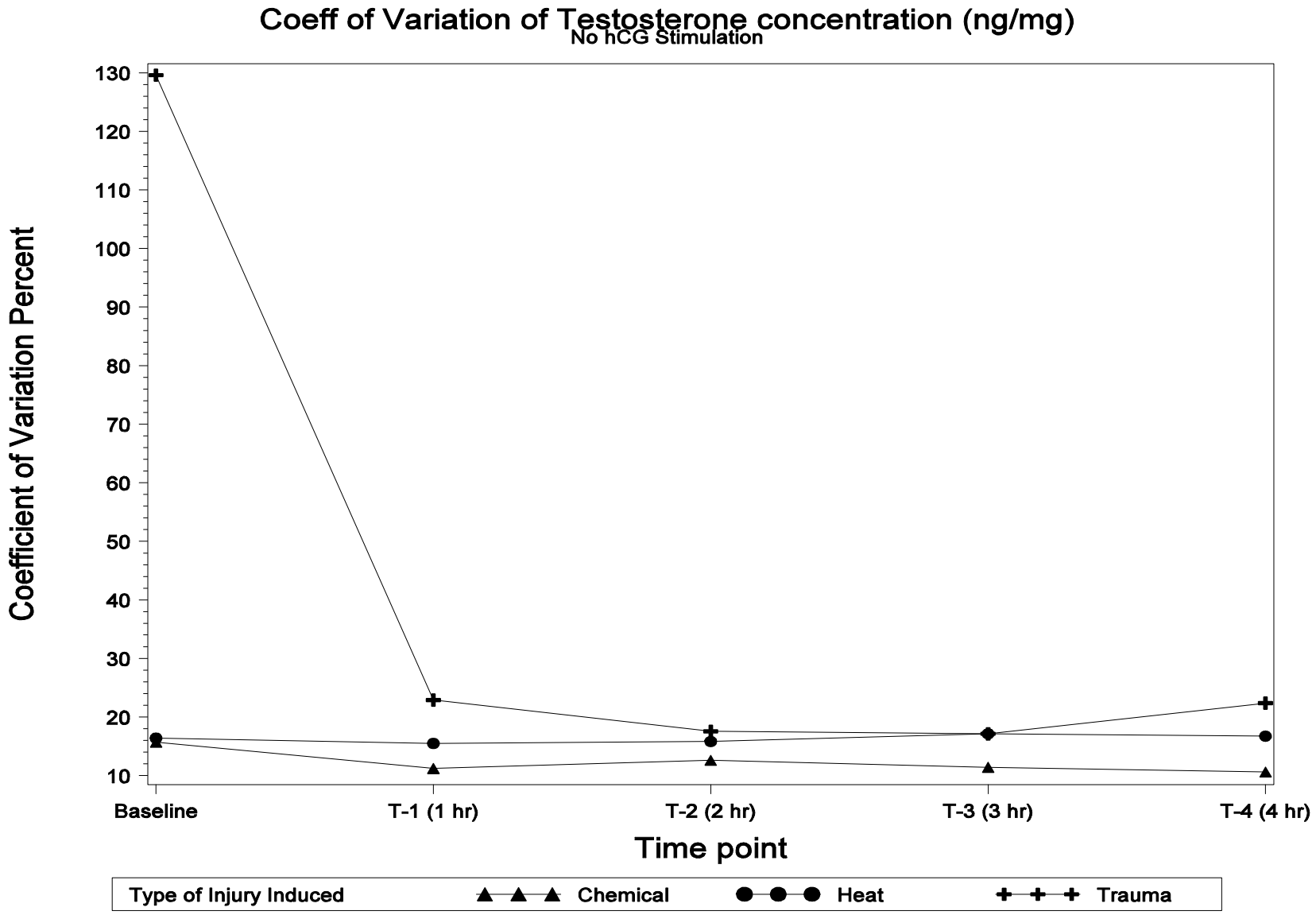

Coeff of Variation of LDH concentration (mU/mg)
with hCG Stimulation
Coefficient of Variation Percent
40
30
20
10
0
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)
Time point
Type of Injury Induced
Chemical
Heat
Trauma

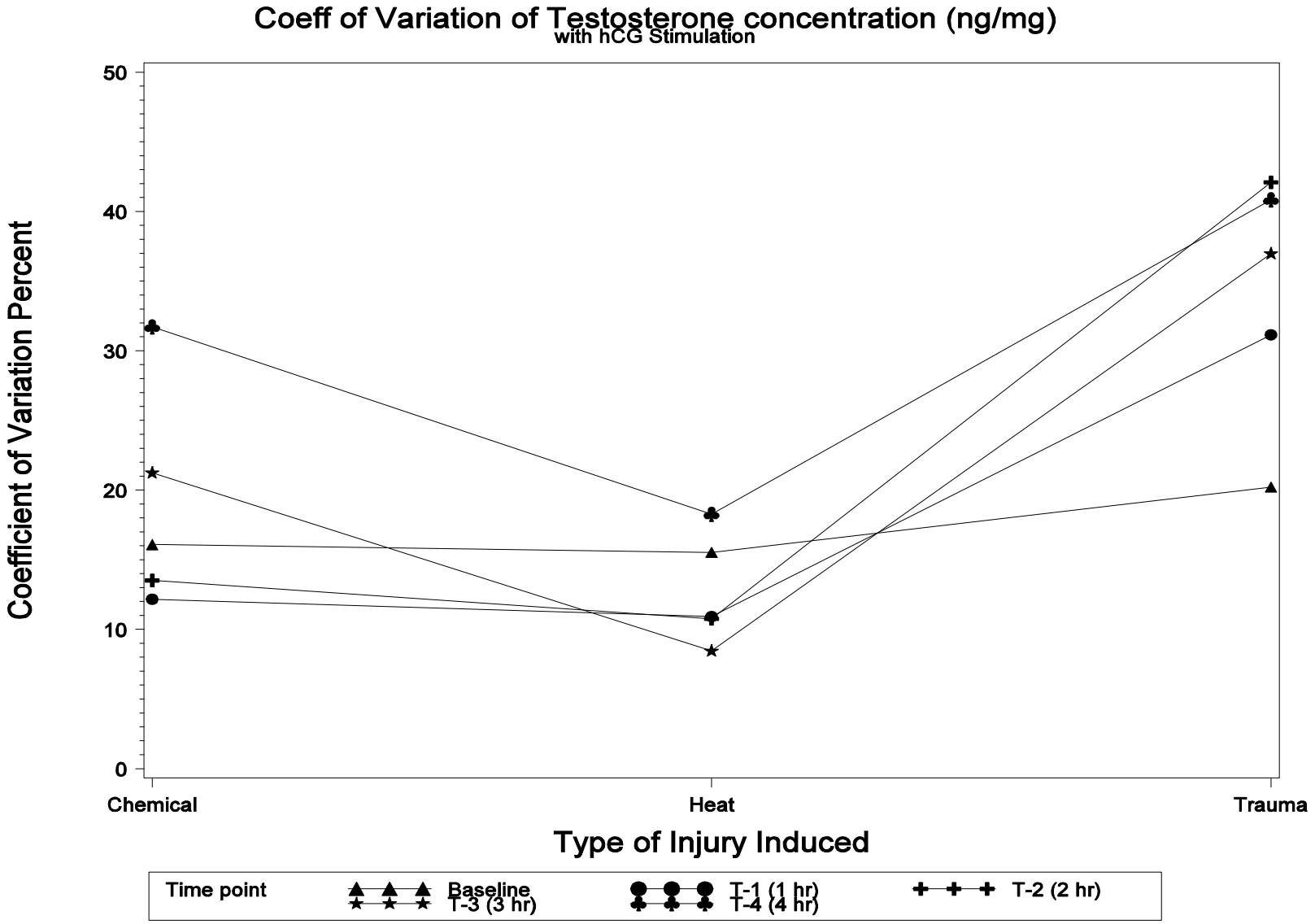

Means of Testosterone concentration (ng/mg)
No hCG Stimulation
Testosterone concentration (ng/mg)
0.13
0.12
0.11
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Chemical
Heat
Trauma
Type of Injury Induced
Time point
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)

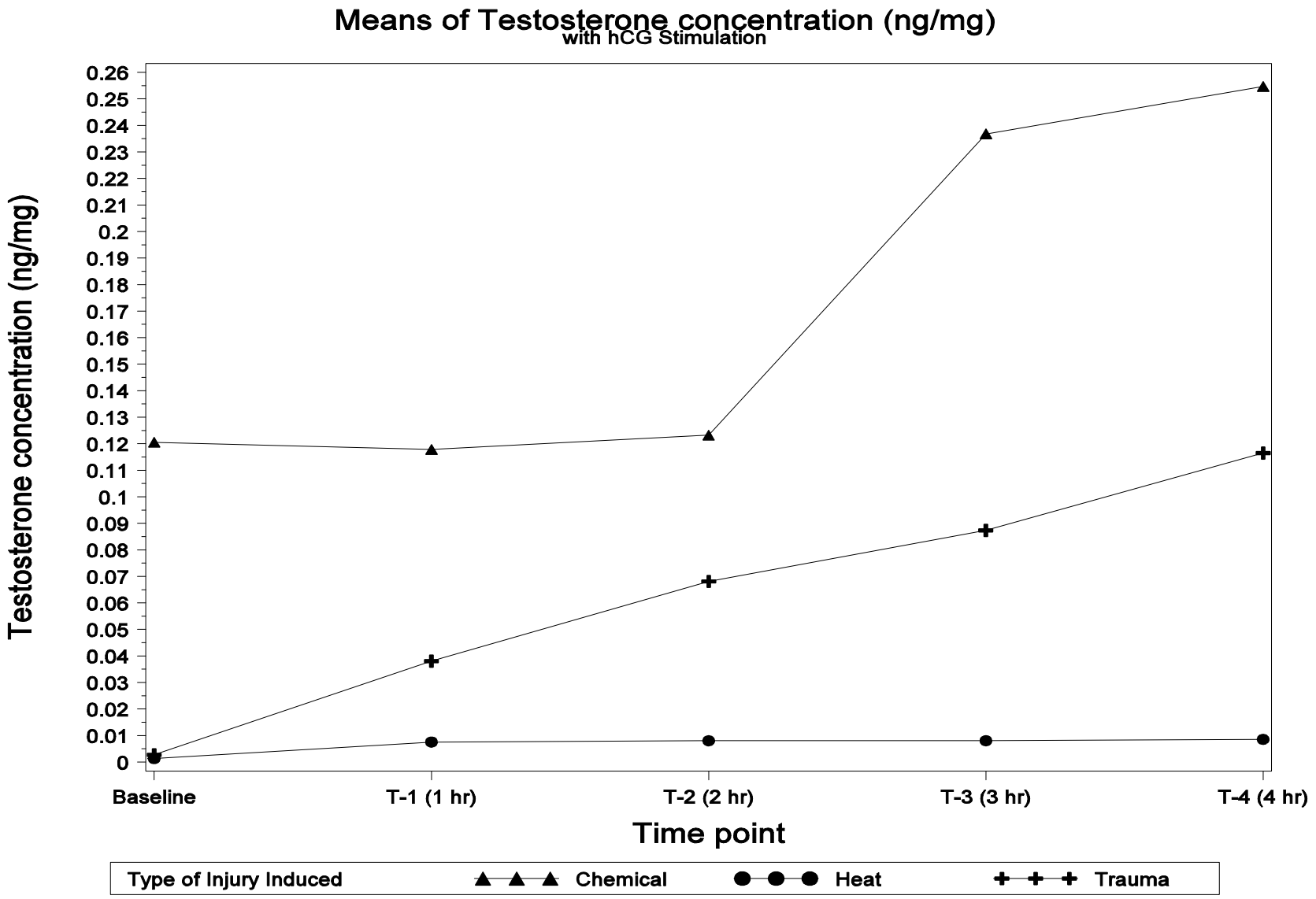

Coeff of Variation of Testosterone concentration (ng/mg)
No hCG Stimulation
Coefficient of Variation Percent
130
120
110
100
90
80
70
60
50
40
30
20
10
Chemical
Heat
Trauma
Type of Injury Induced
Time point
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)


Means of Testosterone concentration (ng/mg)
No hCG Stimulation
Testosterone concentration (ng/mg)
0.13
0.12
0.11
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)
Time point
Type of Injury Induced
Chemical
Heat
Trauma


Means of Testosterone concentration (ng/mg)
with hCG Stimulation
Testosterone concentration (ng/mg)
0.26
0.25
0.24
0.23
0.22
0.21
0.2
0.19
0.18
0.17
0.16
0.15
0.14
0.13
0.12
0.11
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Chemical
Heat
Trauma
Type of Injury Induced
Time point
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)


Coeff of Variation of Testosterone concentration (ng/mg)
No hCG Stimulation
Coefficient of Variation Percent
130
120
110
100
90
80
70
60
50
40
30
20
10
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)
Time point
Type of Injury Induced
Chemical
Heat
Trauma


Coeff of Variation of Testosterone concentration (ng/mg)
with hCG Stimulation
Coefficient of Variation Percent
50
40
30
20
10
0
Chemical
Heat
Trauma
Type of Injury Induced
Time point
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)


Means of Testosterone concentration (ng/mg)
with hCG Stimulation
Testosterone concentration (ng/mg)
0.26
0.25
0.24
0.23
0.22
0.21
0.2
0.19
0.18
0.17
0.16
0.15
0.14
0.13
0.12
0.11
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)
Time point
Type of Injury Induced
Chemical
Heat
Trauma

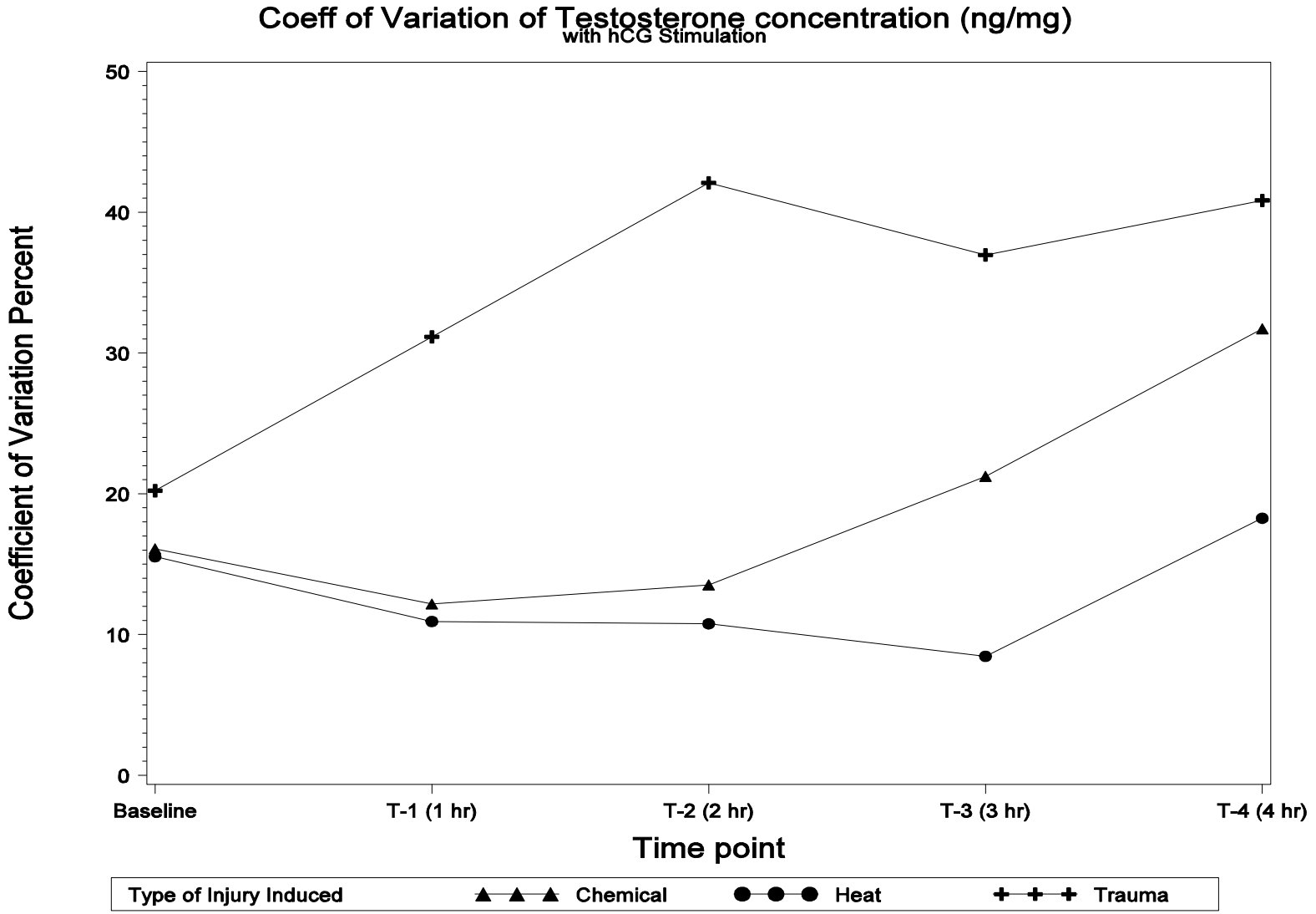

Coeff of Variation of Testosterone concentration (ng/mg)
with hCG Stimulation
Coefficient of Variation Percent
50
40
30
20
10
0
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)
Time point
Type of Injury Induced
Chemical
Heat
Trauma

Table 115. Summary of Injury Experiment: Testosterone Concentrations* (ng/mg) by Sampling Timepoints

Injury Type**	Baseline	1 Hr	2 Hr	3 Hr	4 Hr
Chemical (Triton-X 100)	**0.1051 ±0.0165**	**0.1204 ±0.0135**	**0.1245 ±0.0157**	**0.1144 ±0.0130**	**0.1010 ±0.0107**
Heat (42▾ C)	**0.0013 ±0.0002**	**0.0068 ±0.0010**	**0.0074 ±0.0012**	**0.0071 ±0.0012**	**0.0066 ±0.0011**
Trauma (Mincing)	**0.0059 ±0.0076**	**0.0331 ±0.0076**	**0.0393 ±0.0069**	**0.0384 ±0.0066**	**0.0360 ±0.0081**

* Mean ± standard deviation
**Injuries occurred during the 1 hour incubation period before the baseline sample was taken.
*** No hCG stimulation occurred

Table 116. Summary of Injury Experiment :Testosterone Concentrations* (ng/mg) by Sampling Timepoints***

Injury Type**	Baseline	1 hr	2 hr	3 hr	4 hr
Chemical (Triton-X 100)	0.1206 ±0.0194	0.1178 ±0.0143	0.1233 ±0.0167	0.2368 ±0.0503	0.2547 ±0.0808
Heat (42▾ C)	0.0013 ±0.0002	0.0076 ±0.0008	0.0081 ±0.0009	0.0081 ±0.0007	0.0086 ±0.0016
Trauma (Mincing)	0.0027 ±0.0006	0.0381 ±0.0119	0.0681 ±0.0287	0.0873 ±0.0323	0.1165 ±0.0476

* Mean ± standard deviation
**Injuries occurred during the 1 hour incubation period before the baseline sample was taken.
*** hCG stimulation occurred

Table 117. Summary of Injury Experiment :LDH Concentrations* (mU/mg) by Sampling Timepoints***

Injury Type**	Baseline	1 Hr	2 Hr	3 Hr	4 Hr
Chemical (Triton-X 100)	951.58 ±152.18	1635.10 ±235.21	1399.6 ±279.88	1189.70 ±241.49	970.32 ±197.44
Heat (42▾ C)	403.25 ±232.85	1083.20 ±234.00	1320.00 ±247.17	1558.10 ±248.69	1834.50 ±243.94
Trauma (Mincing)	397.88 ±122.91	811.21 ±131.80	823.43 ±105.27	847.36 ±77.69	940.08 ±67.56

* Mean ± standard deviation
**Injuries occurred during the 1 hour incubation period before the baseline sample was taken.
*** No hCG stimulation occurred

Table 118. Injury Experiment :LDH Concentrations* (mU/mg) by Sampling Timepoints***

Injury Type**	Baseline	1 Hr	2 Hr	3 Hr	4 Hr
Chemical (Triton-X 100)	994.59 ±90.49	1799.90 ±453.24	1555.00 ±460.20	1305.70 ±395.79	1061.90 ±329.34
Heat (42▾ C)	235.67 ±69.37	1323.30 ±141.25	1716.70 ±102.04	2021.90 ±112.60	2343.80 ±147.89
Trauma (Mincing)	504.56 ±201.32	775.14 ±46.10	851.35 ±62.73	884.63 ±78.50	1039.30 ±106.44

Mean ± standard deviation
**Injuries occurred during the 1 hour incubation period before the baseline sample was taken.
*** hCG stimulation occurred

14.0 VEHICLE EFFECT EXPERIMENT

14.1 Statistical Analysis of Phase II Vehicle and Vehicle Concentration Determination

14.1.1 Objective

The objective of the vehicle and vehicle concentration experiment is to determine the effect of various types of vehicles and vehicle concentrations on assay performance. The assay was performed with and without hCG stimulation under three different vehicle types: DMSO, ethanol and Tween 20. Each vehicle was evaluated at each of five different concentrations: 0 (control), 0.5, 1, 5 and 10 percent (v/v). Concentrations of LDH and testosterone for each sample were measured at baseline and at 1, 2, 3 and 4 hours after baseline.

14.1.2 Data

A SAS data set was constructed from the raw Excel data file. Each observation includes concentration measurements for LDH and testosterone, the type of vehicle used, the concentration of the vehicle and the corresponding repeated measurement times. The data used in the analysis are displayed in Tables 153 and 154.

14.1.3 Statistical Analysis Methods and Results

In order to examine the effects of the vehicles and their concentrations on the concentrations of LDH and testosterone, summary statistics were calculated for each vehicle type and each concentration level. Results of the means, standard deviations, sample sizes and coefficients of variation for LDH concentrations are shown in Tables 155 through 170 and the results for testosterone concentrations are shown in Tables 171 through 186. Graphs of the means and coefficients of variation for LDH and testosterone concentrations are presented at the end of the section report.

