Canine reproductive ultrasound examination for predicting future sperm quality

Running title: testis ultrasound to predict semen quality

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The reproductive potential of male animals is commonly evaluated using a breeding soundness examination incorporating B-mode ultrasound examination of the testes and recently Doppler ultrasound examination of the testicular arteries. These techniques may detect testicular normality or pathology, and while some measured parameters are associated with semen quality at the time of ultrasound examination, few studies have investigated the relationship with future semen quality.

We hypothesised that B-mode and Doppler ultrasound measurements would correlate with future semen quality. Within two studies we investigated the relationship between ultrasound measured testicular volume, testicular echogenicity, testicular homogeneity, subjective assessment of the testicular parenchyma, testicular artery resistance index and pulsatility index with subsequent semen quality. Fifty-five normal fertile dogs of which 29 had stable semen quality and 26 had a subsequent decline in semen quality were examined during a six-month period commencing 62 days after the ultrasound examination. Statistical analysis showed that no ultrasound parameters were predictive of future total sperm output or percentage live normal sperm. However, mean testicular echogenicity was positively related to motility ($t = 2.202$, $P = 0.039$).

We conclude that quantitative ultrasound assessment of the appearance of the testicular parenchyma has potential for evaluation of future semen quality in dogs.

Keywords: B-mode, Doppler, ultrasound, dog, sperm motility, fertility

Introduction

A breeding soundness examination is commonly performed to examine the breeding potential of dogs. The procedure includes clinical examination of the reproductive tract, observation of libido, examination of semen quality, and in some cases ultrasound examination of the reproductive tract, and endocrine testing. More recently, Doppler ultrasonography has allowed an additional evaluation of the reproductive organs, with useful information about blood flow and velocity (Freitas et al., 2013; 2015). Our work to date has utilised B-mode ultrasound to characterise the normal appearance of the testes and prostate gland and how these are disturbed in cases of pathology (England, 1991; Souza et al., 2016). Recently, we described digital image analysis of testicular and prostatic ultrasonographic echogenicity and heterogeneity in dogs and their relation to semen quality (Moxon et al., 2015), and we characterised differences of testicular artery blood flow measured using Doppler ultrasonography in pre- and post-pubertal dogs (Souza et al., 2014; Souza et al., 2015a), and in dogs with established infertility (Souza et al., 2015b). In these and other studies (Zelli et al., 2013), measurements have generally been related to semen quality close to the time of ultrasound examination rather than future semen quality as would be expected based upon the time taken for spermatogenesis and sperm maturation. Interestingly, in a limited number of studies in other species, some associations between the ultrasonographic appearance and future semen quality have been established (Ahmadi et al., 2012; Brito et al., 2012; Arteaga et al., 2015). Indeed, Arteaga et al. (2005) found that in bulls, testicular parenchymal pixel intensity measured by ultrasound had a better association with future semen quality than with present semen quality.

The aims of this retrospective clinical study were to examine whether testicular ultrasound appearance and testicular artery blood flow measurements could be used to predict future

characteristics of semen quality. Two studies were performed with a total of 55 individual dogs. In Study 1, testicular volume, testicular echogenicity, testicular homogeneity were measured, and parenchymal appearance was subjectively scored in 24 normal fertile dogs of which 11 had stable semen quality and 13 had a subsequent decline in semen quality of unknown origin. In Study 2, testicular artery resistance index and pulsatility index were measured in 31 dogs of which 18 had stable semen quality and 13 had a subsequent decline in semen quality of unknown origin.

Materials and methods

Study animals

Study 1: Testicular ultrasound appearance

Twenty-four healthy stud dogs of two breeds (18 Labradors and six Golden retrievers) were included. Dogs were aged 1.5 to 7.7 years (mean 4.3 ± 0.4 years) and weighed between 28.4 and 40.6 kg (mean 35.0 ± 0.5 kg). All dogs had proven normal semen quality and were fertile based on achieving at least one pregnancy within six months prior to the study.