Table 119. Vehicle and Vehicle Concentrations Data for No hCG Stimulation

Sample_ID	Timepoint	Vehicle	Vehicle Type	Vehicle Concentration	LDH (mU/mg)	Testosterone (ng/mg)
A	Baseline	0.5% ETOH	ETOH	0.5%	207.7	0.0021
A	T-1 (1 hr)	0.5% ETOH	ETOH	0.5%	708.6	
A	T-2 (2 hr)	0.5% ETOH	ETOH	0.5%	1001.8	0.0166
A	T-3 (3 hr)	0.5% ETOH	ETOH	0.5%	1166.8	0.0199
A	T-4 (4 hr)	0.5% ETOH	ETOH	0.5%	1258.4	0.0205
B	Baseline	1.0% ETOH	ETOH	1.0%	231.7	0.0029
B	T-1 (1 hr)	1.0% ETOH	ETOH	1.0%	842.5	0.0167
B	T-2 (2 hr)	1.0% ETOH	ETOH	1.0%	1369.1	0.0238
B	T-3 (3 hr)	1.0% ETOH	ETOH	1.0%	1448.1	0.0243
B	T-4 (4 hr)	1.0% ETOH	ETOH	1.0%	1542.9	0.0224
C	Baseline	5.0% ETOH	ETOH	5.0%	303.9	0.0026
C	T-1 (1 hr)	5.0% ETOH	ETOH	5.0%	1157.2	0.0122
C	T-2 (2 hr)	5.0% ETOH	ETOH	5.0%	1408.5	0.0137
C	T-3 (3 hr)	5.0% ETOH	ETOH	5.0%	1525.4	0.0150
C	T-4 (4 hr)	5.0% ETOH	ETOH	5.0%	1654.0	0.0125
D	Baseline	10.0% ETOH	ETOH	10.0%	361.2	0.0029
D	T-1 (1 hr)	10.0% ETOH	ETOH	10.0%	679.2	0.0148
D	T-2 (2 hr)	10.0% ETOH	ETOH	10.0%	927.2	0.0156
D	T-3 (3 hr)	10.0% ETOH	ETOH	10.0%	1148.2	0.0160
D	T-4 (4 hr)	10.0% ETOH	ETOH	10.0%	1390.8	0.0150
E	Baseline	0.5% DMSO	DMSO	0.5%	317.1	0.0033
E	T-1 (1 hr)	0.5% DMSO	DMSO	0.5%	764.4	0.0203
E	T-2 (2 hr)	0.5% DMSO	DMSO	0.5%	1030.6	0.0249
E	T-3 (3 hr)	0.5% DMSO	DMSO	0.5%	1313.7	0.0242
E	T-4 (4 hr)	0.5% DMSO	DMSO	0.5%	1528.9	0.0232
F	Baseline	1.0% DMSO	DMSO	1.0%	244.2	0.0022
F	T-1 (1 hr)	1.0% DMSO	DMSO	1.0%	573.2	0.0189
F	T-2 (2 hr)	1.0% DMSO	DMSO	1.0%	759.0	0.0279
F	T-3 (3 hr)	1.0% DMSO	DMSO	1.0%	1003.2	0.0253
F	T-4 (4 hr)	1.0% DMSO	DMSO	1.0%	1380.0	0.0207
G	Baseline	5.0% DMSO	DMSO	5.0%	152.4	0.0016
G	T-1 (1 hr)	5.0% DMSO	DMSO	5.0%	1306.5	0.0239
G	T-2 (2 hr)	5.0% DMSO	DMSO	5.0%	1589.5	0.0279
G	T-3 (3 hr)	5.0% DMSO	DMSO	5.0%	1937.9	0.0264
G	T-4 (4 hr)	5.0% DMSO	DMSO	5.0%	2319.0	0.0286
H	Baseline	10.0% DMSO	DMSO	10.0%	236.7	0.0026
H	T-1 (1 hr)	10.0% DMSO	DMSO	10.0%	1479.3	0.0219
H	T-2 (2 hr)	10.0% DMSO	DMSO	10.0%	2242.6	0.0217
H	T-3 (3 hr)	10.0% DMSO	DMSO	10.0%	3047.3	0.0189
H	T-4 (4 hr)	10.0% DMSO	DMSO	10.0%	3461.5	0.0167

Sample_ID	Timepoint	Vehicle	Vehicle Type	Vehicle Concentration	LDH (mU/mg)	Testosterone (ng/mg)
I	Baseline	0.5% Tween	Tween	0.5%	321.0	0.0023
I	T-1 (1 hr)	0.5% Tween	Tween	0.5%	1444.7	0.0230
I	T-2 (2 hr)	0.5% Tween	Tween	0.5%	1938.2	0.0179
I	T-3 (3 hr)	0.5% Tween	Tween	0.5%	1973.8	0.0174
I	T-4 (4 hr)	0.5% Tween	Tween	0.5%	2098.7	0.0140
J	Baseline	1.0% Tween	Tween	1.0%	304.3	0.0023
J	T-1 (1 hr)	1.0% Tween	Tween	1.0%	1206.6	0.0234
J	T-2 (2 hr)	1.0% Tween	Tween	1.0%	1478.9	0.0232
J	T-3 (3 hr)	1.0% Tween	Tween	1.0%	1804.6	0.0215
J	T-4 (4 hr)	1.0% Tween	Tween	1.0%	2151.6	0.0143
K	Baseline	5.0% Tween	Tween	5.0%	128.5	0.0018
K	T-1 (1 hr)	5.0% Tween	Tween	5.0%	2631.3	0.0344
K	T-2 (2 hr)	5.0% Tween	Tween	5.0%	3240.2	0.0246
K	T-3 (3 hr)	5.0% Tween	Tween	5.0%	3921.8	0.0245
K	T-4 (4 hr)	5.0% Tween	Tween	5.0%	3217.9	0.0245
L	Baseline	10.0% Tween	Tween	10.0%	95.4	0.0023
L	T-1 (1 hr)	10.0% Tween	Tween	10.0%	3784.3	0.0630
L	T-2 (2 hr)	10.0% Tween	Tween	10.0%	4445.8	0.0570
L	T-3 (3 hr)	10.0% Tween	Tween	10.0%	4368.3	0.0580
L	T-4 (4 hr)	10.0% Tween	Tween	10.0%	4004.8	0.0267
M	Baseline	ETOH Control	ETOH	0.0%	215.4	0.0021
M	T-1 (1 hr)	ETOH Control	ETOH	0.0%	491.4	0.0231
M	T-2 (2 hr)	ETOH Control	ETOH	0.0%	668.1	0.0395
M	T-3 (3 hr)	ETOH Control	ETOH	0.0%	938.7	0.0560
M	T-4 (4 hr)	ETOH Control	ETOH	0.0%	1325.2	0.0670
N	Baseline	DMSO Control	DMSO	0.0%	234.9	0.0030
N	T-1 (1 hr)	DMSO Control	DMSO	0.0%	1089.2	0.0247
N	T-2 (2 hr)	DMSO Control	DMSO	0.0%	1377.5	0.0262
N	T-3 (3 hr)	DMSO Control	DMSO	0.0%	1580.4	0.0265
N	T-4 (4 hr)	DMSO Control	DMSO	0.0%	1713.8	0.0254
O	Baseline	Tween Control	Tween	0.0%	174.9	0.0026
O	T-1 (1 hr)	Tween Control	Tween	0.0%	683.1	0.0295
O	T-2 (2 hr)	Tween Control	Tween	0.0%	956.3	0.0346
O	T-3 (3 hr)	Tween Control	Tween	0.0%	1327.9	0.0348
O	T-4 (4 hr)	Tween Control	Tween	0.0%	1590.2	0.0335

Table 120. Vehicle and Vehicle Concentrations Data for hCG Stimulation

Sample ID	Timepoint	Vehicle	Vehicle Type	Vehicle Concentration	LDH (mU/mg)	Testosterone (ng/mg)
AC	Baseline	0.5% ETOH	ETOH	0.5%	217.8	0.0021
AC	T-1 (1 hr)	0.5% ETOH	ETOH	0.5%	876.6	0.0200
AC	T-2 (2 hr)	0.5% ETOH	ETOH	0.5%	1172.5	0.0412
AC	T-3 (3 hr)	0.5% ETOH	ETOH	0.5%	1328.9	0.0629
AC	T-4 (4 hr)	0.5% ETOH	ETOH	0.5%	1591.3	0.0835
BC	Baseline	1.0% ETOH	ETOH	1.0%	332.6	0.0034
BC	T-1 (1 hr)	1.0% ETOH	ETOH	1.0%	548.8	0.0198
BC	T-2 (2 hr)	1.0% ETOH	ETOH	1.0%	765.0	0.0330
BC	T-3 (3 hr)	1.0% ETOH	ETOH	1.0%	936.8	0.0467
BC	T-4 (4 hr)	1.0% ETOH	ETOH	1.0%	1064.3	0.0595
CC	Baseline	5.0% ETOH	ETOH	5.0%	183.1	0.0023
CC	T-1 (1 hr)	5.0% ETOH	ETOH	5.0%	754.7	0.0196
CC	T-2 (2 hr)	5.0% ETOH	ETOH	5.0%	937.8	0.0355
CC	T-3 (3 hr)	5.0% ETOH	ETOH	5.0%	1132.1	0.0613
CC	T-4 (4 hr)	5.0% ETOH	ETOH	5.0%	1320.8	0.1082
DC	Baseline	10.0% ETOH	ETOH	10.0%	229.3	0.0026
DC	T-1 (1 hr)	10.0% ETOH	ETOH	10.0%	715.1	0.0206
DC	T-2 (2 hr)	10.0% ETOH	ETOH	10.0%	1031.7	0.0210
DC	T-3 (3 hr)	10.0% ETOH	ETOH	10.0%	1250.0	0.0265
DC	T-4 (4 hr)	10.0% ETOH	ETOH	10.0%	1452.0	0.0309
EC	Baseline	0.5% DMSO	DMSO	0.5%	168.8	0.0019
EC	T-1 (1 hr)	0.5% DMSO	DMSO	0.5%	906.0	0.0169
EC	T-2 (2 hr)	0.5% DMSO	DMSO	0.5%	1249.3	0.0268
EC	T-3 (3 hr)	0.5% DMSO	DMSO	0.5%	1525.0	0.0409
EC	T-4 (4 hr)	0.5% DMSO	DMSO	0.5%	1778.3	0.0542
FC	Baseline	1.0% DMSO	DMSO	1.0%	285.7	0.0020
FC	T-1 (1 hr)	1.0% DMSO	DMSO	1.0%	1074.3	0.0361
FC	T-2 (2 hr)	1.0% DMSO	DMSO	1.0%	1537.1	0.0916
FC	T-3 (3 hr)	1.0% DMSO	DMSO	1.0%	1662.9	0.1271
FC	T-4 (4 hr)	1.0% DMSO	DMSO	1.0%	1874.3	0.2407
GC	Baseline	5.0% DMSO	DMSO	5.0%	157.3	0.0019
GC	T-1 (1 hr)	5.0% DMSO	DMSO	5.0%	971.9	0.0172
GC	T-2 (2 hr)	5.0% DMSO	DMSO	5.0%	1219.1	0.0270
GC	T-3 (3 hr)	5.0% DMSO	DMSO	5.0%	1544.9	0.0418
GC	T-4 (4 hr)	5.0% DMSO	DMSO	5.0%	1977.5	0.0470
HC	Baseline	10.0% DMSO	DMSO	10.0%	135.2	0.0022
HC	T-1 (1 hr)	10.0% DMSO	DMSO	10.0%	723.1	0.0362
HC	T-2 (2 hr)	10.0% DMSO	DMSO	10.0%	1469.7	0.0388
HC	T-3 (3 hr)	10.0% DMSO	DMSO	10.0%	2369.2	0.0467
HC	T-4 (4 hr)	10.0% DMSO	DMSO	10.0%	3004.1	0.0521

Sample ID	Timepoint	Vehicle	Vehicle Type	Vehicle Concentration	LDH (mU/mg)	Testosterone (ng/mg)
IC	Baseline	0.5% Tween	Tween	0.5%	148.8	0.0017
IC	T-1 (1 hr)	0.5% Tween	Tween	0.5%	1002.8	0.0180
IC	T-2 (2 hr)	0.5% Tween	Tween	0.5%	1603.3	0.0226
IC	T-3 (3 hr)	0.5% Tween	Tween	0.5%	1840.2	0.0217
IC	T-4 (4 hr)	0.5% Tween	Tween	0.5%	2099.2	0.0243
JC	Baseline	1.0% Tween	Tween	1.0%	242.1	0.0024
JC	T-1 (1 hr)	1.0% Tween	Tween	1.0%	962.5	0.0237
JC	T-2 (2 hr)	1.0% Tween	Tween	1.0%	1302.6	0.0225
JC	T-3 (3 hr)	1.0% Tween	Tween	1.0%	1608.1	0.0232
JC	T-4 (4 hr)	1.0% Tween	Tween	1.0%	1907.8	0.0184
KC	Baseline	5.0% Tween	Tween	5.0%	93.5	0.0013
KC	T-1 (1 hr)	5.0% Tween	Tween	5.0%	2523.4	0.0238
KC	T-2 (2 hr)	5.0% Tween	Tween	5.0%	5461.4	0.0367
KC	T-3 (3 hr)	5.0% Tween	Tween	5.0%	5654.2	0.0273
KC	T-4 (4 hr)	5.0% Tween	Tween	5.0%	5566.6	0.0372
LC	Baseline	10.0% Tween	Tween	10.0%	106.1	0.0026
LC	T-1 (1 hr)	10.0% Tween	Tween	10.0%	2882.7	0.0868
LC	T-2 (2 hr)	10.0% Tween	Tween	10.0%	4352.0	0.0408
LC	T-3 (3 hr)	10.0% Tween	Tween	10.0%	4374.3	0.0447
LC	T-4 (4 hr)	10.0% Tween	Tween	10.0%	4379.9	0.0417
MC	Baseline	ETOH Control	ETOH	0.0%	222.3	0.0021
MC	T-1 (1 hr)	ETOH Control	ETOH	0.0%	803.9	0.0146
MC	T-2 (2 hr)	ETOH Control	ETOH	0.0%	935.0	0.0213
MC	T-3 (3 hr)	ETOH Control	ETOH	0.0%	1214.4	0.0290
MC	T-4 (4 hr)	ETOH Control	ETOH	0.0%	1413.9	0.0344
NC	Baseline	DMSO Control	DMSO	0.0%	488.2	0.0082
NC	T-1 (1 hr)	DMSO Control	DMSO	0.0%	1418.7	0.0285
NC	T-2 (2 hr)	DMSO Control	DMSO	0.0%	1596.8	0.0291
NC	T-3 (3 hr)	DMSO Control	DMSO	0.0%	1614.0	0.0341
NC	T-4 (4 hr)	DMSO Control	DMSO	0.0%	1660.0	0.0310
OC	Baseline	Tween Control	Tween	0.0%	254.5	0.0035
OC	T-1 (1 hr)	Tween Control	Tween	0.0%	735.3	0.0639
OC	T-2 (2 hr)	Tween Control	Tween	0.0%	1402.7	0.1699
OC	T-3 (3 hr)	Tween Control	Tween	0.0%	2013.6	0.1944
OC	T-4 (4 hr)	Tween Control	Tween	0.0%	2177.6	0.2324

Table 121. Means of LDH concentration (mU/mg) by Vehicle Type
No hCG Stimulation

Timepoint	DMSO	ETOH	Tween
Baseline	237.1	264.0	204.8
T-1 (1 hr)	1,042.5	775.8	1,950.0
T-2 (2 hr)	1,399.8	1,075.0	2,411.9
T-3 (3 hr)	1,776.5	1,245.5	2,679.3
T-4 (4 hr)	2,080.7	1,434.3	2,612.6

Table 122. Standard Deviations of LDH concentration (mU/mg) by Vehicle Type
No hCG Stimulation

Timepoint	DMSO	ETOH	Tween
Baseline	58.4	66.4	102.6
T-1 (1 hr)	374.2	247.3	1,249.2
T-2 (2 hr)	568.7	312.5	1,417.4
T-3 (3 hr)	789.3	239.4	1,368.0
T-4 (4 hr)	850.5	161.9	978.1

Table 123. Sample sizes of LDH concentration (mU/mg) by Vehicle Type
No hCG Stimulation

Timepoint	DMSO	ETOH	Tween
Baseline	**5**	**5**	**5**
T-1 (1 hr)	**5**	**5**	**5**
T-2 (2 hr)	**5**	**5**	**5**
T-3 (3 hr)	**5**	**5**	**5**
T-4 (4 hr)	**5**	**5**	**5**

Table 124. Coeff of Variation of LDH concentration (mU/mg) by Vehicle Type
No hCG Stimulation

Timepoint	DMSO	ETOH	Tween
Baseline	24.6	25.1	50.1
T-1 (1 hr)	35.9	31.9	64.1
T-2 (2 hr)	40.6	29.1	58.8
T-3 (3 hr)	44.4	19.2	51.1
T-4 (4 hr)	40.9	11.3	37.4

Table 125. Means of LDH Concentration (mU/mg) by Vehicle Concentration
No hCG Stimulation

Timepoint	0.0%	0.5%	1.0%	5.0%	10.0%
Baseline	208.4	282.0	260.1	194.9	231.1
T-1 (1 hr)	754.6	972.6	874.1	1,698.3	1,980.9
T-2 (2 hr)	1,000.6	1,323.5	1,202.4	2,079.4	2,538.5
T-3 (3 hr)	1,282.3	1,484.8	1,418.6	2,461.7	2,854.6
T-4 (4 hr)	1,543.1	1,628.7	1,691.5	2,397.0	2,952.4

Table 126. Standard Deviations of LDH concentration (mU/mg) by Vehicle Concentration
No hCG Stimulation

Timepoint	0.0%	0.5%	1.0%	5.0%	10.0%
Baseline	30.6	64.3	38.8	95.1	133.0
T-1 (1 hr)	305.2	409.8	317.9	811.4	1,612.1
T-2 (2 hr)	356.7	532.5	387.8	1,009.3	1,777.8
T-3 (3 hr)	323.2	429.9	401.5	1,281.2	1,618.7
T-4 (4 hr)	198.5	428.9	406.7	784.9	1,379.3

Table 127. Sample sizes of LDH Concentration (mU/mg) by Vehicle Concentration
No hCG Stimulation

Timepoint	0.0%	0.5%	1.0%	5.0%	10.0%
Baseline	3	3	3	3	3
T-1 (1 hr)	3	3	3	3	3
T-2 (2 hr)	3	3	3	3	3
T-3 (3 hr)	3	3	3	3	3
T-4 (4 hr)	3	3	3	3	3

Table 128. Coeff of Variation of LDH Concentration (mU/mg) by Vehicle Concentration
No hCG Stimulation

Timepoint	0.0%	0.5%	1.0%	5.0%	10.0%
Baseline	14.7	22.8	14.9	48.8	57.6
T-1 (1 hr)	40.4	42.1	36.4	47.8	81.4
T-2 (2 hr)	35.7	40.2	32.3	48.5	70.0
T-3 (3 hr)	25.2	29.0	28.3	52.0	56.7
T-4 (4 hr)	12.9	26.3	24.0	32.7	46.7

Table 129. Means of LDH Concentration (mU/mg) by Vehicle Type with hCG Stimulation

Timepoint	DMSO	ETOH	Tween
Baseline	247.1	237.0	169.0
T-1 (1 hr)	1,018.8	739.8	1,621.3
T-2 (2 hr)	1,414.4	968.4	2,824.4
T-3 (3 hr)	1,743.2	1,172.4	3,098.1
T-4 (4 hr)	2,058.8	1,368.4	3,226.2

Table 130. Standard Deviations of LDH Concentration (mU/mg) by Vehicle Type with hCG Stimulation

Timepoint	DMSO	ETOH	Tween
Baseline	146.9	56.3	75.4
T-1 (1 hr)	257.6	122.6	1,000.8
T-2 (2 hr)	170.9	149.3	1,943.9
T-3 (3 hr)	354.2	149.5	1,812.5
T-4 (4 hr)	541.3	195.9	1,652.0

Table 131. Sample sizes of LDH Concentration (mU/mg) by Vehicle Type with hCG Stimulation

Timepoint	DMSO	ETOH	Tween
Baseline	5	5	5
T-1 (1 hr)	5	5	5
T-2 (2 hr)	5	5	5
T-3 (3 hr)	5	5	5
T-4 (4 hr)	5	5	5

Table 132. Coeff of Variation of LDH Concentration (mU/mg) by Vehicle Type with hCG Stimulation

Timepoint	DMSO	ETOH	Tween
Baseline	59.5	23.8	44.6
T-1 (1 hr)	25.3	16.6	61.7
T-2 (2 hr)	12.1	15.4	68.8
T-3 (3 hr)	20.3	12.8	58.5
T-4 (4 hr)	26.3	14.3	51.2

Table 133. Means of LDH Concentration (mU/mg) by Vehicle Concentration with hCG Stimulation

Timepoint	0.0%	0.5%	1.0%	5.0%	10.0%
Baseline	321.7	178.5	286.8	144.6	156.9
T-1 (1 hr)	986.0	928.5	861.9	1,416.7	1,440.3
T-2 (2 hr)	1,311.5	1,341.7	1,201.6	2,539.5	2,284.4
T-3 (3 hr)	1,614.0	1,564.7	1,402.6	2,777.1	2,664.5
T-4 (4 hr)	1,750.5	1,822.9	1,615.5	2,955.0	2,945.3

Table 134. Standard Deviations of LDH Concentration (mU/mg) by Vehicle Concentration with hCG Stimulation

Timepoint	0.0%	0.5%	1.0%	5.0%	10.0%
Baseline	145.1	35.5	45.3	46.2	64.4
T-1 (1 hr)	376.4	66.0	276.8	964.6	1,249.2
T-2 (2 hr)	340.2	229.8	395.9	2,534.4	1,803.9
T-3 (3 hr)	399.6	258.0	404.3	2,500.2	1,582.9
T-4 (4 hr)	389.8	256.9	477.6	2,285.5	1,464.8

Table 135. Sample sizes of LDH Concentration (mU/mg) by Vehicle Concentration with hCG Stimulation

Timepoint	0.0%	0.5%	1.0%	5.0%	10.0%
Baseline	3	3	3	3	3
T-1 (1 hr)	3	3	3	3	3
T-2 (2 hr)	3	3	3	3	3
T-3 (3 hr)	3	3	3	3	3
T-4 (4 hr)	3	3	3	3	3

Table 136. Coeff of Variation of LDH Concentration (mU/mg) by Vehicle Concentration with hCG Stimulation

Timepoint	0.0%	0.5%	1.0%	5.0%	10.0%
Baseline	45.1	19.9	15.8	31.9	41.0
T-1 (1 hr)	38.2	7.1	32.1	68.1	86.7
T-2 (2 hr)	25.9	17.1	32.9	99.8	79.0
T-3 (3 hr)	24.8	16.5	28.8	90.0	59.4
T-4 (4 hr)	22.3	14.1	29.6	77.3	49.7

Table 137. Means of Testosterone Concentration (ng/mg) by Vehicle Type No hCG Stimulation

Timepoint	DMSO	ETOH	Tween
Baseline	0.0025	0.0025	0.0023
T-1 (1 hr)	0.0219	0.0162	0.0346
T-2 (2 hr)	0.0257	0.0218	0.0315
T-3 (3 hr)	0.0243	0.0262	0.0312
T-4 (4 hr)	0.0229	0.0275	0.0226

Table 138. Standard Deviations of Testosterone Concentration (ng/mg) by Vehicle Type No hCG Stimulation

Timepoint	DMSO	ETOH	Tween
Baseline	0.0006	0.0004	0.0003
T-1 (1 hr)	0.0024	0.0042	0.0165
T-2 (2 hr)	0.0026	0.0106	0.0155
T-3 (3 hr)	0.0032	0.0171	0.0163
T-4 (4 hr)	0.0045	0.0225	0.0084

Table 139. Sample sizes of Testosterone Concentration (ng/mg) by Vehicle Type No hCG Stimulation

Timepoint	DMSO	ETOH	Tween
Baseline	5	5	5
T-1 (1 hr)	5	5	5
T-2 (2 hr)	5	5	5
T-3 (3 hr)	5	5	5
T-4 (4 hr)	5	5	5

Table 140. Coeff of Variation of Testosterone Concentration (ng/mg) by Vehicle Type No hCG Stimulation

Timepoint	DMSO	ETOH	Tween
Baseline	25.4	15.9	12.3
T-1 (1 hr)	11.0	26.1	47.7
T-2 (2 hr)	10.1	48.4	49.3
T-3 (3 hr)	13.0	65.0	52.1
T-4 (4 hr)	19.7	81.7	37.2

Table 141. Means of Testosterone Concentration (ng/mg) by Vehicle Concentration No hCG Stimulation

Timepoint	0.0%	0.5%	1.0%	5.0%	10.0%
Baseline	0.0026	0.0025	0.0025	0.0020	0.0026
T-1 (1 hr)	0.0258	0.0191	0.0197	0.0235	0.0332
T-2 (2 hr)	0.0334	0.0198	0.0250	0.0221	0.0314
T-3 (3 hr)	0.0391	0.0205	0.0237	0.0220	0.0309
T-4 (4 hr)	0.0420	0.0192	0.0191	0.0219	0.0195

Table 142. Standard Deviations of Testosterone Concentration (ng/mg) by Vehicle Concentration No hCG Stimulation

Timepoint	0.0%	0.5%	1.0%	5.0%	10.0%
Baseline	0.0004	0.0007	0.0004	0.0005	0.0003
T-1 (1 hr)	0.0033	0.0046	0.0034	0.0111	0.0260
T-2 (2 hr)	0.0067	0.0044	0.0025	0.0074	0.0224
T-3 (3 hr)	0.0152	0.0034	0.0020	0.0061	0.0235
T-4 (4 hr)	0.0221	0.0048	0.0043	0.0084	0.0063

Table 143. Sample sizes of Testosterone Concentration (ng/mg) by Vehicle Concentration No hCG Stimulation

Timepoint	0.0%	0.5%	1.0%	5.0%	10.0%
Baseline	3	3	3	3	3
T-1 (1 hr)	3	3	3	3	3
T-2 (2 hr)	3	3	3	3	3
T-3 (3 hr)	3	3	3	3	3
T-4 (4 hr)	3	3	3	3	3

Table 144. Coeff of Variation of Testosterone Concentration (ng/mg) by Vehicle Concentration No hCG Stimulation

Timepoint	0.0%	0.5%	1.0%	5.0%	10.0%
Baseline	17.4	25.6	14.3	24.4	11.5
T-1 (1 hr)	12.8	24.0	17.4	47.3	78.4
T-2 (2 hr)	20.1	22.4	10.1	33.6	71.2
T-3 (3 hr)	38.9	16.7	8.2	27.7	75.9
T-4 (4 hr)	52.6	24.7	22.3	38.3	32.3

Table 145. Means of Testosterone Concentration (ng/mg) by Vehicle Type with hCG Stimulation

Timepoint	DMSO	ETOH	Tween
Baseline	0.0032	0.0025	0.0023
T-1 (1 hr)	0.0270	0.0189	0.0432
T-2 (2 hr)	0.0427	0.0304	0.0585
T-3 (3 hr)	0.0581	0.0452	0.0623
T-4 (4 hr)	0.0850	0.0633	0.0708

Table 146. Standard Deviations of Testosterone Concentration (ng/mg) by Vehicle Type with hCG Stimulation

Timepoint	DMSO	ETOH	Tween
Baseline	0.0028	0.0005	0.0009
T-1 (1 hr)	0.0096	0.0024	0.0305
T-2 (2 hr)	0.0278	0.0089	0.0628
T-3 (3 hr)	0.0388	0.0172	0.0744
T-4 (4 hr)	0.0875	0.0328	0.0908

Table 147. Sample sizes of Testosterone Concentration (ng/mg) by Vehicle Type with hCG Stimulation

Timepoint	DMSO	ETOH	Tween
Baseline	5	5	5
T-1 (1 hr)	5	5	5
T-2 (2 hr)	5	5	5
T-3 (3 hr)	5	5	5
T-4 (4 hr)	5	5	5

Table 148. Coeff of Variation of Testosterone Concentration (ng/mg) by Vehicle Type with hCG Stimulation

Timepoint	DMSO	ETOH	Tween
Baseline	85.8	21.6	37.2
T-1 (1 hr)	35.4	12.9	70.5
T-2 (2 hr)	65.1	29.4	107.3
T-3 (3 hr)	66.8	38.1	119.6
T-4 (4 hr)	103.0	51.9	128.3

Table 149. Means of Testosterone Concentration (ng/mg) by Vehicle Concentration with hCG Stimulation

Timepoint	0.0%	0.5%	1.0%	5.0%	10.0%
Baseline	0.0046	0.0019	0.0026	0.0018	0.0025
T-1 (1 hr)	0.0356	0.0183	0.0265	0.0202	0.0478
T-2 (2 hr)	0.0734	0.0302	0.0490	0.0331	0.0336
T-3 (3 hr)	0.0858	0.0418	0.0657	0.0434	0.0393
T-4 (4 hr)	0.0992	0.0540	0.1062	0.0641	0.0416

Table 150. Standard Deviations of Testosterone Concentration (ng/mg) by Vehicle Concentration with hCG Stimulation

Timepoint	0.0%	0.5%	1.0%	5.0%	10.0%
Baseline	0.0032	0.0002	0.0007	0.0005	0.0002
T-1 (1 hr)	0.0254	0.0015	0.0085	0.0034	0.0346
T-2 (2 hr)	0.0836	0.0098	0.0372	0.0053	0.0109
T-3 (3 hr)	0.0941	0.0206	0.0545	0.0171	0.0112
T-4 (4 hr)	0.1153	0.0296	0.1183	0.0384	0.0106

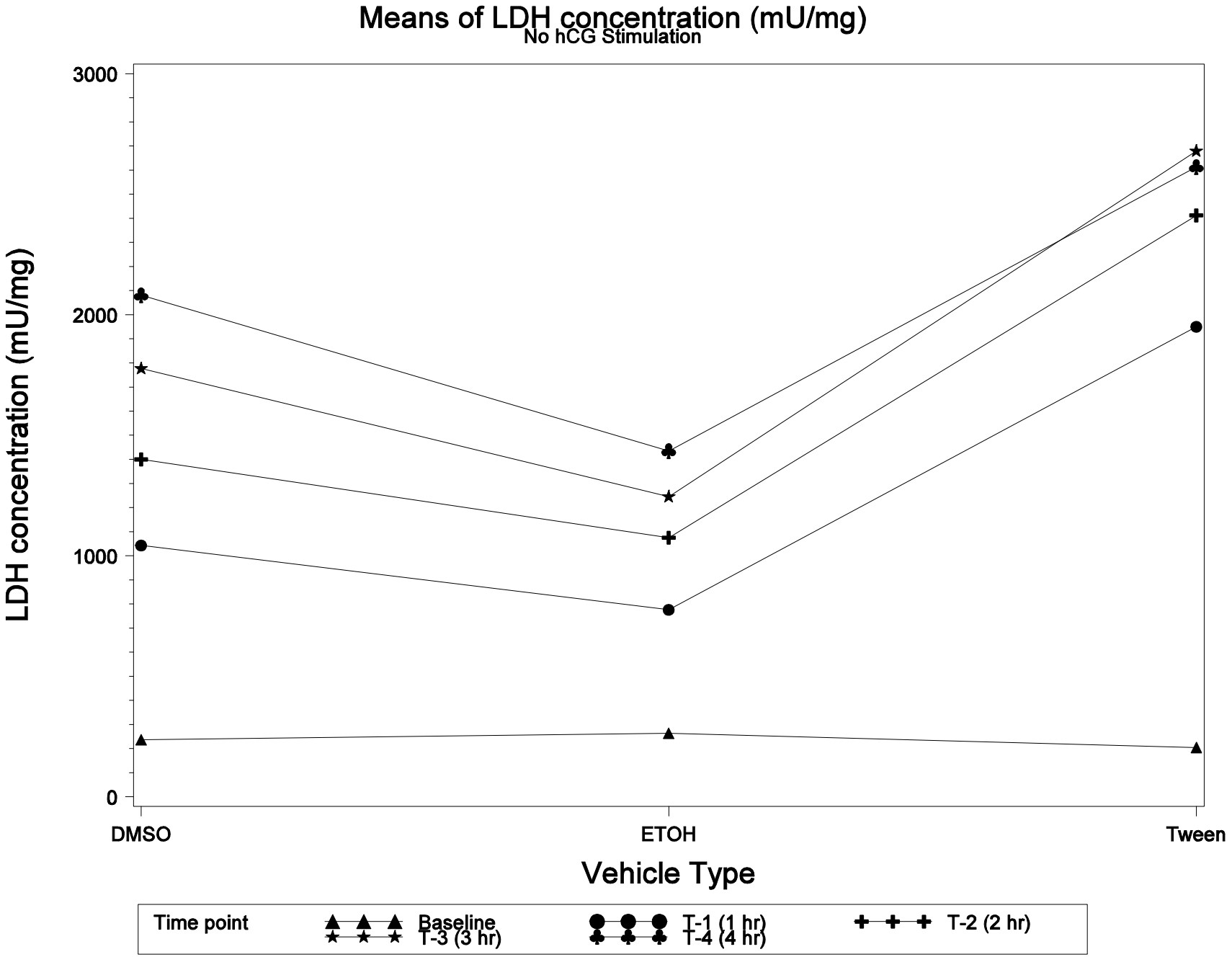
Table 151. Sample sizes of Testosterone Concentration (ng/mg) by Vehicle Concentration with hCG Stimulation

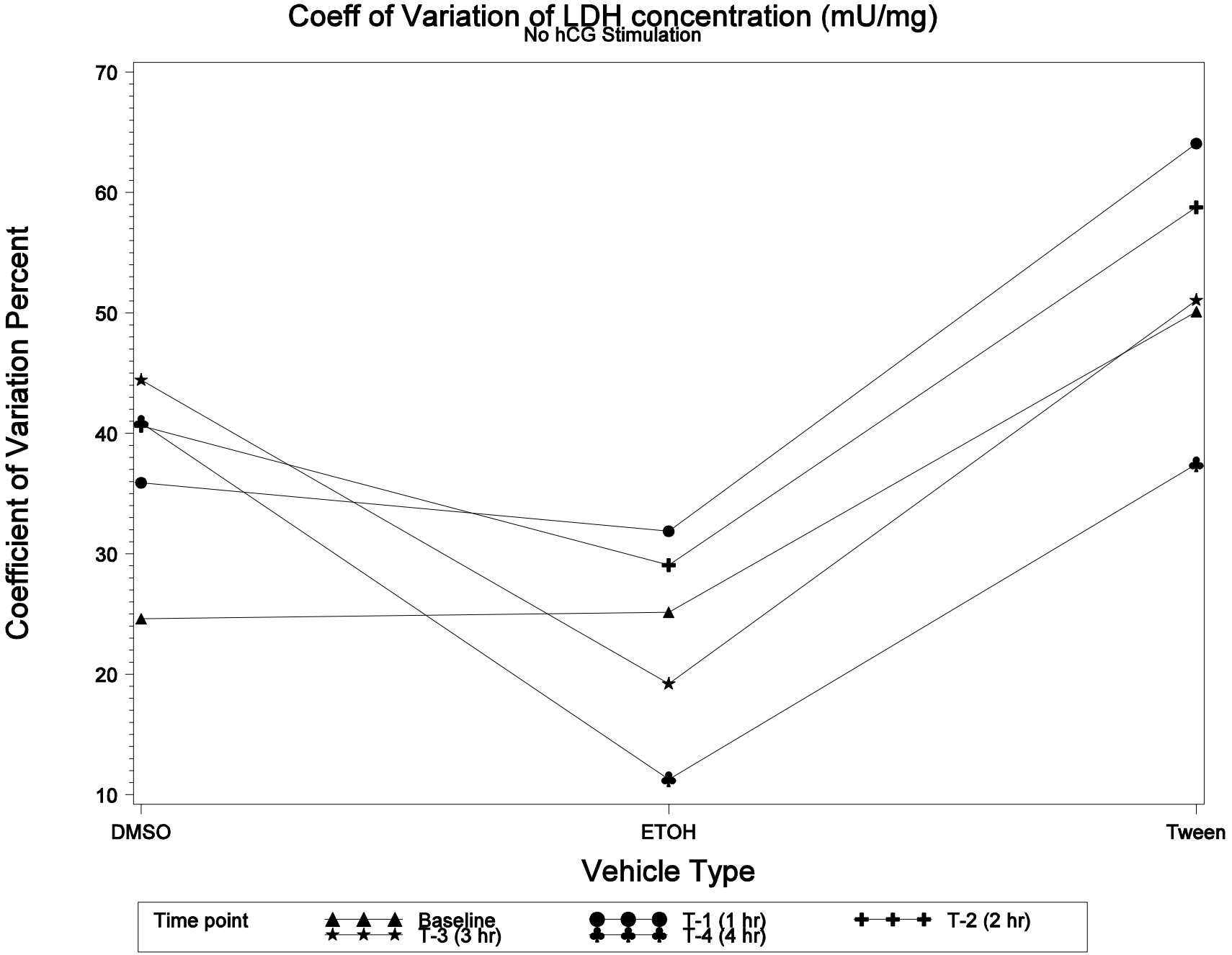
Timepoint	0.0%	0.5%	1.0%	5.0%	10.0%
Baseline	3	3	3	3	3
T-1 (1 hr)	3	3	3	3	3
T-2 (2 hr)	3	3	3	3	3
T-3 (3 hr)	3	3	3	3	3
T-4 (4 hr)	3	3	3	3	3

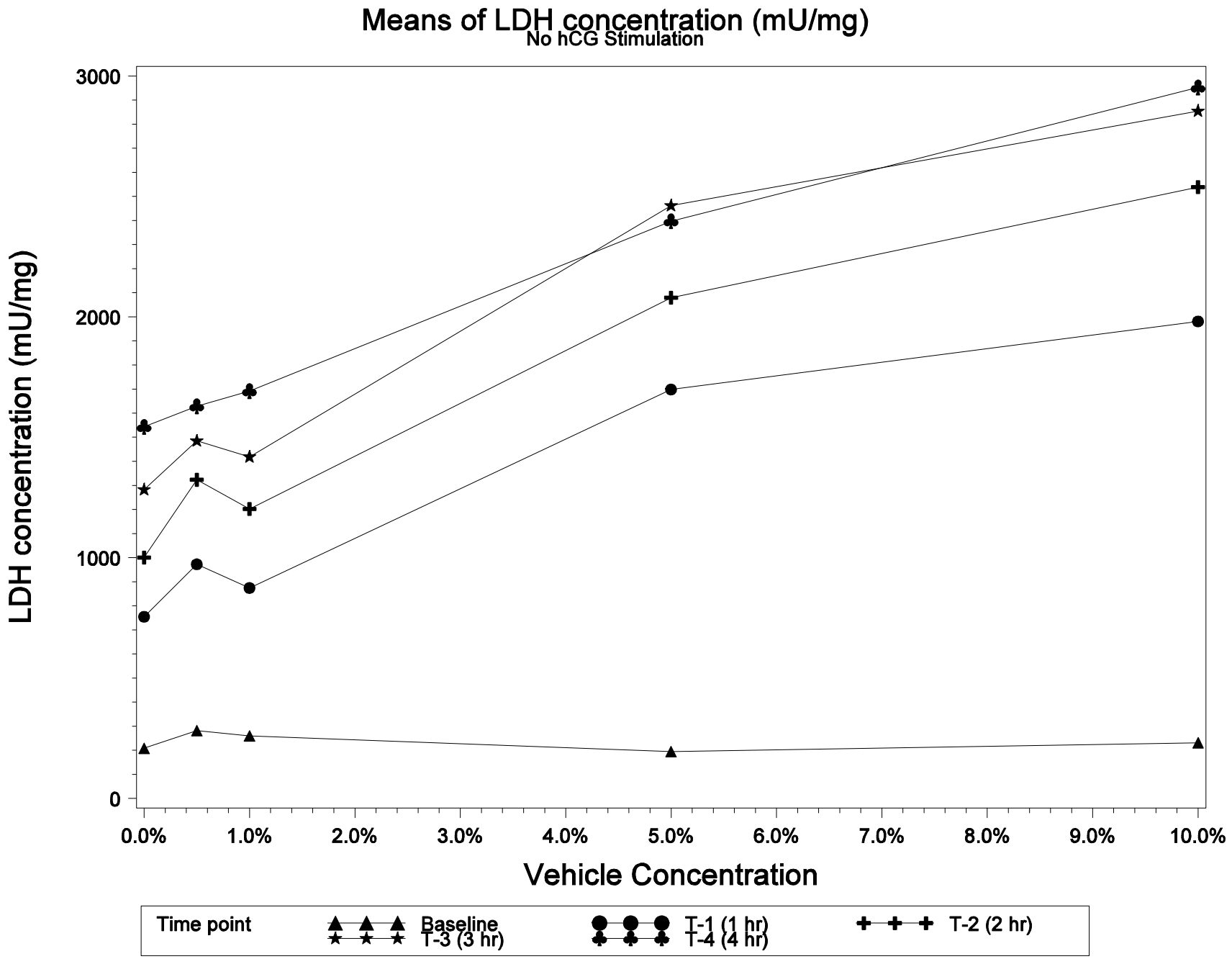
Table 152. Coeff of Variation of Testosterone Concentration (ng/mg) by Vehicle Concentration with hCG Stimulation

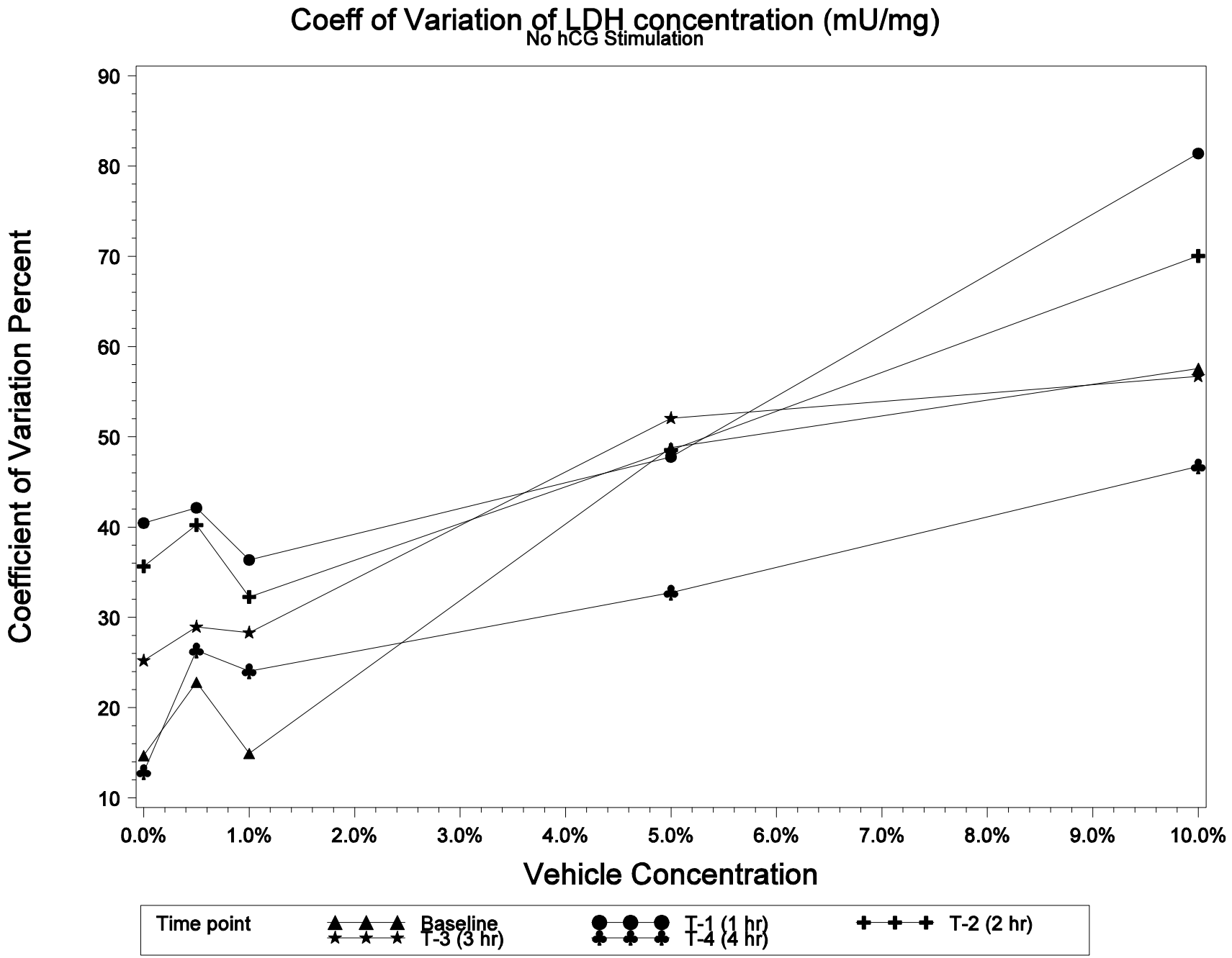
Timepoint	0.0%	0.5%	1.0%	5.0%	10.0%
Baseline	70.1	10.8	27.2	28.7	7.8
T-1 (1 hr)	71.3	8.4	32.0	16.6	72.4
T-2 (2 hr)	113.8	32.3	76.0	16.0	32.5
T-3 (3 hr)	109.6	49.3	83.0	39.3	28.4
T-4 (4 hr)	116.2	54.8	111.4	59.9	25.4