Study 2: Testicular artery blood flow

Thirty-one healthy stud dogs of two breeds (21 Labradors and 10 Golden retrievers) were included. Dogs were aged 1.5 to 6.9 years (mean 4.3 ± 0.3 years) and weighed between 28.1 and 39.8 kg (mean 33.7 ± 0.6 kg). All dogs had proven normal semen quality and were fertile in the six months prior to the study.

Ultrasound examination

Study 1: Testicular volume and testicular ultrasound appearance

Real time B-mode ultrasonography (Pandion 300s, Pie Data, UK) with a 10 MHz mechanical-sector transducer was used to evaluate testicular volume and perform objective and subjective assessment of testicular echogenicity and homogeneity. To measure testicular volume, the testes were imaged in the sagittal, transverse and dorsal planes. The mediastinum was used as a reference point for measuring the testicular length, width and height using electronic callipers (Souza et al., 2014). Testicular volume was calculated using the formula for an ellipse; volume = length x width x height x 0.5236 (Paltiel et al., 2002). Total testicular volume (TTV) was calculated by adding together the volumes of each testis.

Mean testicular echogenicity (MTE) (calculated as the mean of nine values for testicular echogenicity) and mean testicular homogeneity (MTH) (calculated as the standard deviation of mean echogenicity) at the tissue level were determined using a semi-automated method as previously described (Moxon et al., 2015). Briefly, two reference points (one in the near field and one in the far field) were selected on the hyperechoic capsule of the testes (being selected as the most echogenic structures identifiable) and a computer macro then randomly placed nine sampling regions of interest (each 2.0 mm^2) over the testicular parenchyma (avoiding the central mediastinum). Within each region of interest, the mean pixel intensity (PI) was measured. Of the two reference points the highest measurement of mean PI (most echogenic) was used to calculate the echogenicity of the comparative region of interest as a percentage of the highest mean pixel intensity using the following formula: Percentage echogenicity = (Mean PI of capsule/Mean PI of testicular parenchyma) × 100. This methodology therefore related all echogenicity measurements to a standard echogenic structure that would be consistent between dogs. The mean of the nine echogenicity values for testicular echogenicity were reported as mean echogenicity. Heterogeneity of testicular echogenicity (MTH) was calculated as the standard deviation of the mean echogenicity of the regions of interest for

each organ. Using this method low values (low variation between regions of interest) represented more homogenous tissues, whilst high values for heterogeneity (high variation between the regions of interest) represented tissues that were less homogenous. These measurements were at the tissue level rather than reporting visible gross changes.

Subjective assessment of testicular parenchymal echotexture was performed on recorded digital images of the left and right testes. Each testis was classified into one of six categories by the first author according to the echotexture of the testicular parenchyma. The image classifications were; (1) hypoechoic, (2) normal echogenicity parenchyma with hypoechoic/ anechoic cysts, (3) normal echogenicity, (4) normal echogenicity parenchyma with echogenic stippling, (5) hyperechoic parenchyma or (6) multiple changes. For data evaluation the highest score from each testis was recorded as the testicular parenchymal echotexture for each dog.

Study 2: Testicular artery blood flow

Doppler ultrasonography (M-Turbo, SonoSite®, UK) with a 5 to 8 MHz micro-convex array transducer was used to measure peak systolic velocity (PSV) and end diastolic velocity (EDV). Dogs were restrained in the standing position and the transducer was placed at the neck of the scrotum to identify the tortuous distal (looping) region of the supra-testicular artery (Carrillo et al., 2012). The diameters of the testicular arteries were measured in the longitudinal plane. The colour gain was adjusted to reduce any excess colour noise and the pulsed Doppler gate was positioned within the lumen of the vessel. Three waves of a cardiac cycle were used to measure mean testicular artery values for PSV and EDV, and these were used by the machine software to calculate testicular artery resistance index (RI) and pulsatility index (PI). This was repeated for each testis. The sample gate was 1 mm across all

regions. The angle of insonation used was less than 60^{o}. The same operator performed each examination. The mean of the two RI and two PI values from each testis were calculated and used for analysis.

Semen evaluation

Immediately following ultrasound examination, semen was collected by digital manipulation in the absence of a teaser bitch and evaluated using World Health Organisation standard methods as described by England (1991). Only dogs with normal semen quality (Total sperm output [TSO] $\geq 200 \times 10^6$ sperm; Percentage total live normal sperm [TLNS] $\geq 60\%$; Percentage normal forward progressive motility [NFPM] $\geq 60\%$) were retained in the study. Semen collection and evaluation was then undertaken between 1 and 7 further times for each dog for the purposes of routine monitoring, for cryopreservation or insemination or when there was concern that quality was declining. Samples collected within a six-month period commencing 62 days after ultrasound examination were used for statistical analysis.

Statistical analysis

All data were examined for normality and were described as mean ± one S.E. Semen quality (TSO, TLNS, NFPM) after the initial assessment was calculated as a mean from all subsequent collections (when there was more than one collection) and these values were used for analysis. The difference in TLNS and NFPM between initial and subsequent semen collections was determined using students t-tests or Wilcoxon signed-rank tests for paired data.

For testicular ultrasound appearance, multiple regressions were used to determine whether TTV, MTE, MTH and subjective appearance of the parenchyma were related to future semen

quality. Three multiple regressions were run; one for each semen quality measure (TSO, TLNS, NFPM). Age and breed were included in the regressions and normality and co-linearity were examined. For testicular artery blood flow, relationships between RI, PI, and TSO, TLNS and NFPM were investigated using linear regression with TSO, TLNS and NFPM as dependant variables. Data were analysed using XLStat (Addinsoft, USA) and SPSS (version 20). Results were considered significant when $P<0.05$.

Results

Study 1: Testicular volume and testicular ultrasound appearance and future semen quality

At the time of ultrasound examination TSO ranged from 200 x 10^6 to 2,470 x 10^6 (mean 741.4 ± 106.4 x 10^6); TLNS ranged from 64% to 87% (mean 75.7% ± 1.2%); NFPM ranged from 70.0% to 95.0% (mean 87.3% ± 1.5%). At subsequent evaluations, semen quality was stable for 11/24 dogs but declined for 13/24 dogs; of the latter, four dogs had poor quality semen at subsequent evaluations (TLNS < 60%; NFPM < 60%). For the 13 dogs with semen quality that declined, TNLS ($P = 0.04$) and NFPM ($P = 0.002$) were significantly lower at subsequent evaluations that at the initial semen collected. In the monitoring period after ultrasound examination, the range of the means for TSO was 336.0 x 10^6 to 3,234.0 x 10^6 (mean 1198.8 ± 138.8 x 10^6), for TLNS was 46.0% to 95.0% (mean 76.1% ± 2.7%) and for NFPM was 50.0% to 90.0% (mean 70.4% ± 1.7%).

TTV for the 24 dogs ranged from 36.1 to 60.5 cm^3 (mean 49.9 ± 1.5 cm^3), MTE ranged from 45.3 to 96.9% (mean 61.3 ± 1.9%) and MTH ranged from 4.3 to 9.8% (mean 6.6 ± 0.3%). Sixteen dogs had two testes that appeared normal and were classified as normal echogenicity (3) and eight dogs were classified as not normal (scores 2, 4, 5 and 6) (Tables 1 and 2).

The multiple regression models for prediction of future semen quality (mean TSO, mean TLNS, mean NFPM) included all ultrasound parameters (TTV, MTE, MTH and subjective appearance). There were no ultrasound parameters that were predictive of future mean TSO or future mean TLNS. However, following model simplification, the model for NFPM predicted 27.9% of the variation ($F = 4.062$, $DF = 2$, $P = 0.032$), MTE was positively related to motility ($t = 2.202$, $P = 0.039$). Although the effect of age was not significant ($t = -1.949$, $p = 0.065$), age was retained in the final model as the effect of MTE on NFPM was lost on removal.