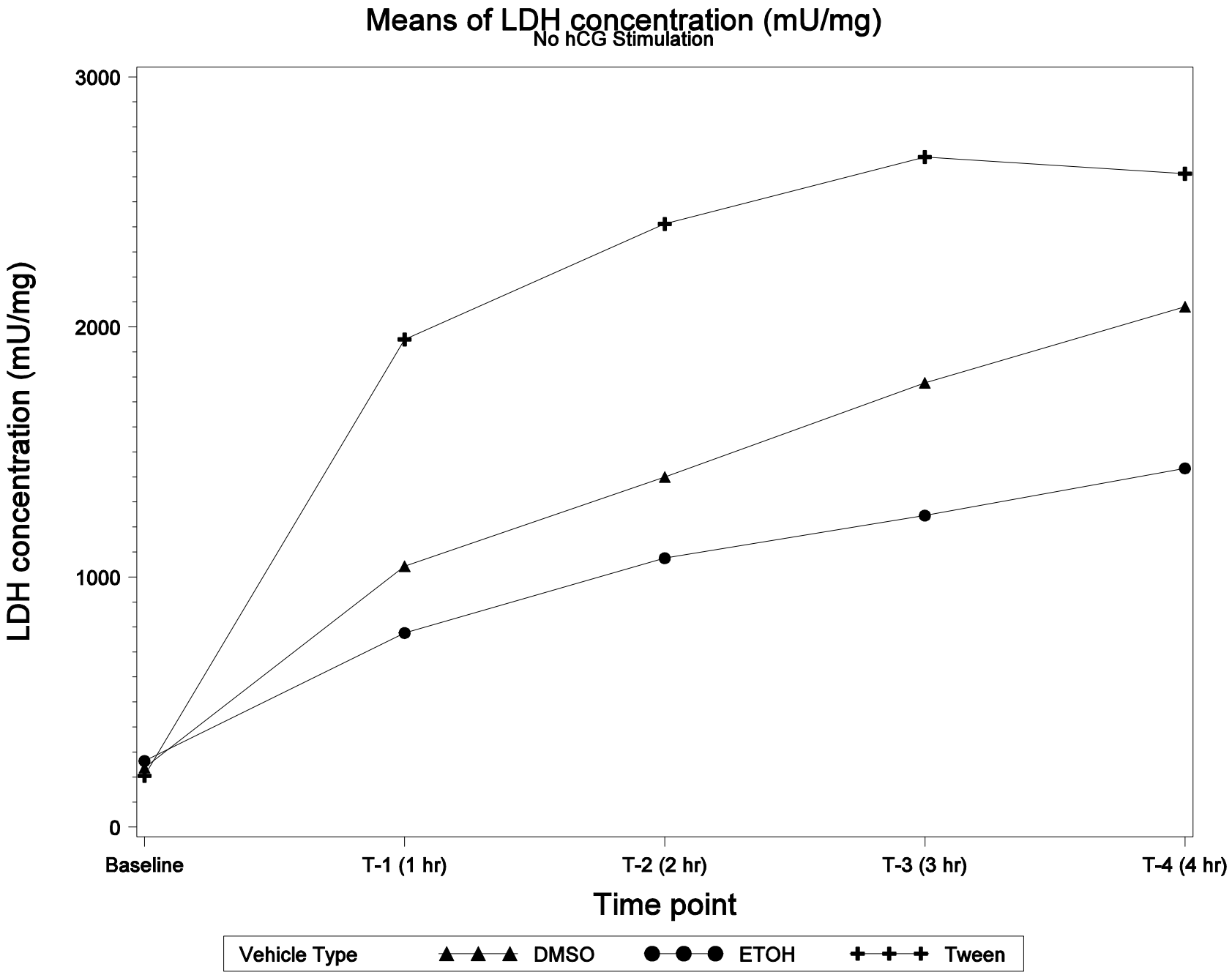
Section **14.0 VEHICLE EFFECT EXPERIMENT**

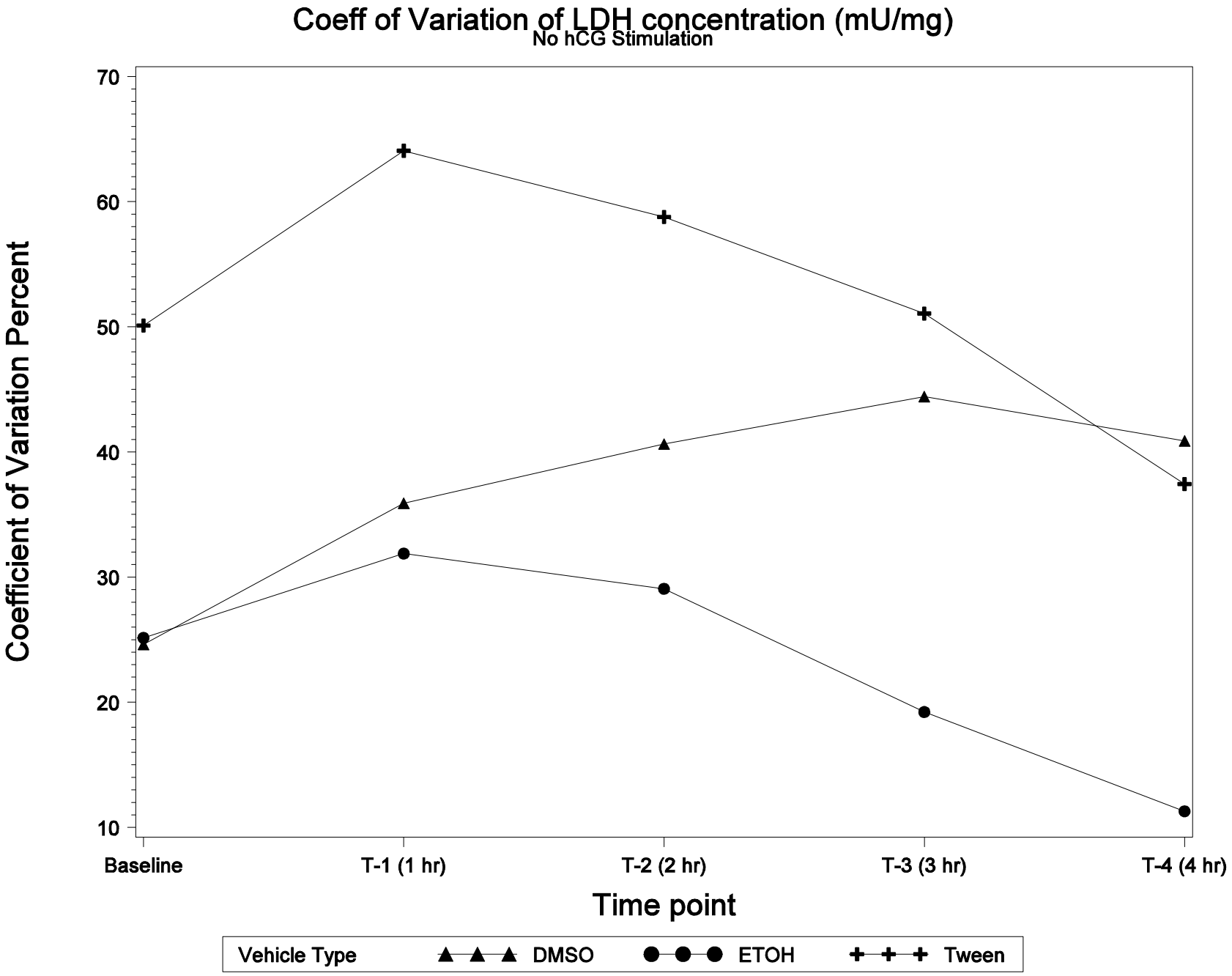

Means of LDH concentration (mU/mg)
No hCG Stimulation
LDH concentration (mU/mg)
3000
2000
1000
0
DMSO
ETOH
Tween
Vehicle Type
Time point
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)

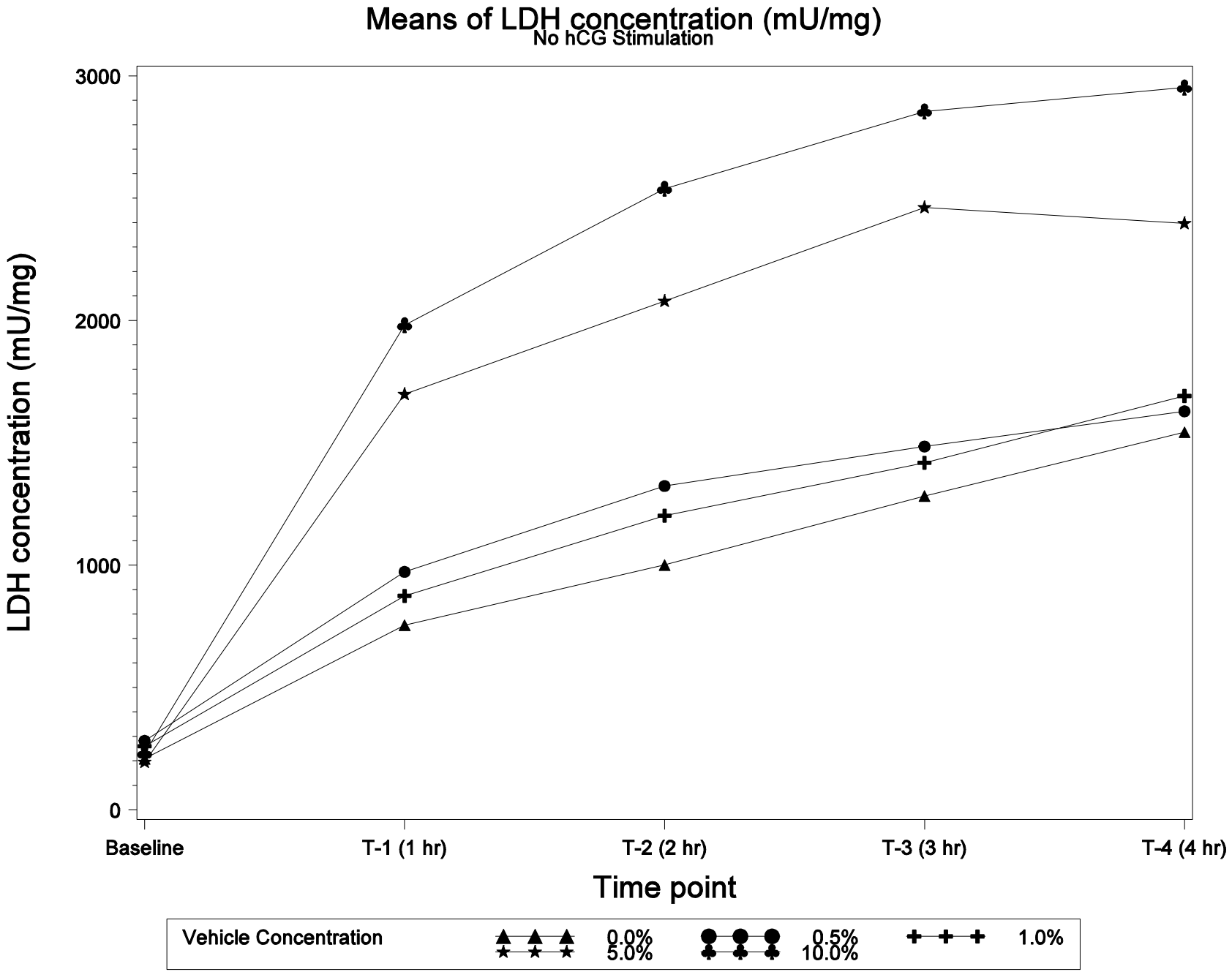

Coeff of Variation of LDH concentration (mU/mg)
No hCG Stimulation
Coefficient of Variation Percent
70
60
50
40
30
20
10
DMSO
ETOH
Tween
Vehicle Type
Time point
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)

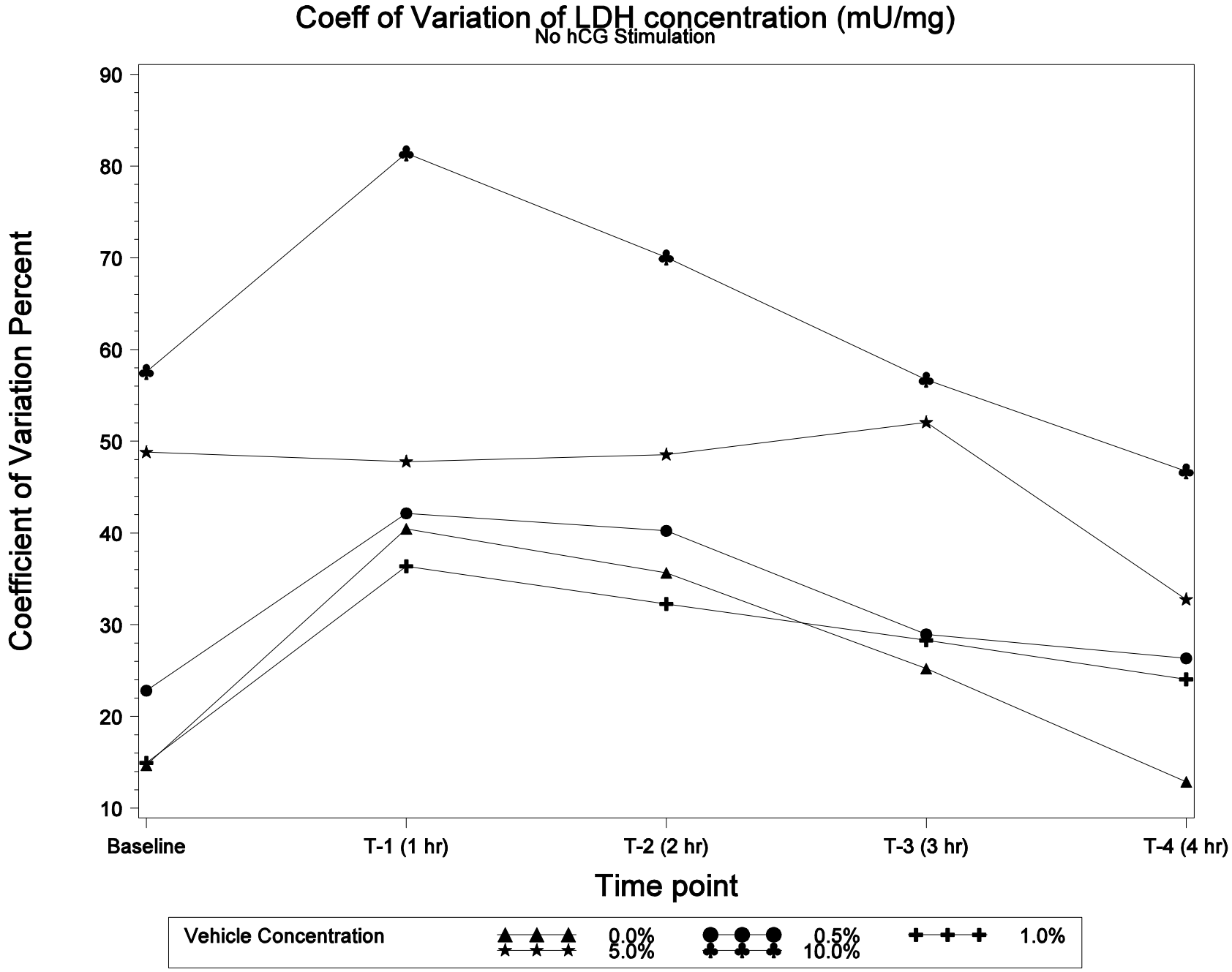

Means of LDH concentration (mU/mg)
No hCG Stimulation
LDH concentration (mU/mg)
3000
2000
1000
0
0.0%
1.0%
2.0%
3.0%
4.0%
5.0%
6.0%
7.0%
8.0%
9.0%
10.0%
Vehicle Concentration
Time point
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)

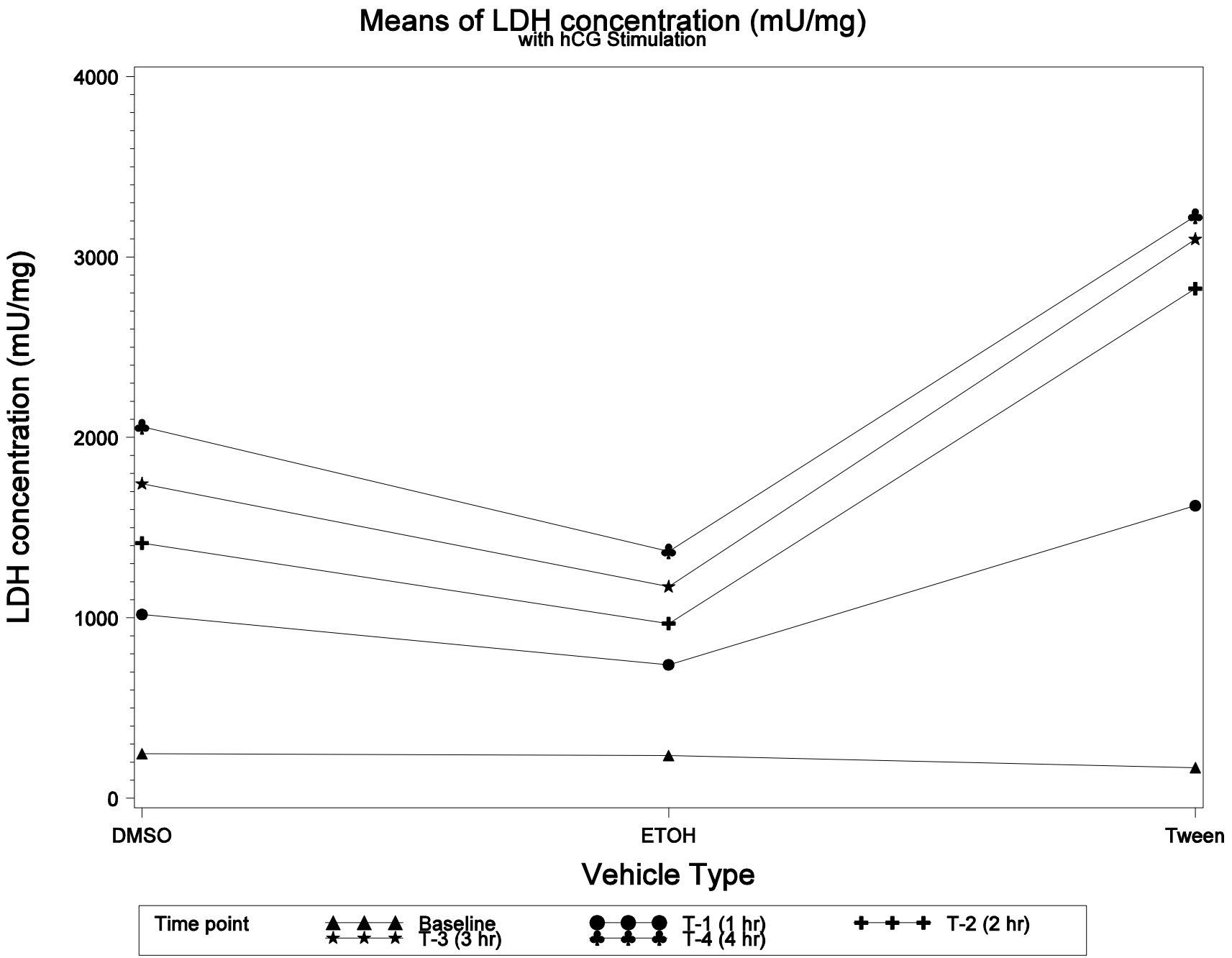

Coeff of Variation of LDH concentration (mU/mg)
No hCG Stimulation
Coefficient of Variation Percent
90
80
70
60
50
40
30
20
10
0.0%
1.0%
2.0%
3.0%
4.0%
5.0%
6.0%
7.0%
8.0%
9.0%
10.0%
Vehicle Concentration
Time point
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)

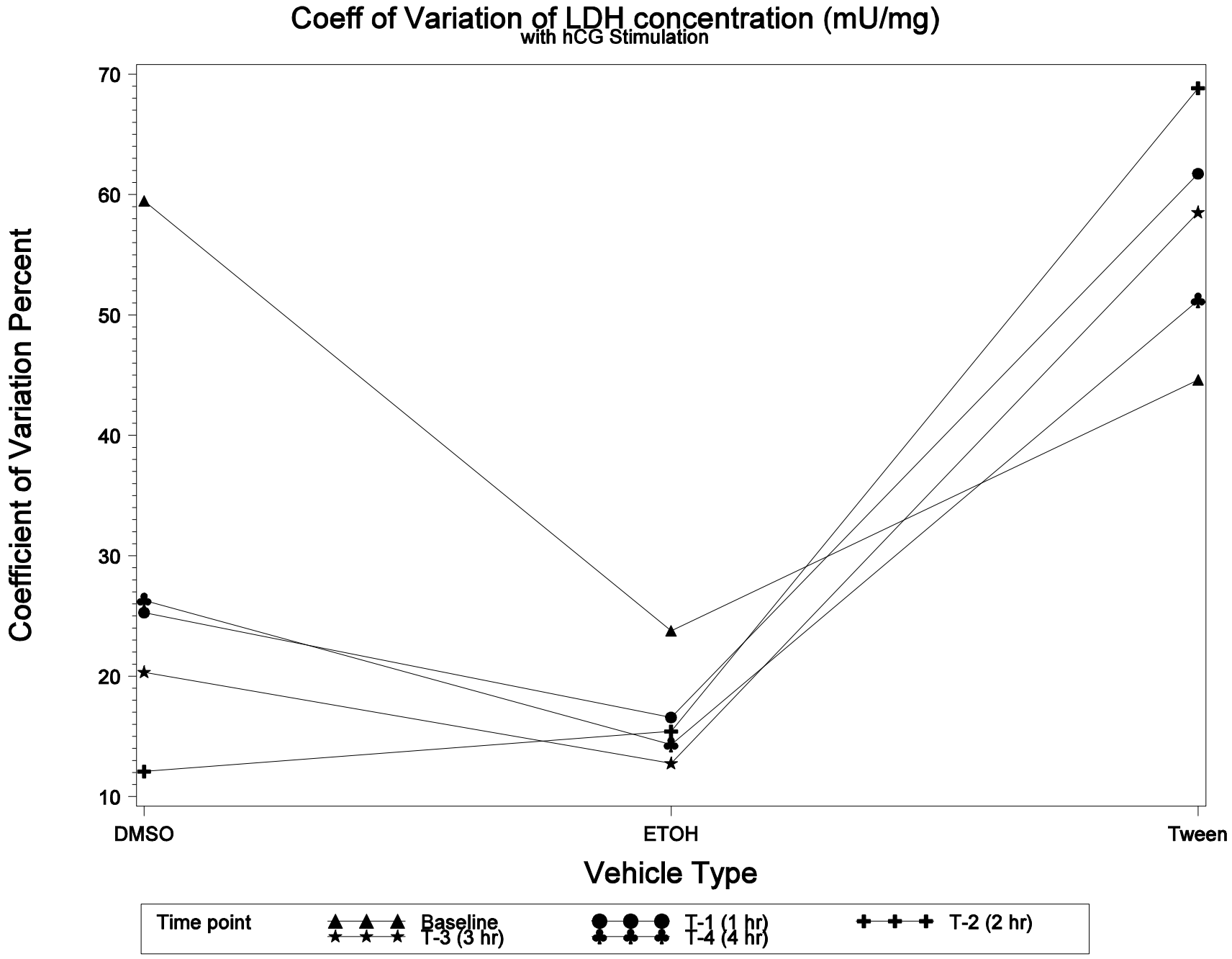

Means of LDH concentration (mU/mg)
No hCG Stimulation
LDH concentration (mU/mg)
3000
2000
1000
0
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)
Time point
Vehicle Type
DMSO
ETOH
Tween

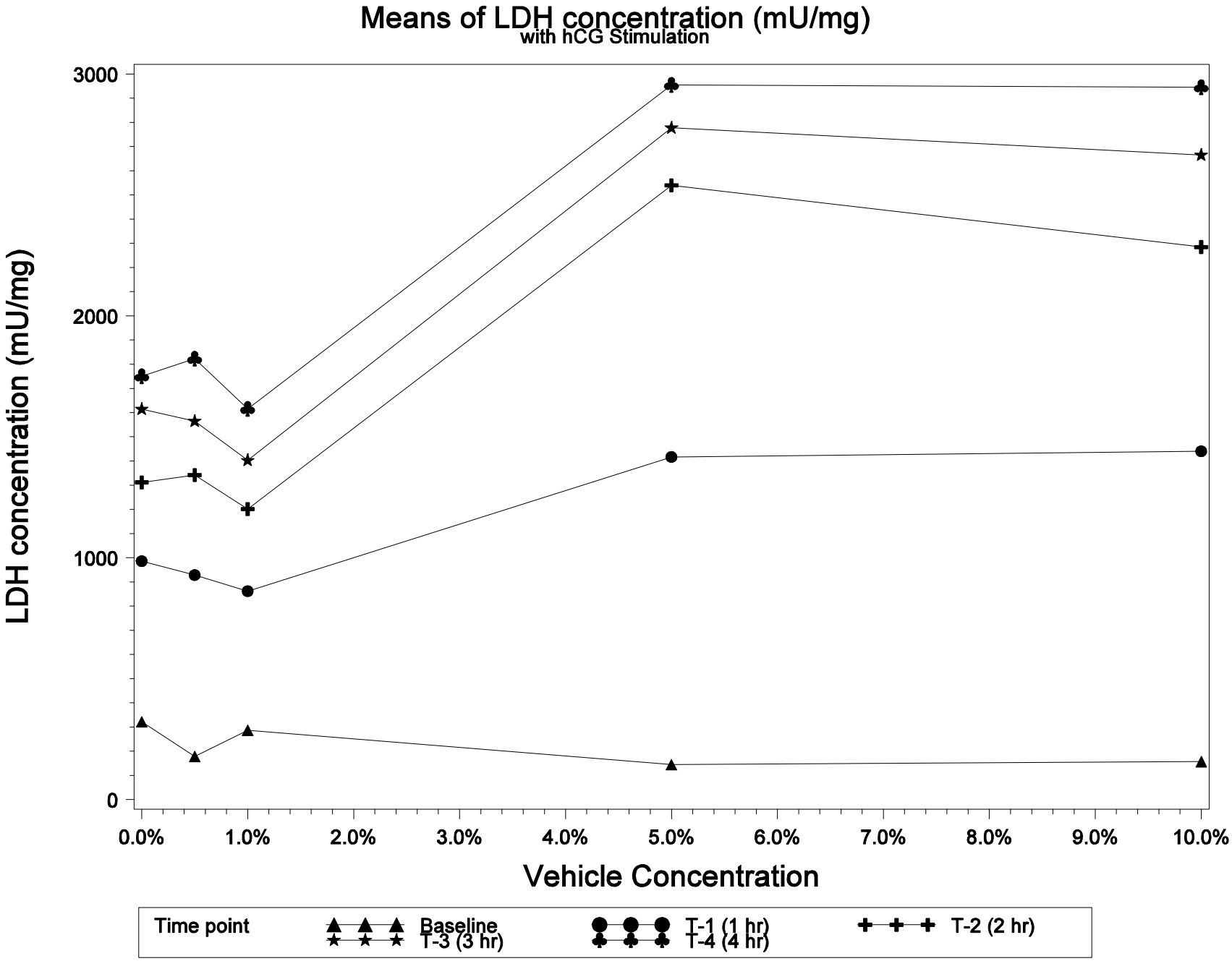

Coeff of Variation of LDH concentration (mU/mg)
No hCG Stimulation
Coefficient of Variation Percent
70
60
50
40
30
20
10
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)
Time point
Vehicle Type
DMSO
ETOH
Tween

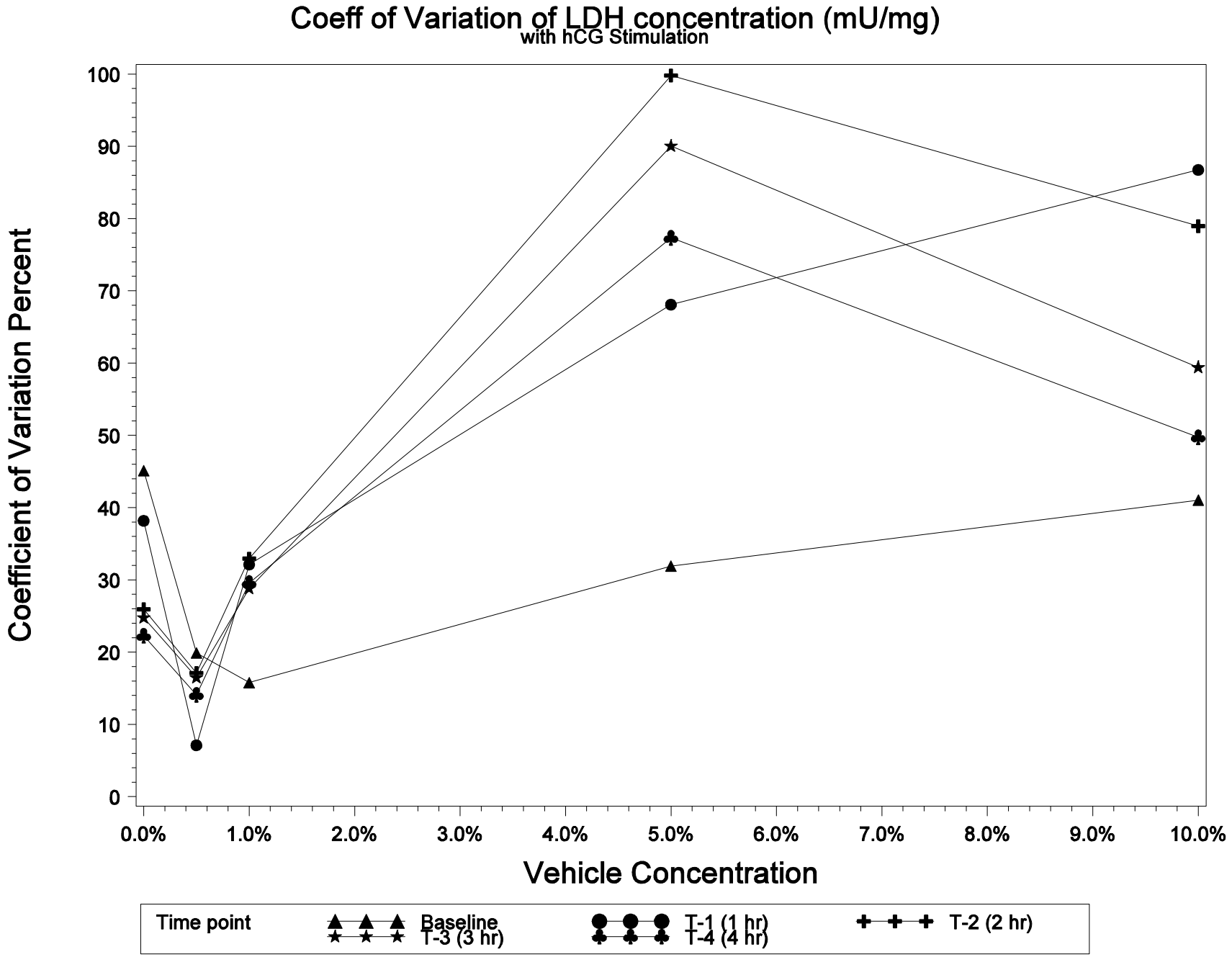

Means of LDH concentration (mU/mg)
No hCG Stimulation
LDH concentration (mU/mg)
3000
2000
1000
0
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)
Time point
Vehicle Concentration
0.0%
0.5%
1.0%
5.0%
10.0%

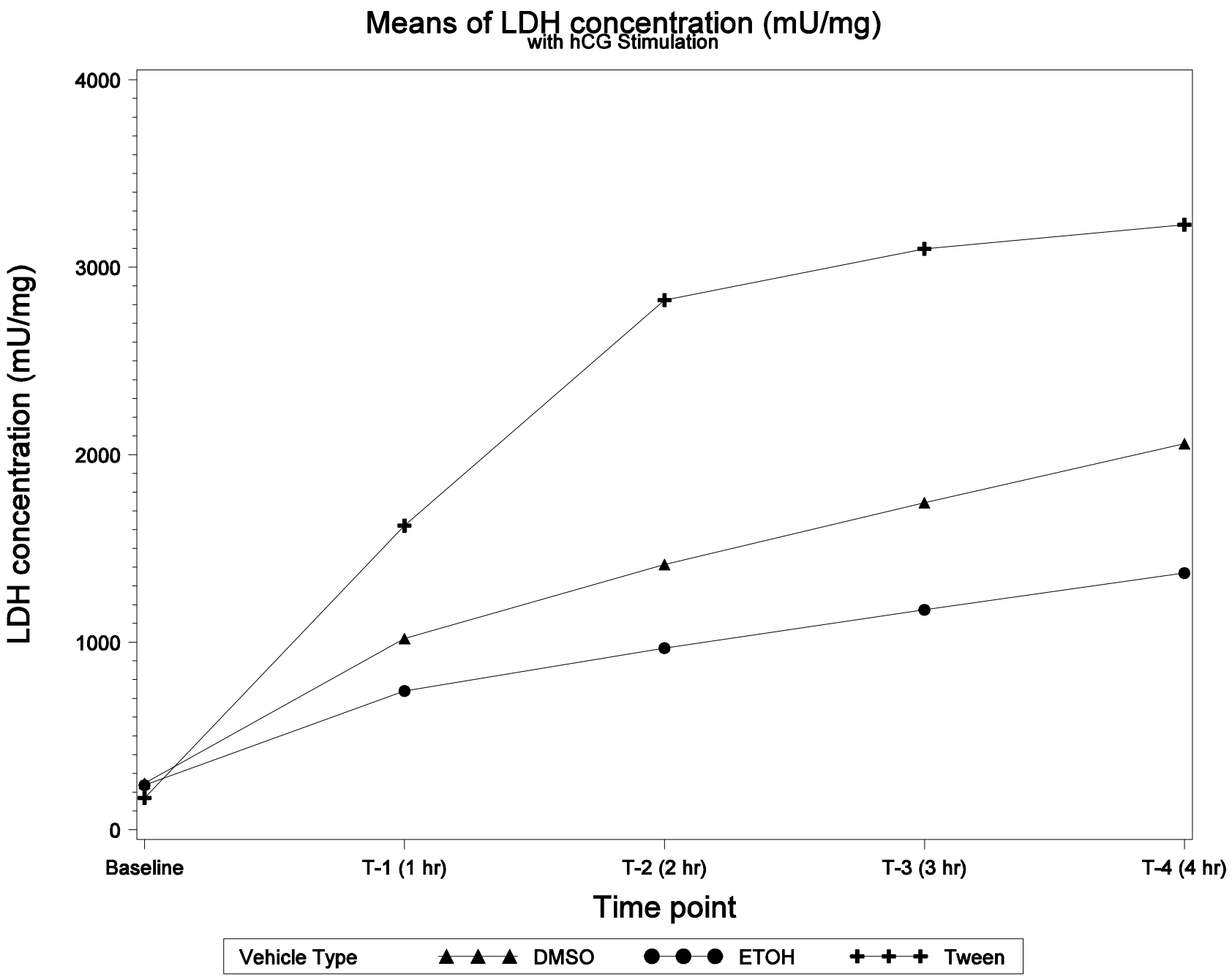

Coeff of Variation of LDH concentration (mU/mg)
No hCG Stimulation
Coefficient of Variation Percent
90
80
70
60
50
40
30
20
10
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)
Time point
Vehicle Concentration
0.0%
0.5%
1.0%
5.0%
10.0%

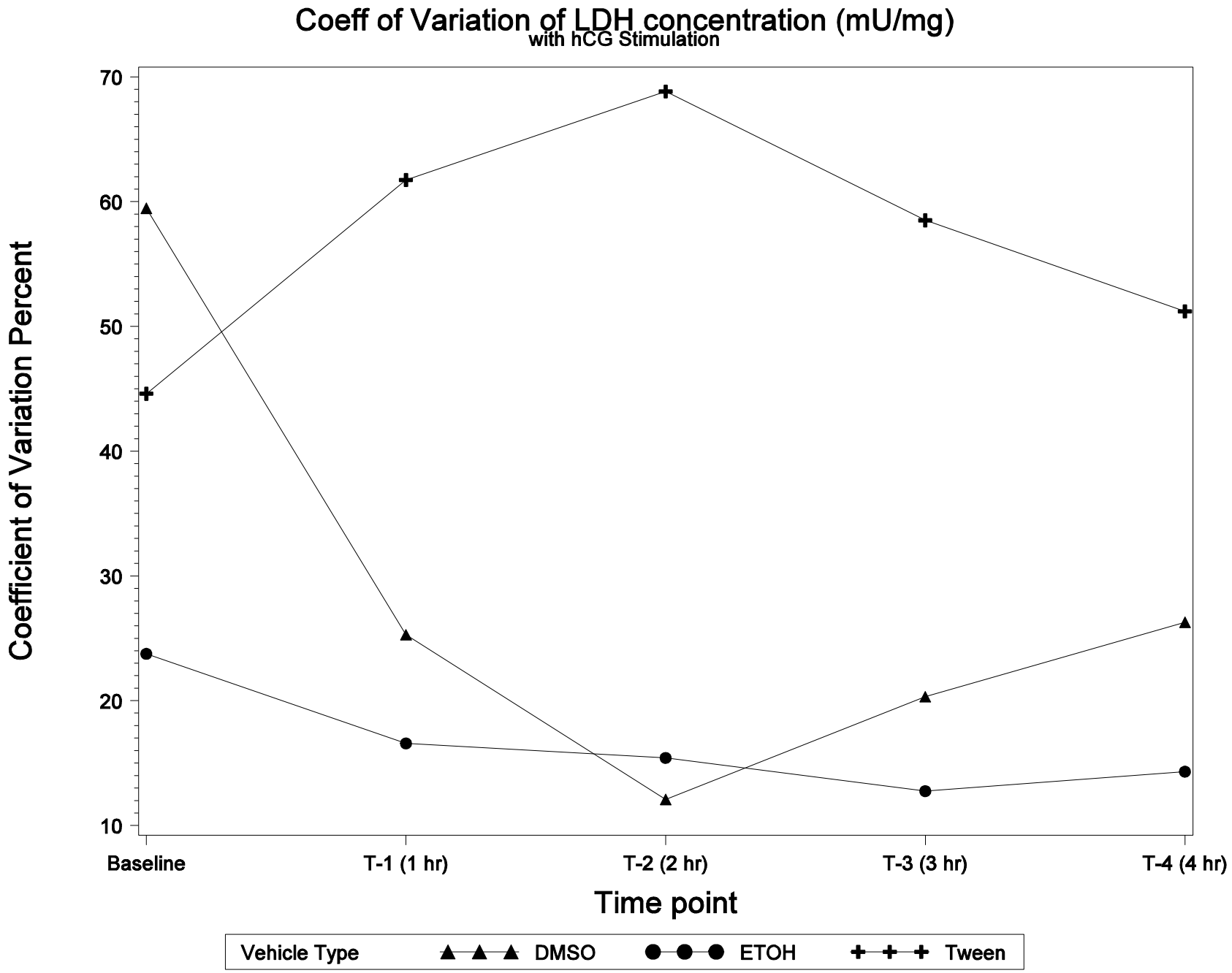

Means of LDH concentration (mU/mg)
with hCG Stimulation
LDH concentration (mU/mg)
4000
3000
2000
1000
0
DMSO
ETOH
Tween
Vehicle Type
Time point
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)

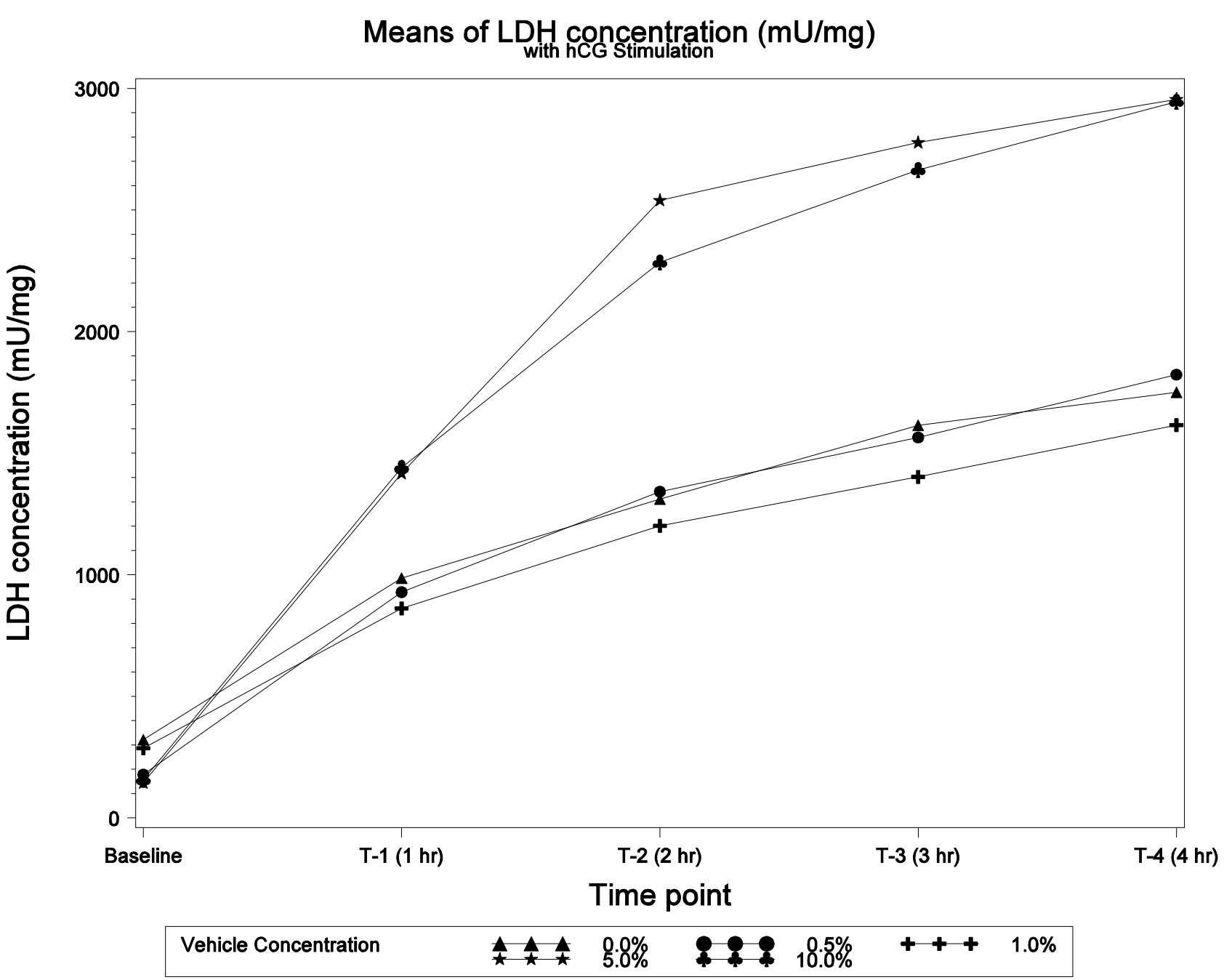

Coeff of Variation of LDH concentration (mU/mg)
with hCG Stimulation
Coefficient of Variation Percent
70
60
50
40
30
20
10
DMSO
ETOH
Tween
Vehicle Type
Time point
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)

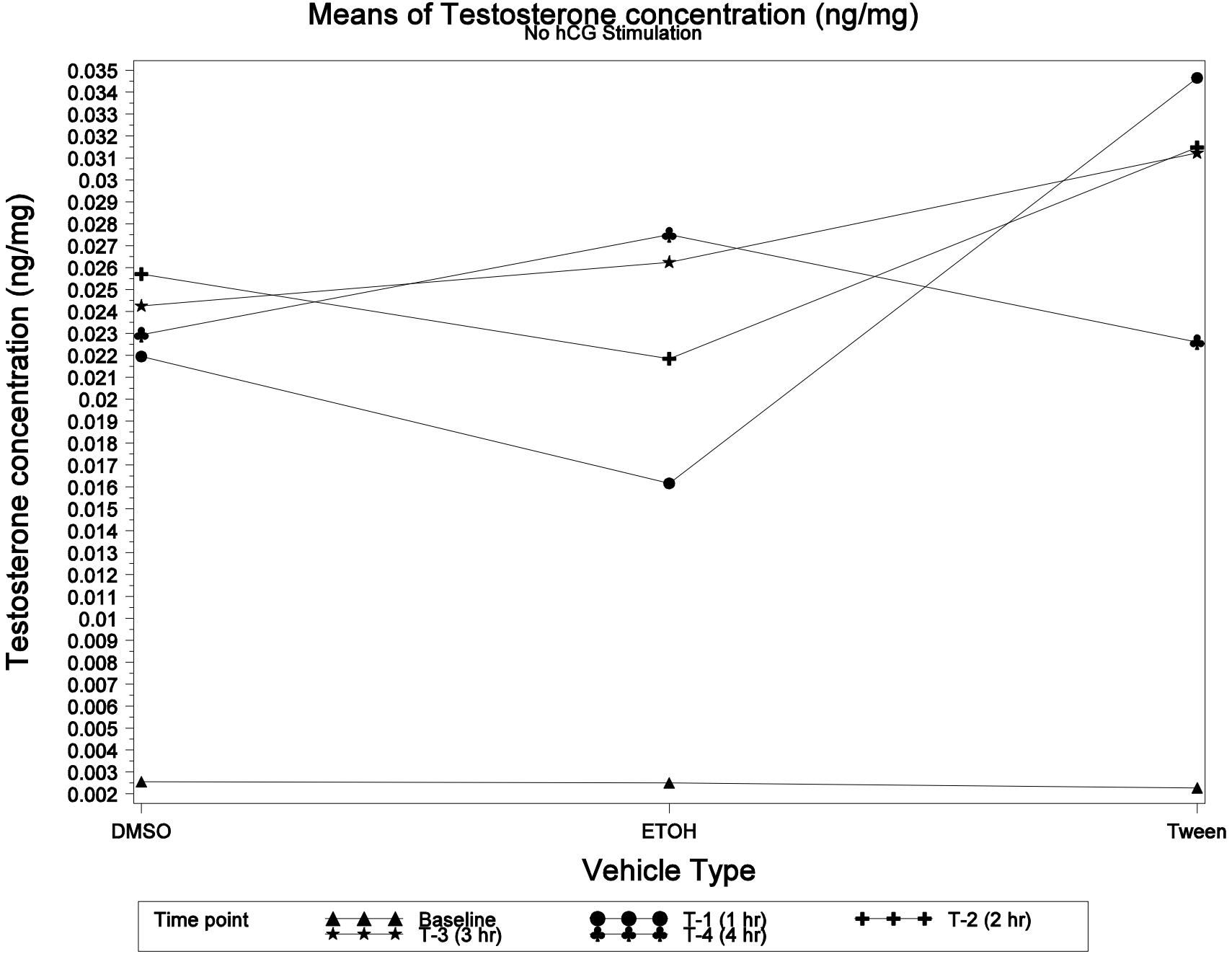

Means of LDH concentration (mU/mg)
with hCG Stimulation
LDH concentration (mU/mg)
3000
2000
1000
0
0.0%
1.0%
2.0%
3.0%
4.0%
5.0%
6.0%
7.0%
8.0%
9.0%
10.0%
Vehicle Concentration
Time point
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)