Study 2: Testicular artery blood flow and future semen quality

At the time of ultrasound examination TSO ranged from 200 x 10^6 to 2,448 x 10^6 (mean 986.5 ± 95.3 x 10^6); TLNS ranged from 62.0% to 95.0% (mean 83.6% ± 1.4%); NFPM ranged from 60 to 90% (mean 79.2% ± 1.4%). At subsequent evaluations, semen quality was stable for 18/31 dogs but declined for 13/31 dogs; of the latter, four dogs had poor quality semen at subsequent evaluations (TLNS < 60%; NFPM < 60%). For the 13 dogs with semen quality that declined, TNLS ($P = 0.002$) and NFPM ($P = 0.002$) were significantly lower at subsequent evaluations that at the initial semen collected. In the monitoring period after ultrasound examination, the range of the means for TSO was 122.5 x 10^6 to 2,750.0 x 10^6 (mean 849.2 ± 86.5), for TLNS was 15.5% to 93.0% (mean 71.8% ± 3.4%) and for NFPM was 20.0% to 85.0% (mean 71.0% ± 2.5%).

Testicular artery RI ranged from 0.17 to 0.58 (mean 0.40 ± 0.02) and testicular artery PI ranged from 0.19 to 1.02 (mean 0.58 ± 0.04) (Table 3). Linear regression showed that there was no relationship between RI or PI at ultrasound examination and subsequent mean semen TSO (RI = $R^2 = 0.002$, $P = 0.796$, PI = $R^2 = 0.006$, $P = 0.687$) TLNS (RI = $R^2 = 0.008$, P =

0.635, PI = R^2 = 0.010, P = 0.601) or NFPM (RI = R^2 = 0.001, P = 0.840, PI = R^2 = 0.002, P = 0.819).

Discussion

B-mode and Doppler ultrasound examination of the reproductive tract of the dog provides important and useful information about the state of the testes at the time of the examination (Zelli et al., 2013; Moxon et al., 2015; Souza et al., 2015b). Studies to date have commonly evaluated testicular echogenicity and heterogeneity, and testicular artery blood flow (Zelli et al., 2013; Moxon et al., 2015; Souza et al., 2015b). The findings are perhaps not surprising since previous work has shown that testicular parenchymal pixel intensity is associated histologically with seminiferous tubule height, the proportion of tubules with a lumen, and the size of the lumen (Evans et al., 1996; Giffin et al., 2009), and because blood flow through the testicular artery is related to the rate of spermatogenesis (Kay et al., 1992). Interestingly, in the present study we found a relationship between mean testicular echogenicity and future semen quality in that higher testicular echogenicity at the tissue level was associated with increased mean normal forward progressive motility in the subsequent monitoring period; findings somewhat similar to those seen in the bull (Arteaga et al., 2005). The biological reason for this is uncertain, however we postulate that this may be associated with a uniform diameter of the seminiferous tubules rather than distortion of tubules sometimes seen cases of pathology. There were however no associations between several ultrasonographic characteristics and future semen quality; notably there was no relationship between total testicular volume, mean testicular heterogeneity, subjective appearance of the testicular parenchyma, testicular artery resistance index and testicular artery pulsatility index. The lack of association between testicular volume and future semen quality is not surprising since although this parameter is often purported to be predictive of current semen quality (Zelli et

al., 2013) this is not always the case (Souza et al., 2015b). Furthermore, relationships between testicular artery blood flow are likely to relate more to current, rather than future, semen quality since endothelial thickening and changes in blood flow occur secondarily to testicular disease (Pinggera et al., 2008), while primary restriction of testicular artery diameter results in rapid testicular changes (Kay et al., 1992). It was somewhat surprising that in the present study no association was found between testicular parenchymal heterogeneity or subjective appearance of the testicular parenchyma and subsequent semen quality, since this has been observed in rams (Ahmadi et al., 2012). Interestingly, Ahmadi et al. (2012) found stronger associations between epididymal echotexture and future semen quality than they did between testicular echotexture and future semen quality. This is perhaps unsurprising given the function of the epididymis as a sperm storage reservoir and site for final sperm maturation, but importantly the study of Ahmadi et al. (2012) investigated semen quality 60 days after ultrasound examination; an interval spanning one spermatogenic cycle length. It is plausible that in the present study the method of calculating mean semen quality throughout the six-month monitoring period (spanning 1-3 spermatogenic cycles [Soares et al., 2009]) masked subtle changes in semen quality, although semen quality did decline in 26 of the 55 dogs across the two studies.