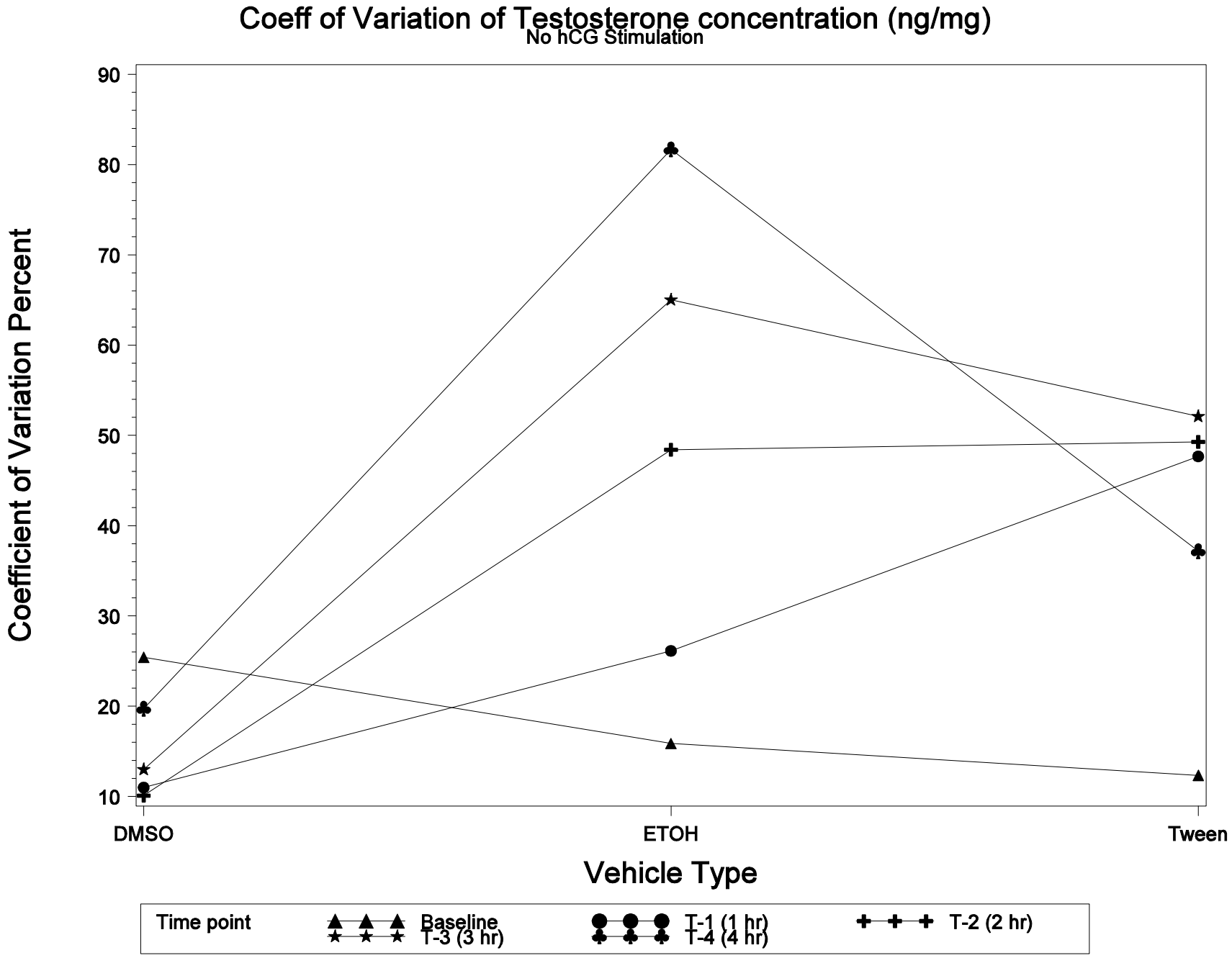

Coeff of Variation of LDH concentration (mU/mg)
with hCG Stimulation
Coefficient of Variation Percent
100
90
80
70
60
50
40
30
20
10
0
0.0%
1.0%
2.0%
3.0%
4.0%
5.0%
6.0%
7.0%
8.0%
9.0%
10.0%
Vehicle Concentration
Time point
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)

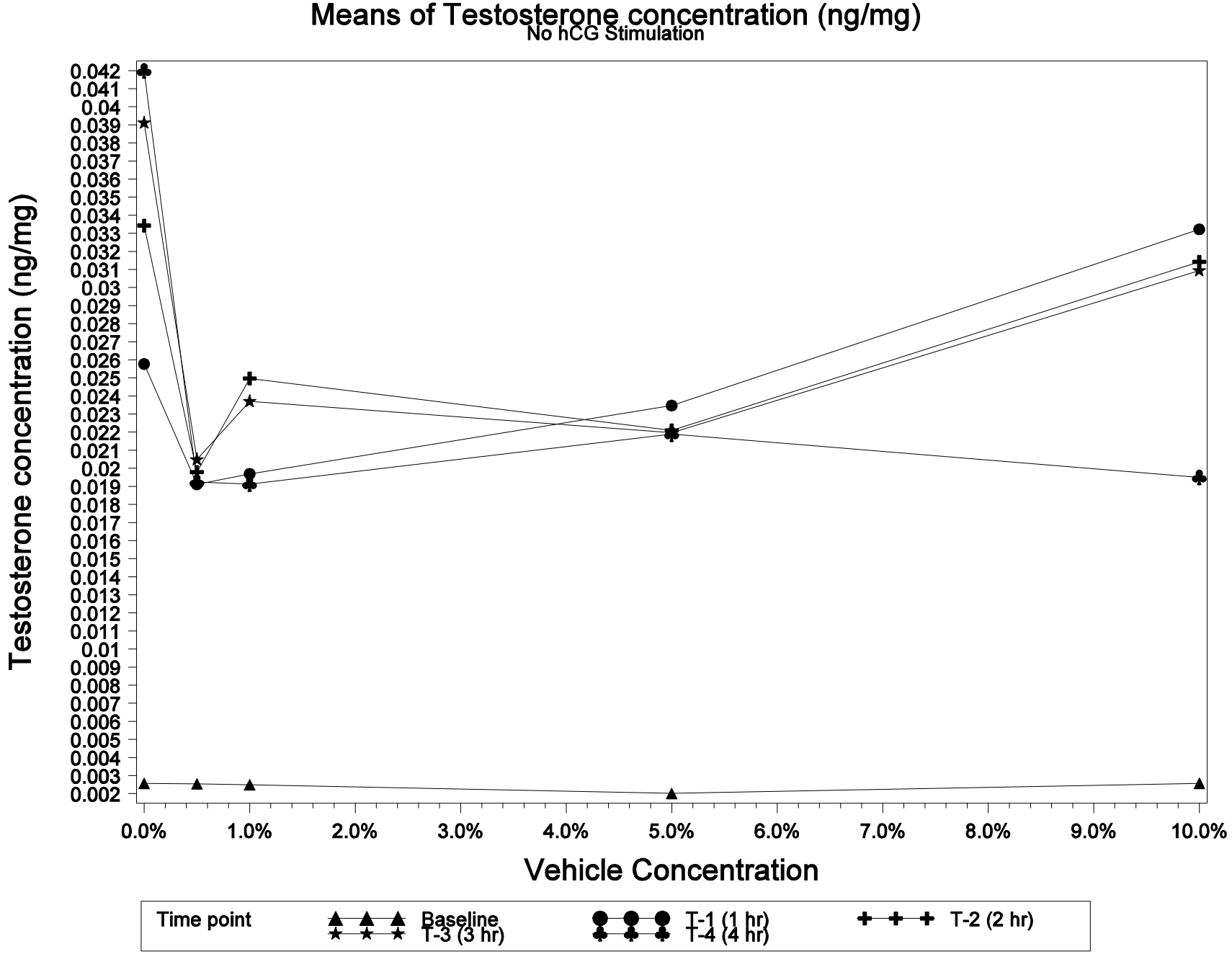

Means of LDH concentration (mU/mg)
with hCG Stimulation
LDH concentration (mU/mg)
4000
3000
2000
1000
0
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)
Time point
Vehicle Type
DMSO
ETOH
Tween

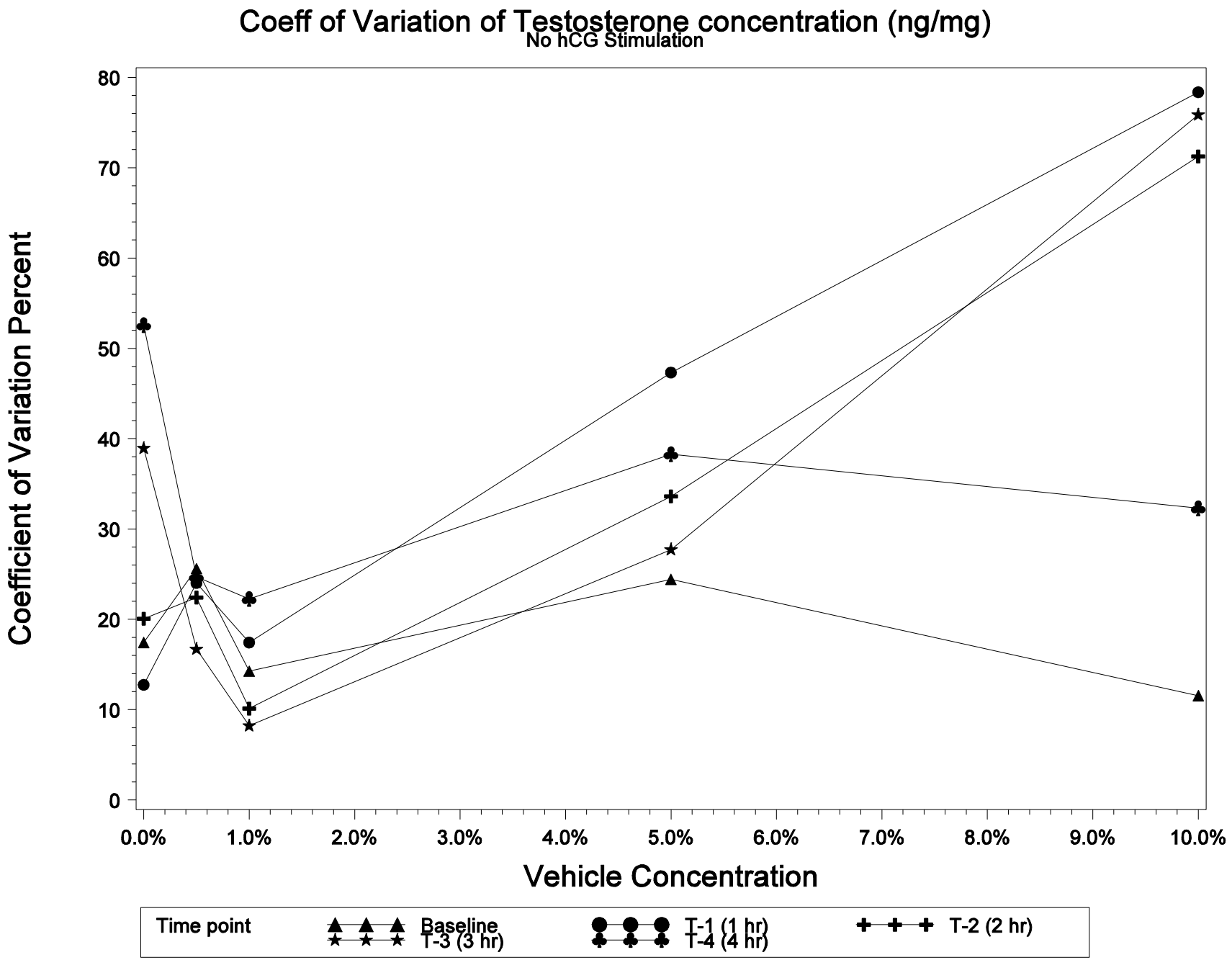

Coeff of Variation of LDH concentration (mU/mg)
with hCG Stimulation
Coefficient of Variation Percent
70
60
50
40
30
20
10
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)
Time point
Vehicle Type
DMSO
ETOH
Tween

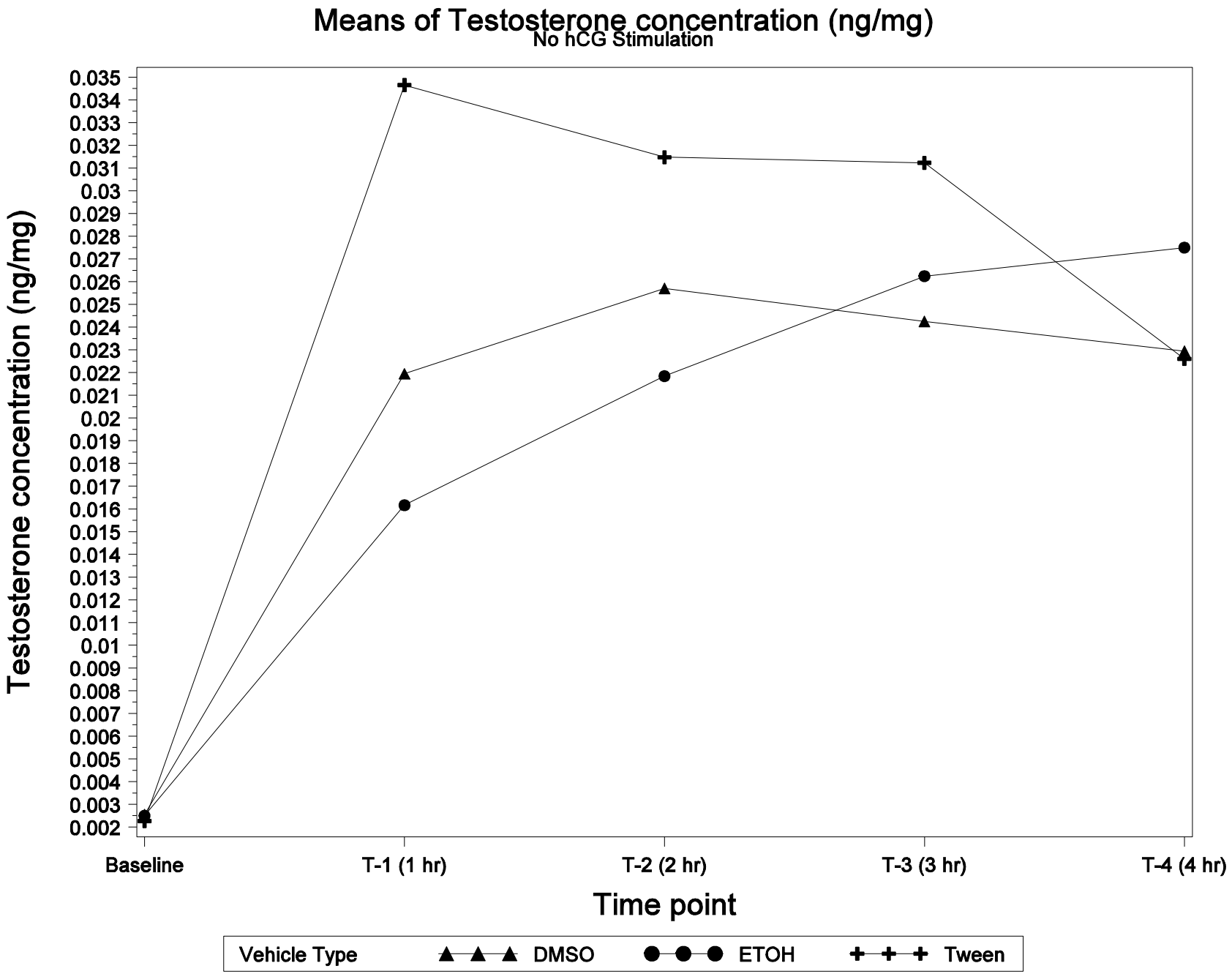

Means of LDH concentration (mU/mg)
with hCG Stimulation
LDH concentration (mU/mg)
3000
2000
1000
0
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)
Time point
Vehicle Concentration
0.0%
5.0%
0.5%
10.0%
1.0%

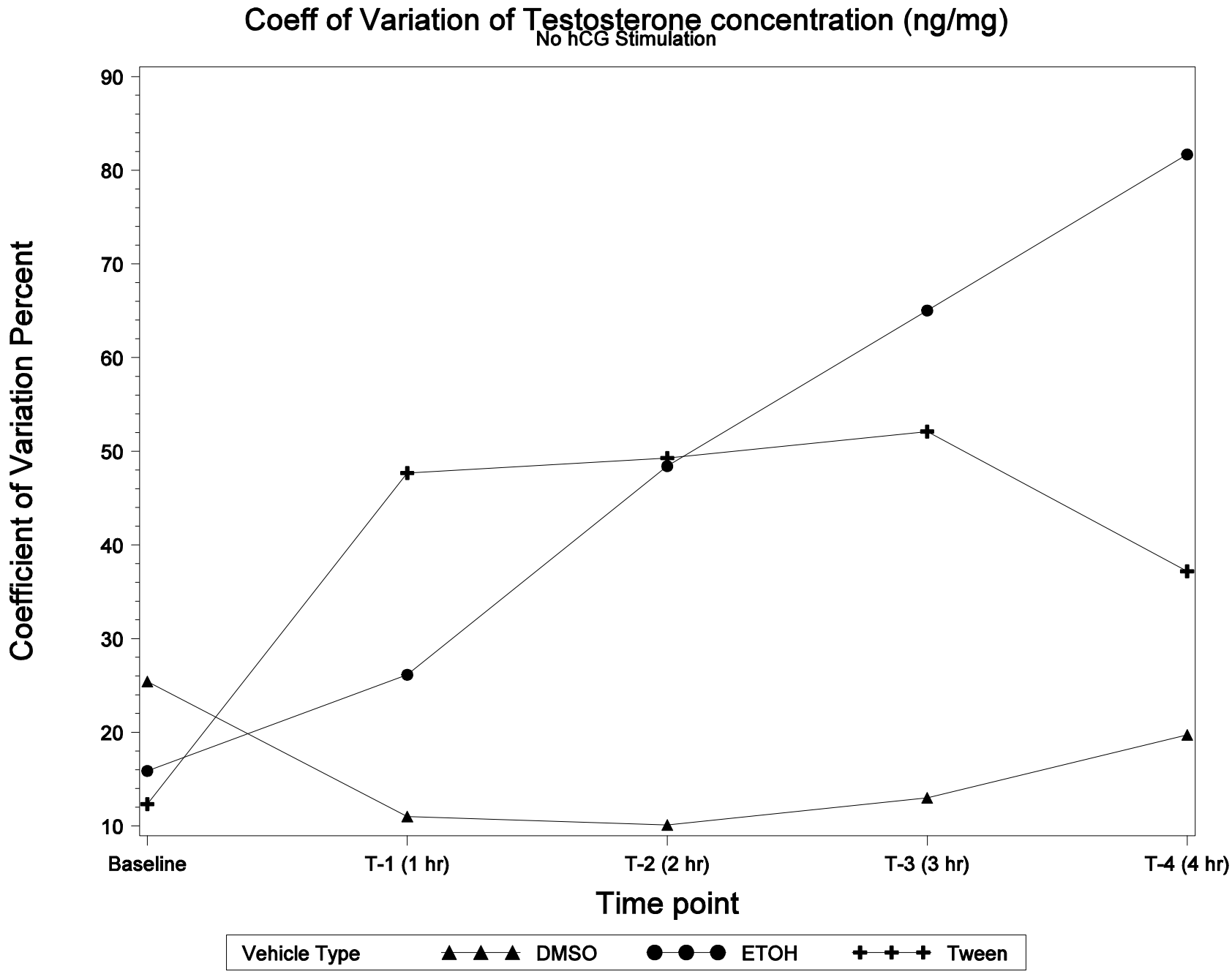

Means of Testosterone concentration (ng/mg)
No hCG Stimulation
Testosterone concentration (ng/mg)
0.035
0.034
0.033
0.032
0.031
0.03
0.029
0.028
0.027
0.026
0.025
0.024
0.023
0.022
0.021
0.02
0.019
0.018
0.017
0.016
0.015
0.014
0.013
0.012
0.011
0.01
0.009
0.008
0.007
0.006
0.005
0.004
0.003
0.002
DMSO
ETOH
Tween
Vehicle Type
Time point
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)

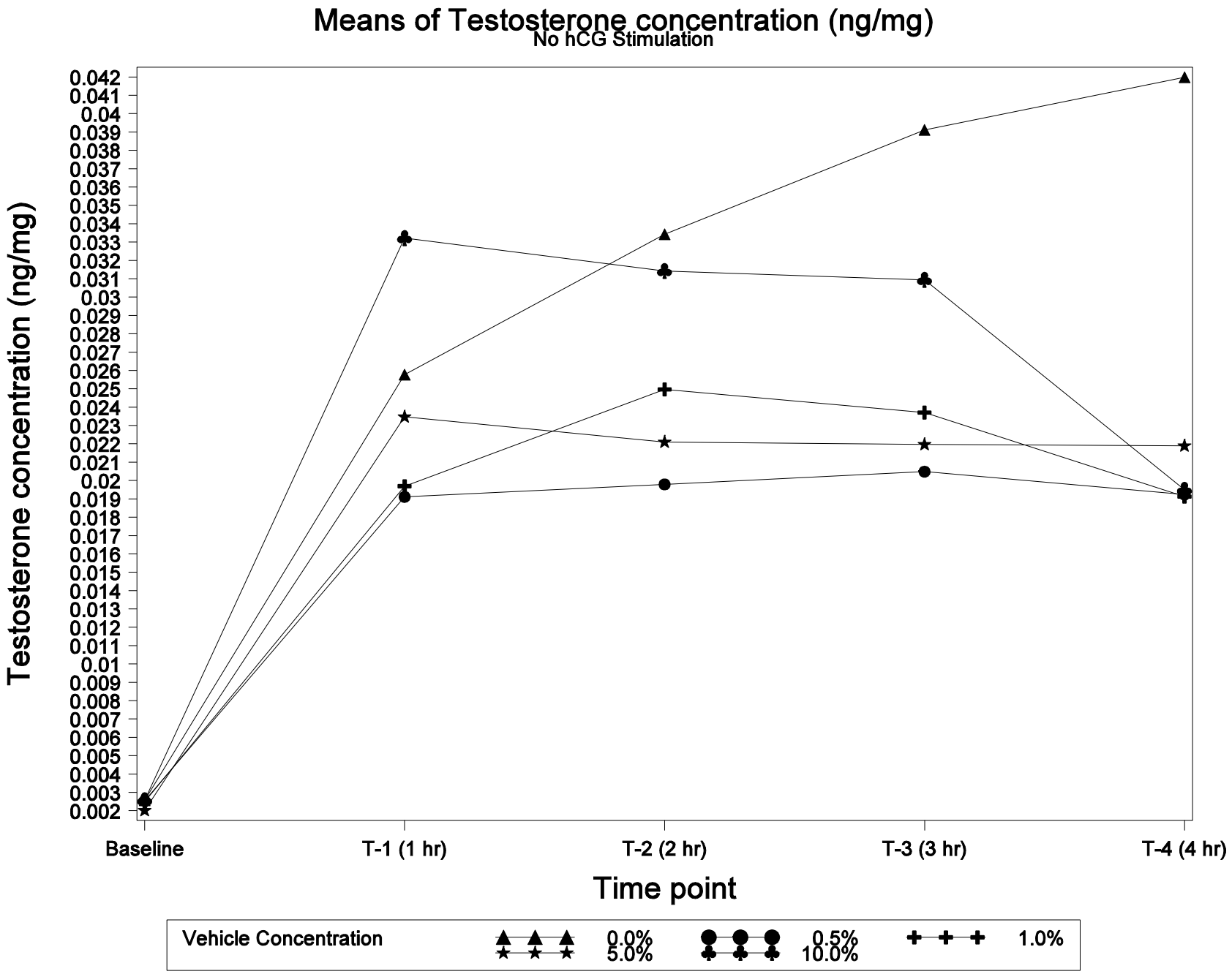

Coeff of Variation of Testosterone concentration (ng/mg)
No hCG Stimulation
Coefficient of Variation Percent
90
80
70
60
50
40
30
20
10
DMSO
ETOH
Tween
Vehicle Type
Time point
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)

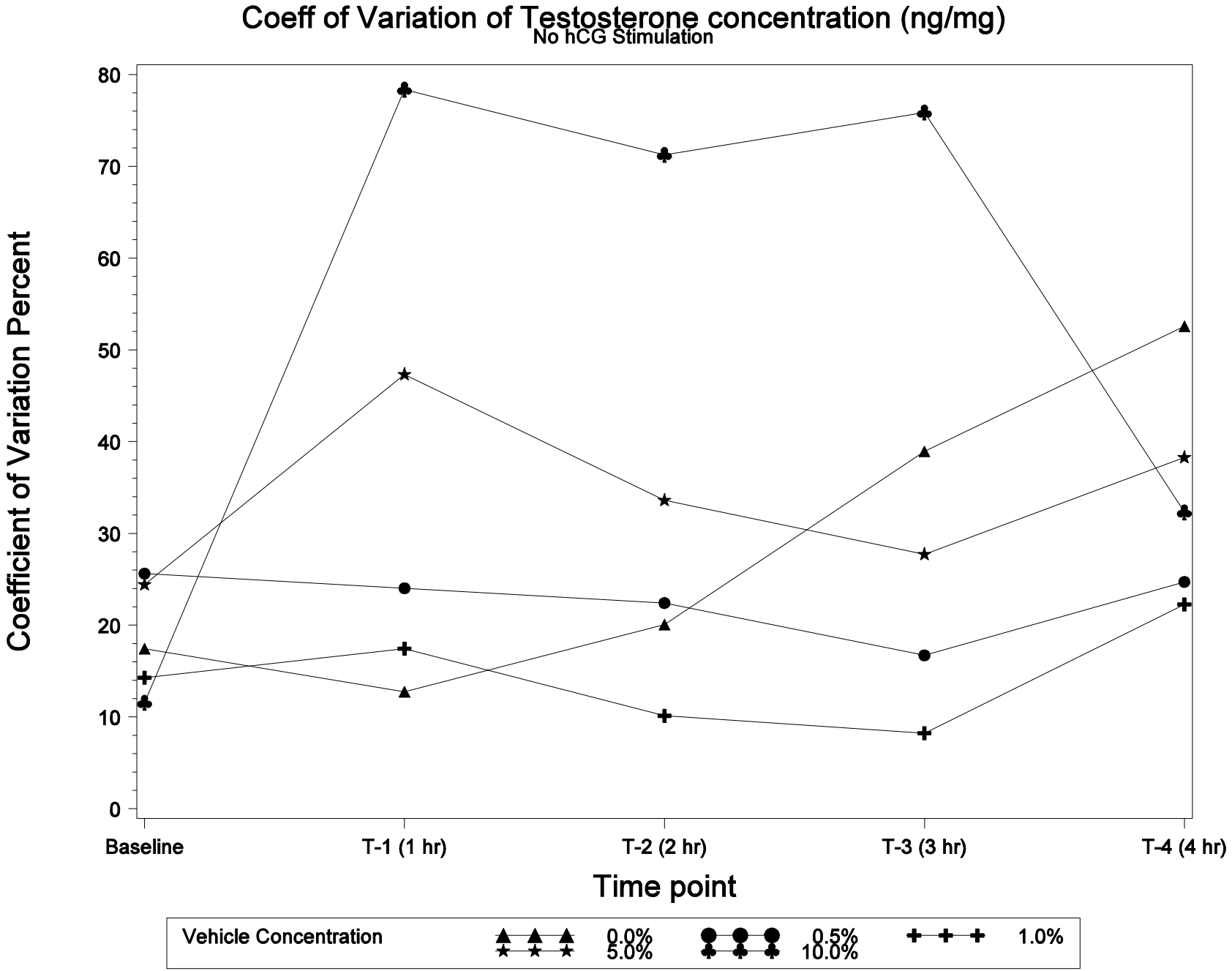

Means of Testosterone concentration (ng/mg)
No hCG Stimulation
Testosterone concentration (ng/mg)
0.042
0.041
0.04
0.039
0.038
0.037
0.036
0.035
0.034
0.033
0.032
0.031
0.03
0.029
0.028
0.027
0.026
0.025
0.024
0.023
0.022
0.021
0.02
0.019
0.018
0.017
0.016
0.015
0.014
0.013
0.012
0.011
0.01
0.009
0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.0%
1.0%
2.0%
3.0%
4.0%
5.0%
6.0%
7.0%
8.0%
9.0%
10.0%
Vehicle Concentration
Time point
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)

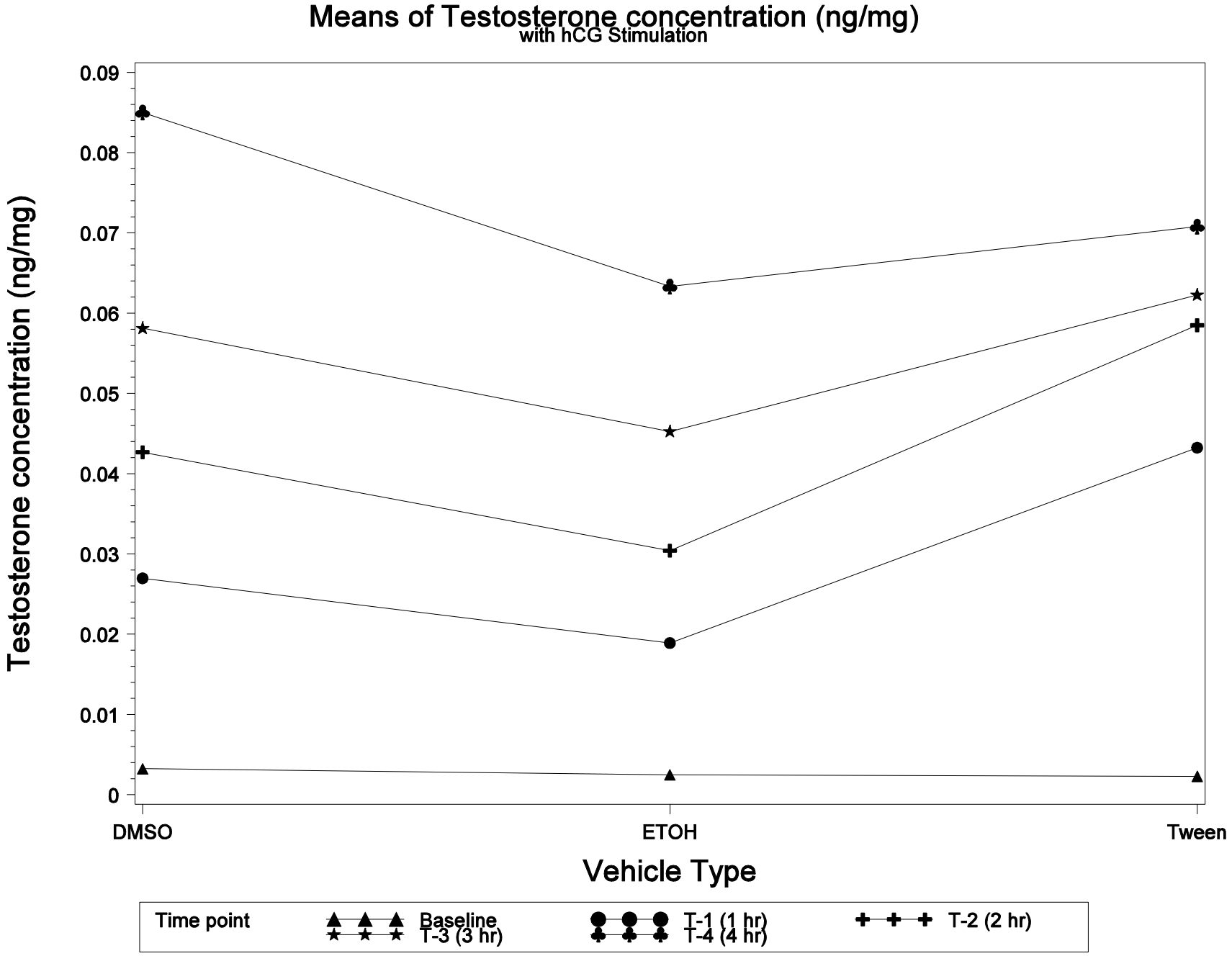

Coeff of Variation of Testosterone concentration (ng/mg)
No hCG Stimulation
Coefficient of Variation Percent
80
70
60
50
40
30
20
10
0
0.0%
1.0%
2.0%
3.0%
4.0%
5.0%
6.0%
7.0%
8.0%
9.0%
10.0%
Vehicle Concentration
Time point
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)

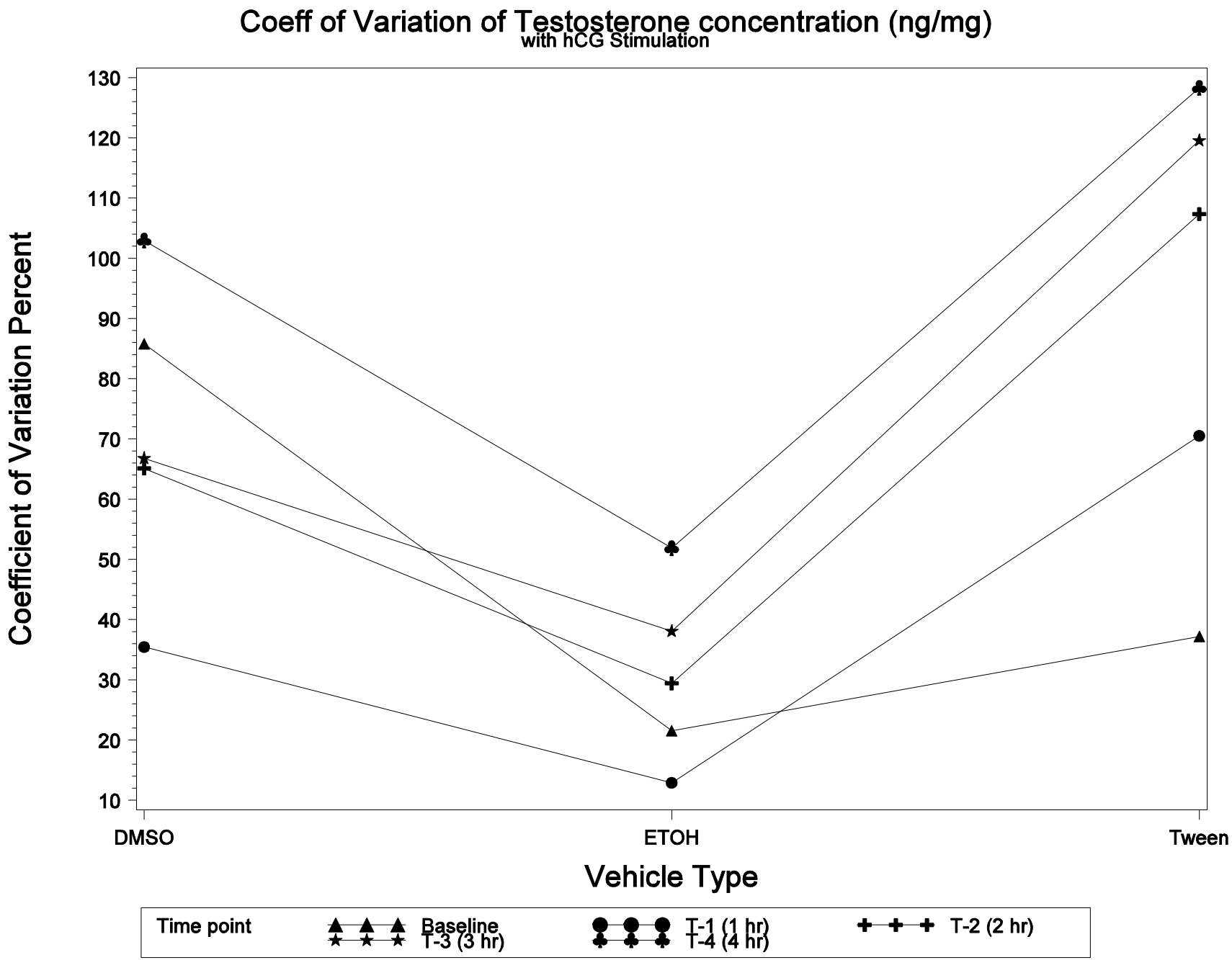

Means of Testosterone concentration (ng/mg)
No hCG Stimulation
Testosterone concentration (ng/mg)
0.035
0.034
0.033
0.032
0.031
0.03
0.029
0.028
0.027
0.026
0.025
0.024
0.023
0.022
0.021
0.02
0.019
0.018
0.017
0.016
0.015
0.014
0.013
0.012
0.011
0.01
0.009
0.008
0.007
0.006
0.005
0.004
0.003
0.002
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)
Time point
Vehicle Type
DMSO
ETOH
Tween

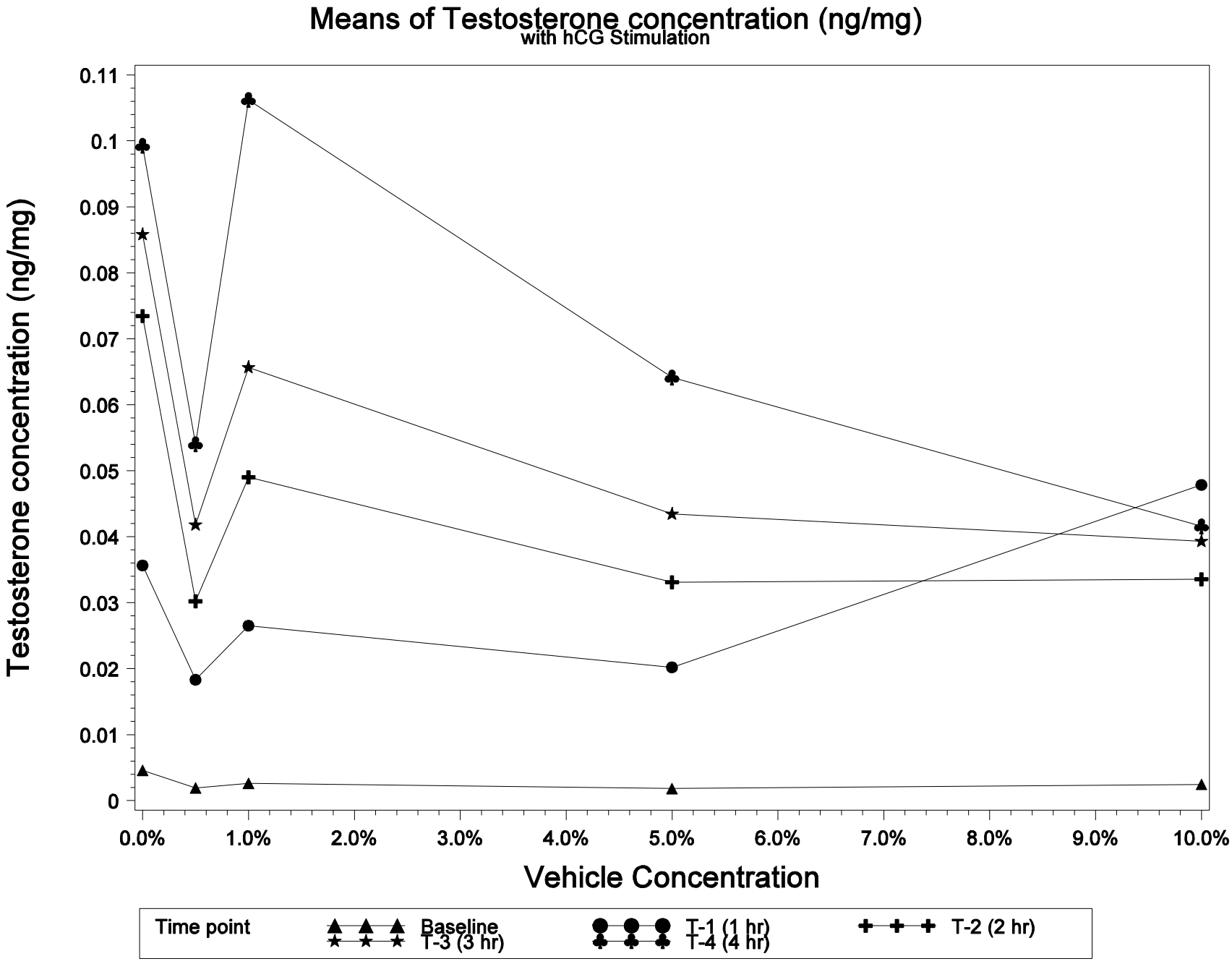

Coeff of Variation of Testosterone concentration (ng/mg)
No hCG Stimulation
Coefficient of Variation Percent
90
80
70
60
50
40
30
20
10
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)
Time point
Vehicle Type
DMSO
ETOH
Tween

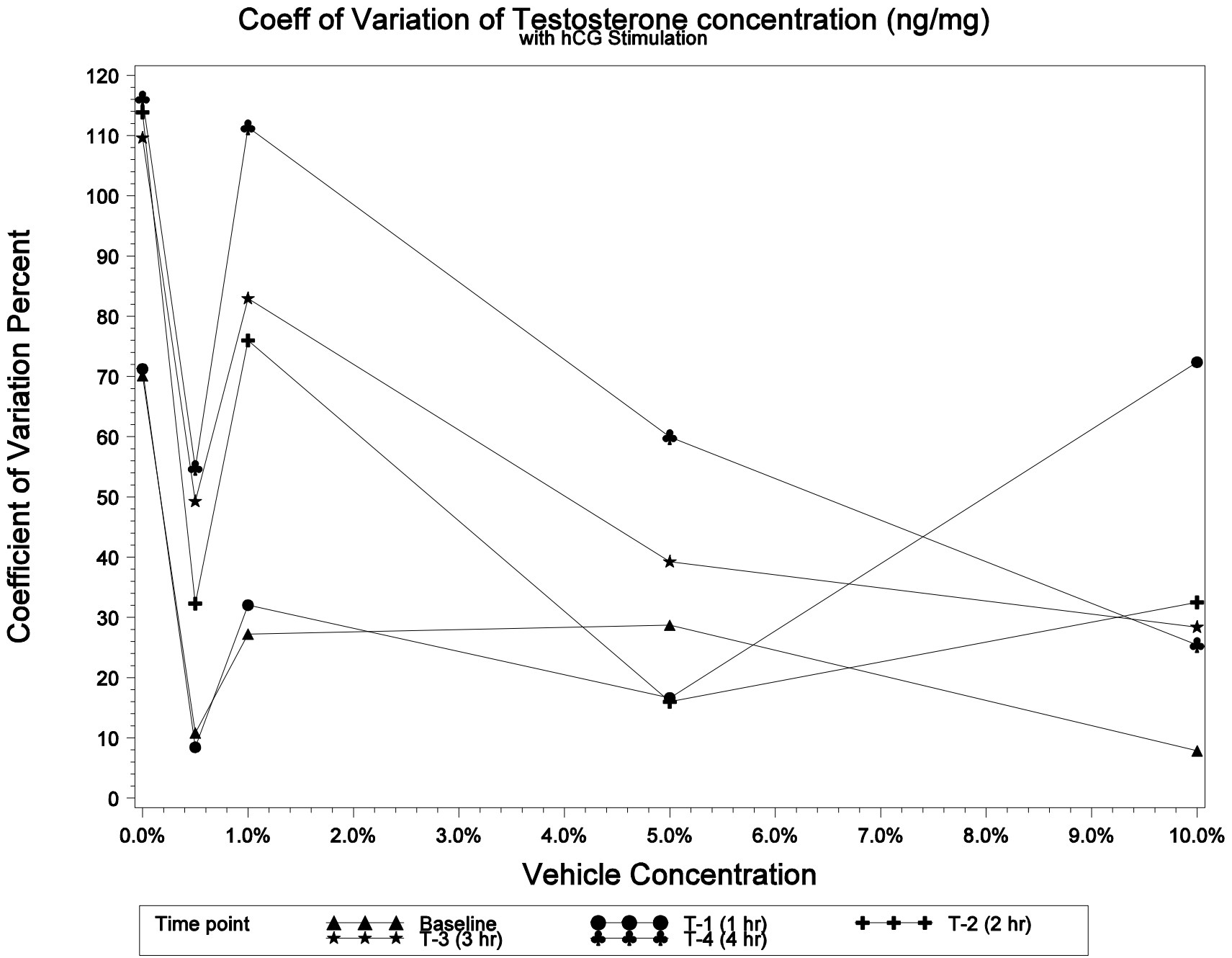

Means of Testosterone concentration (ng/mg)
No hCG Stimulation
Testosterone concentration (ng/mg)
0.042
0.041
0.04
0.039
0.038
0.037
0.036
0.035
0.034
0.033
0.032
0.031
0.03
0.029
0.028
0.027
0.026
0.025
0.024
0.023
0.022
0.021
0.02
0.019
0.018
0.017
0.016
0.015
0.014
0.013
0.012
0.011
0.01
0.009
0.008
0.007
0.006
0.005
0.004
0.003
0.002
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)
Time point
Vehicle Concentration
0.0%
0.5%
1.0%
5.0%
10.0%

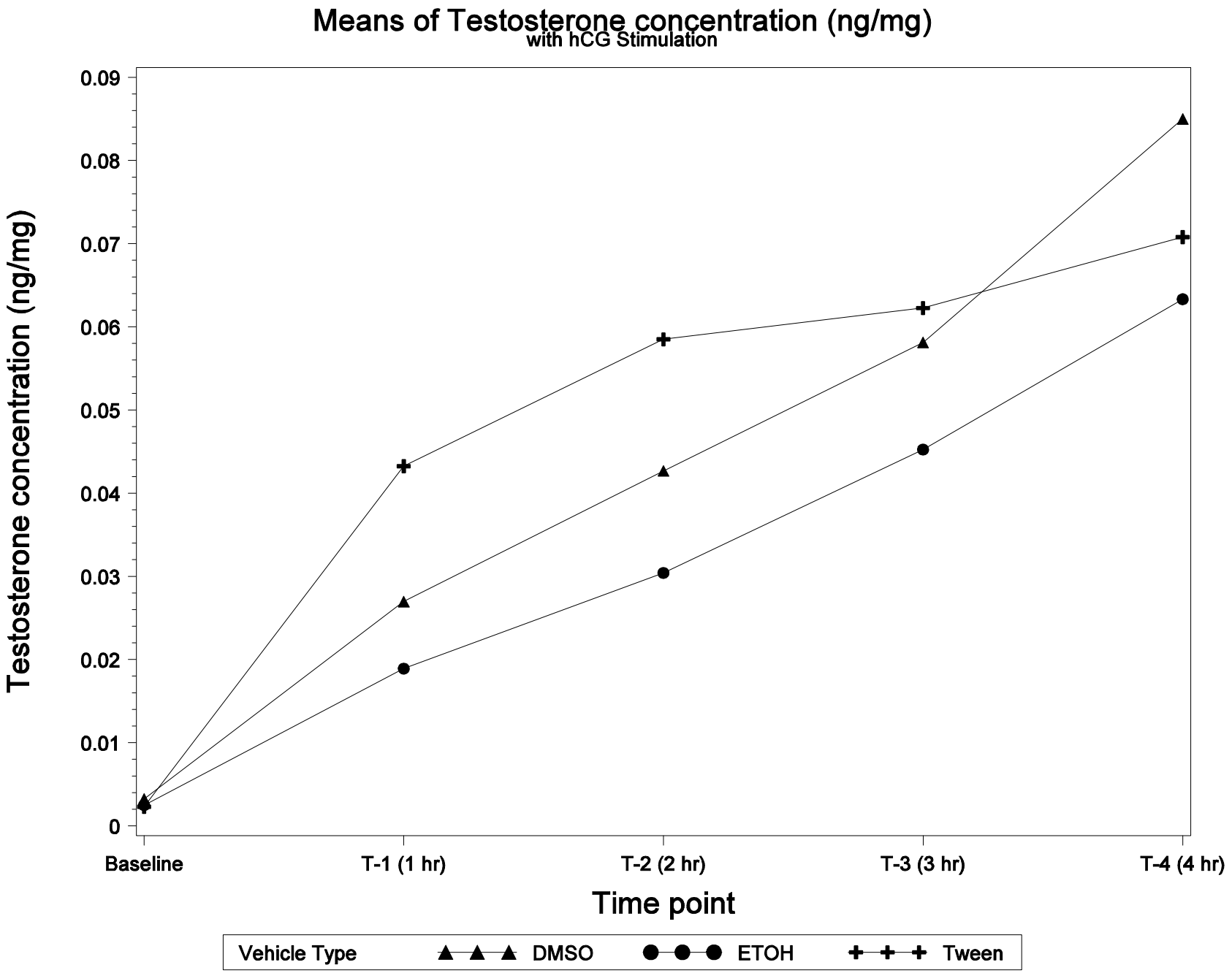

Coeff of Variation of Testosterone concentration (ng/mg)
No hCG Stimulation
Coefficient of Variation Percent
80
70
60
50
40
30
20
10
0
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)
Time point
Vehicle Concentration
0.0%
0.5%
1.0%
5.0%
10.0%


Means of Testosterone concentration (ng/mg)
with hCG Stimulation
Testosterone concentration (ng/mg)
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
DMSO
ETOH
Tween
Vehicle Type
Time point
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)


Coeff of Variation of Testosterone concentration (ng/mg)
with hCG Stimulation
Coefficient of Variation Percent
130
120
110
100
90
80
70
60
50
40
30
20
10
DMSO
ETOH
Tween
Vehicle Type
Time point
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)


Means of Testosterone concentration (ng/mg)
with hCG Stimulation
Testosterone concentration (ng/mg)
0.11
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
0.0%
1.0%
2.0%
3.0%
4.0%
5.0%
6.0%
7.0%
8.0%
9.0%
10.0%
Vehicle Concentration
Time point
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)


Coeff of Variation of Testosterone concentration (ng/mg)
with hCG Stimulation
Coefficient of Variation Percent
120
110
100
90
80
70
60
50
40
30
20
10
0
0.0%
1.0%
2.0%
3.0%
4.0%
5.0%
6.0%
7.0%
8.0%
9.0%
10.0%
Vehicle Concentration
Time point
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)


Means of Testosterone concentration (ng/mg)
with hCG Stimulation
Testosterone concentration (ng/mg)
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)
Time point
Vehicle Type
DMSO
ETOH
Tween

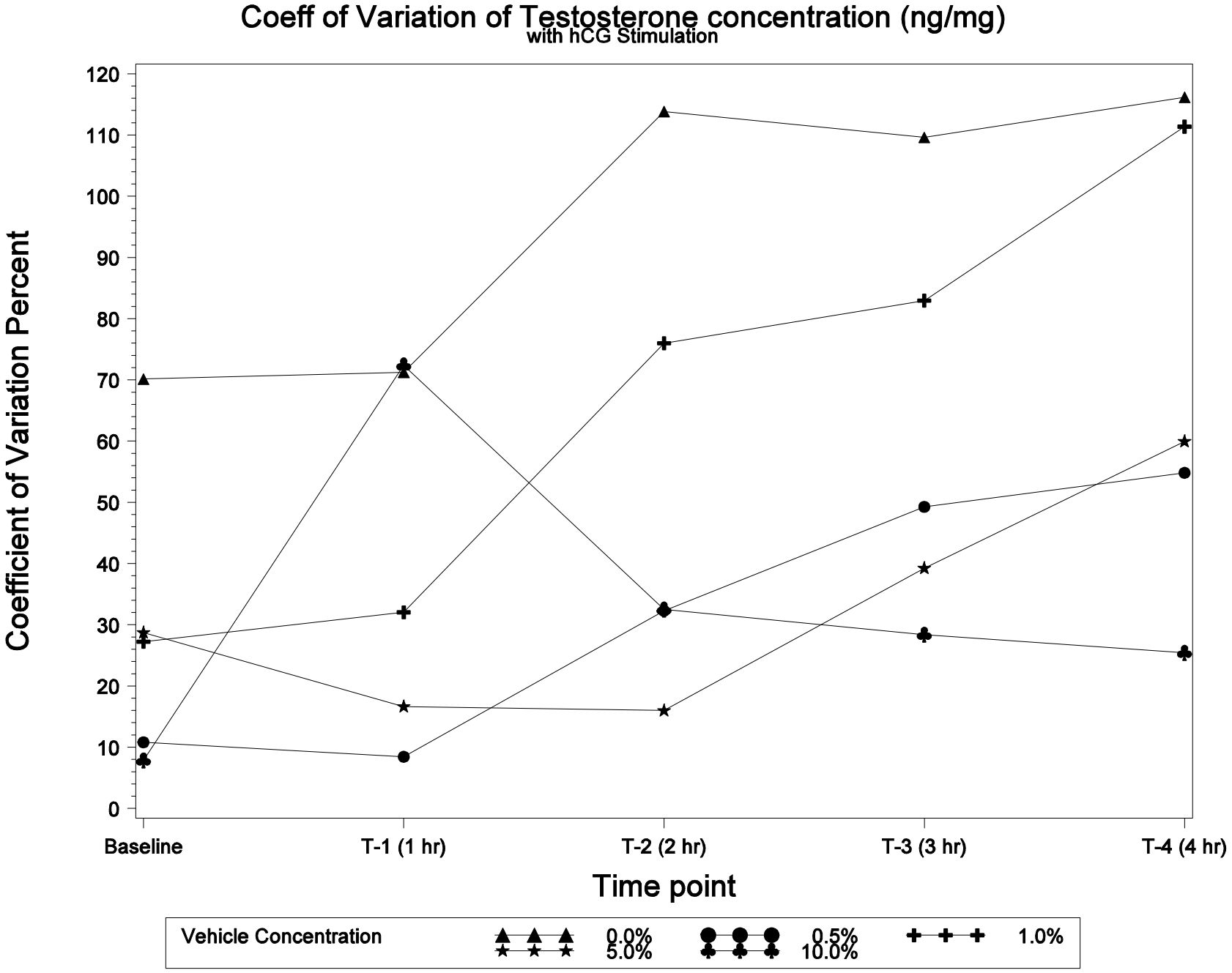

Coeff of Variation of Testosterone concentration (ng/mg)
with hCG Stimulation
Coefficient of Variation Percent
120
110
100
90
80
70
60
50
40
30
20
10
0
Baseline
T-1 (1 hr)
T-2 (2 hr)
T-3 (3 hr)
T-4 (4 hr)
Time point
Vehicle Concentration
0.0%
0.5%
1.0%
5.0%
10.0%

Table 153. Summary of Vehicle Experiment :LDH Concentrations* (mU/mg) by Vehicle Type**

Timepoint	DMSO	ETOH	Tween 20
Baseline	237.1 ±58.4	264.0 ±66.4	204.8 ±102.6
1 hr	1,042.5 ±374.2	775.8 ±247.3	1,950.0 ±1,249.2
2 hr	1,399.8 ± 568.7	1,075.0 ± 312.5	2,411.9 ± 1,417.4
3 hr	1,776.5 ± 789.3	1,245.5 ±239.4	2,679.3 ± 1,368.0
4 hr	2,080.7 ±850.5	1,434.3 ±161.9	2,612.6 ±978.1

* Mean ± standard deviation
** No hCG stimulation occurred

Table 154. Summary of Vehicle Experiment :LDH Concentrations* (mU/mg) by Vehicle Type**

Timepoint	DMSO	ETOH	Tween 20
Baseline	247.1 ±146.9	237.0 ±56.3	169.0 ±75.4
1 hr	1,018.8 ±257.6	739.8 ±122.6	1,621.3 ±1,000.8
2 hr	1,414.4 ± 170.9	968.4 ± 149.3	2,824.4 ± 1,943.9
3 hr	1,743.2 ± 354.2	1,172.4 ± 149.5	3,098.1 ± 1,812.5
4 hr	2,058.8 ±541.3	1,368.4 ±195.9	3,226.2 ±1,652.0

* Mean ± standard deviation
** hCG stimulation occurred

Table 155. Summary of Vehicle Experiment :LDH Concentrations* (mU/mg) by Vehicle Concentration**

Timepoint	0.0%	0.5%	1.0%	5.0%	10.0%
Baseline	208.4 ± 30.6	282.0 ± 64.3	260.1 ± 38.8	194.9 ± 95.1	231.1 ±133.0
1 hr	754.5 ± 305.2	972.6 ± 409.8	874.1 ±317.9	1,698.3 ±811.4	1,980.9 ±1,612.1
2 hr	1,000.6 ± 356.7	1,323.5 ± 532.5	1,202.4 ± 387.8	2,079.4 ± 1,009.3	2,538.5 ± 1,777.8
3 hr	1,282.3 ± 323.2	1,484.8 ± 429.9	1,418.6 ± 401.5	2,461.7 ± 1,281.2	2,854.6 ± 1,618.7
4 hr	1,543.1 ± 198.5	1,628.7 ± 428.9	1,691.5 ±406.7	2,397.0 ±784.9	2,952.4 ±1,379.3

* Mean ± standard deviation

** No hCG stimulation occurred

Table 156. Summary of Vehicle Experiment :LDH Concentrations* (mU/mg) by Vehicle Concentration**

Timepoint	0.0%	0.5%	1.0%	5.0%	10.0%
Baseline	321.7 ± 145.1	178.5 ± 35.5	286.8 ± 45.3	144.6 ± 46.2	156.9 ±64.4
1 hr	986.0 ± 376.4	928.5 ± 66.0	861.9 ±276.8	1,416.7 ±964.6	1,440.3 ±1,249.2
2 hr	1,311.5 ± 340.2	1,341.7 ± 229.8	1,201.6 ± 395.9	2,539.5 ± 2,534.4	2,284.4 ± 1,803.9
3 hr	1,614.0 ± 399.6	1,564.7 ± 258.0	1,402.6 ± 404.3	2,777.1 ± 2,500.2	2,664.5 ± 1,582.9
4 hr	1,750.5 ± 389.8	1,822.9 ± 256.9	1,615.5 ± 477.6	2,955.0 ±2,285.5	2,945.3 ± 1,464.8

* Mean ± standard deviation
** hCG stimulation occurred

Table 157. Summary of Vehicle Experiment :Testosterone Concentrations* (ng/mg) by Vehicle Type**

Timepoint	DMSO	ETOH	Tween 20
Baseline	0.0025 ± 0.0006	0.0025 ±0.0004	0.0023 ±0.0003
1 hr	0.0219 ± 0.0024	0.0162 ± 0.0042	0.0346 ± 0.0165
2 hr	0.0257 ± 0.0026	0.0218 ± 0.0106	0.0315 ± 0.0155
3 hr	0.0243 ± 0.0032	0.0262 ± 0.0171	0.0312 ± 0.0163
4 hr	0.0229 ± 0.0045	0.0275 ± 0.0225	0.0226 ± 0.0084

* Mean ± standard deviation
** No hCG stimulation occurred

Table 158. Summary of Vehicle Experiment :Testosterone Concentrations* (ng/mg) by Vehicle Type**

Timepoint	DMSO	ETOH	Tween 20
Baseline	0.0032 ± 0.0028	0.0025 ±0.0005	0.0023 ±0.0009
1 hr	0.0270 ± 0.0096	0.0189 ± 0.0024	0.0432 ± 0.0305
2 hr	0.0427 ± 0.0278	0.0304 ± 0.0089	0.0585 ± 0.0628
3 hr	0.0581 ± 0.0388	0.0452 ± 0.0172	0.0623 ± 0.0744
4 hr	0.0850 ± 0.0875	0.0633 ± 0.0328	0.0708 ± 0.0908

* Mean ± standard deviation
** hCG stimulation occurred

Table 159. Summary of Vehicle Experiment :Testosterone Concentrations* (ng/mg) by Vehicle Concentration**

Timepoint	0.0%	0.5%	1.0%	5.0%	10.0%
Baseline	0.0026 ± 0.0004	0.0025 ± 0.0007	0.0025 ± 0.0004	0.0020 ± 0.0005	0.0026 ±0.0003
1 hr	0.0258 ± 0.0033	0.0191 ± 0.0046	0.0197 ± 0.0034	0.0235 ±0.0111	0.0332 ± 0.0260
2 hr	0.0334 ± 0.0067	0.0198 ± 0.0044	0.0250 ± 0.0025	0.0221 ± 0.0074	0.0314 ± 0.0224
3 hr	0.0391 ± 0.0152	0.0205 ± 0.0034	0.0237 ± 0.0020	0.0220 ± 0.0061	0.0309 ± 0.0235
4 hr	0.0420 ± 0.0221	0.0192 ± 0.0048	0.0191 ± 0.0043	0.0219 ± 0.0084	0.0195 ± 0.0063

* Mean ± standard deviation
** No hCG stimulation occurred

Table 160. Summary of Vehicle Experiment :Testosterone Concentrations* (ng/mg) by Vehicle Concentration**

Timepoint	0.0%	0.5%	1.0%	5.0%	10.0%
Baseline	0.0046 ± 0.0032	0.0019 ± 0.0002	0.0026 ± 0.0007	0.0018 ± 0.0005	0.0025 ±0.0002
1 hr	0.0356 ± 0.0254	0.0189 ± 0.0015	0.0265 ± 0.0085	0.0202 ±0.0034	0.0478 ± 0.0346
2 hr	0.0734 ± 0.0836	0.0302 ± 0.0098	0.0490 ± 0.0372	0.0331 ± 0.0053	0.0336 ± 0.0109
3 hr	0.0858 ± 0.0941	0.0418 ± 0.0206	0.0657 ± 0.0545	0.0434 ± 0.0171	0.0393 ± 0.0112
4 hr	0.0992 ± 0.1153	0.0540 ± 0.0296	0.1062 ± 0.1183	0.0641 ± 0.0384	0.0416 ± 0.0106

* Mean ± standard deviation
** hCG stimulation occurred

15.0 DISCUSSION

15.1. Incubation Factors Experiments

The test tube results had smaller root mean squared errors (RMSEs) than vial results both with and without hCG. This may be the result of the tissue not breaking up as much since there is less space for tissue:media interaction than in the vial. For the cases with no hCG, the shaker speed was the dominant factor. However, for situations with hCG, incubation volume and fragment size were important for the incubations in vials but shaker speed and incubation temperature also became important for test tubes.

Vials consistently resulted in higher testosterone levels than test tubes. The optimum values occurred at the extremes of the experimental regions in all cases. The optimum fragment size generally fell in the middle portion of its range. For vials, the optimal fragment size with no hCG was around 80 mg, while with hCG the optimal size was approximately 200 mg. Therefore it is felt that 100-175 mg of testicular tissue is a range that would be appropriate to use in the slice testis assay when vials are used. The fragment size for test tubes appeared to be optimal at 130-170 mg.

The optimal hCG concentration fell in the middle of the range of tested concentrations. Although that range was 0.05-0.08 for some cases, it was felt that the 0.1 concentration would be the most easily adapted for a screening assay. The concentration for hCG concentration in the assay has to be calculated for the final concentration in the volume of media to be used in each vial and the 0.1 concentration is more easily calculated and weighed for a general assay.

The optimal temperature fell between 34 and 37°C and was approximately 36°C . When shaker speed was treated as a continuous factor, the optimal speed was low (135 rpm) with vials with no hCG, but high (200 rpm) with the other cases. It is felt that the overall optimal speed is around 175 rpm based on our studies and those of others. We found that 200 rpm is difficult to maintain in the incubator, especially over the 24 hour period without movement of the shaker itself and results in dissociation of the testicular fragments.

The optimal volume of the media for test tubes was at the lower extreme of 2.5 ml but in all other cases it was approximately 4 ml. Therefore we kept the incubation volume at 5.0 ml for ease of the other technical operations that had to be performed in later assays. This included larger aliquots for LDH determinations than had originally been planned.

15.2 Organ Preparation Factors Experiments

In the organ preparation series of experiments, the use of warm and cold Delbecco's Phosphate Buffered Saline (DPBS) as well as cold media was evaluated as well as the time the tissue spent in these solutions , the aliquot volume and the hCG concentration. In the cases with

hCG, there were very small degrees of freedom for error, resulting in difficulty in testing for the significance of factors. In the cases with no hCG, there were somewhat smaller RMSEs for cold media than for cold or warm buffered saline. For the cold solutions, time delay (the time from placing the tissue in the holding solution until placing it in the media to start the incubations) was the dominant factor as would be expected. Baseline was important for the buffered saline cases.

Cold buffered saline cases yielded slightly higher testosterone values than cold media and the cold media values were slightly higher than the warm buffered saline. The time delays were inconsistent when considered across time points. Aliquot volumes were also inconsistent and often at the extremes of the regions. The LDH experiments performed with the optimized factors appeared to confirm that the time delay should not be more than one hour before the starting assay incubation. These data combined with the results from the previous experiments to determine the hCG concentration to be used in the optimized assay. The cold DPBS appeared to be the best solution to collect the testes in prior to the initial assay incubation.

16.0 CONCLUSIONS

Based upon the incubation experiments, the optimized assay factors that yielded the maximum testosterone concentrations were the use of scintillation vials with testicular fragments of 100 to 175 mg. This fragment size should allow for at least 8-10 fragments per testis for use in the pre-validation of the sliced testis assay. The volume of incubation media should be 4-5 ml.

It was difficult to determine the optimal factors from the testis preparation experiments. There should have been more determinations using hCG. The small number used made the degrees of freedom for error very small. The cases without hCG however showed that cold buffered saline with no more than one hour of delay before the first assay incubation in media 199 was optimal. The aliquot volume can be less than 0.5 ml (the optimal appeared to be around 0.3ml) but the larger volume is necessary if more than one determination is to be made from the aliquot. When smaller aliquots were used, there was not enough sample if repeat determinations for testosterone were necessary.

Therefore, the optimal assay was run using Delbecco's PBS for collection of testes for a period less than one hour. The testis fragment was then placed in a scintillation vial with 5 ml of media 199 and incubated at 36°C for one hour. The media was then replaced with fresh media and the baseline samples were taken. Then media removed was replaced with the same volume of media or media plus hCG. The sample was then re-incubated and hourly samples were taken. The optimal assay was run as part of the 24 hour sampling assay also and the sampling after 4 hours was not optimal in terms of the rise in both testosterone and LDH concentration. This probably is an indication of cell death and release of testosterone.

The injury experiment showed the differences in LDH and testosterone concentration with the different types of injury. Chemical injury with Triton X-100 caused the cell to lyse resulting in high LDH values and the cellular testosterone was released at the one hour time point. Heat (42°C) caused the LDH to continually rise but the testosterone remained low indicating that the cellular testosterone production was minimal (probably due to denaturation of enzymatic proteins) during incubation. The trauma of mincing the tissue resulted in both the testosterone and LDH concentrations maximal at the 1 hour time point. Only the heated sample showed no additional testosterone release after hCG stimulation.

The vehicle experiment should help us to determine the influence of vehicle concentration influence on the assay system. The testosterone assays for these experiments need to also include vehicle blanks to determine if the vehicle itself is affecting the assay.

It should be noted that all of these optimization experiments were performed on control testicular fragments (albeit $\pm$ hCG) . There is no guarantee that the same optimized conditions established in this work would necessarily hold for testicular tissue incubated with LH, FSH or an anti-androgen with the mechanism of action of inhibition of testosterone biosynthesis.

APPENDIX 1

LDH Validation and Verification with Media 199 without Phenol Red

(See Draft Letter Report for Phase I)