The present study is limited in scope in that the decline in semen quality was substantial in only eight dogs, but offers a tantalising insight into the possibility that ultrasound can be used to quantitatively assess pixel intensity representing physical properties of the testicular parenchyma which are related to future semen quality. Further studies in this area and extending to examination of epididymal appearance are warranted.

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Table 1. The data for the 24 individual dogs showing each dog's age, breed, initial semen collection results (total sperm output [TSO], total live normal sperm [TLNS], normal forward progressive motility [NFPM]), total testicular volume (TTV) mean testicular echogenicity (MTE), mean testicular homogeneity (MTH), score for subjective appearance of the testes, number of subsequent semen collections and subsequent semen collection results (shown as a mean value where there was more than one subsequent collection in the six-month period). Four dogs were semen quality declined to a poor value are highlighted in bold.

Dog		Initial semen collection								Subsequent semen collections				
Dog no.	Breed	Age	TSO (x 10^6)	TLNS (%)	NFPM (%)	TTV (cm^3)	MTE (%)	MTH (%)	Subj. appearance score	N	Days from initial collection	TSO (x 10^6)	TLNS (%)	NFPM (%)
1	Lab	5.42	360.0	87.0	80.0	36.26	56.69	4.65	2	1	219	375.0	92.0	70.0
2	Lab	1.65	749.0	74.0	90.0	40.67	52.24	7.68	3	1	128	720.0	82.0	75.0
3	Lab	2.28	390.0	75.0	90.0	48.68	61.99	5.85	6	1	224	480.0	89.0	90.0
4	**GRet**	**5.56**	**660.0**	**75.0**	**95.0**	49.21	59.98	4.32	3	**1**	**171**	**912.0**	**52.0**	**50.0**
5	**Lab**	**5.17**	**2470.0**	**79.0**	**95.0**	43.08	59.46	8.23	3	**1**	**105**	**1417.0**	**56.0**	**70.0**
6	**Lab**	**5.30**	**592.5**	**77.0**	**95.0**	54.26	45.27	7.82	4	**1**	**171**	**336.0**	**60.0**	**50.0**
7	Lab	4.30	272.5	84.0	85.0	54.51	54.69	6.27	4	1	133	625.0	95.0	70.0
8	Lab	1.52	200.0	64.0	80.0	56.12	63.67	6.69	3	1	181	510.0	85.0	65.0
9	Lab	2.06	301.0	71.0	85.0	60.45	53.35	5.71	4	1	186	1012.0	72.0	70.0
10	**GRet**	**6.58**	**320.0**	**69.0**	**70.0**	59.40	64.02	6.39	2	**1**	**198**	**1060.0**	**46.0**	**70.0**
11	Lab	1.81	580.0	68.0	90.0	37.05	64.95	7.15	3	1	171	1170.0	82.0	75.0
12	GRet	4.98	1425.0	78.0	95.0	58.52	54.51	8.43	3	1	174	2016.0	82.0	75.0
13	Lab	5.93	1029.0	70.0	70.0	59.59	96.90	7.63	3	1	95	897.0	71.0	80.0
14	Lab	5.62	1040.0	79.0	95.0	54.89	57.54	9.80	3	1	153	3234.0	82.0	75.0
15	Lab	5.09	465.0	73.0	80.0	44.80	62.52	4.52	3	2	161,238	1366.0	62.5	72.5
16	Lab	3.60	592.0	82.0	90.0	58.50	59.40	8.05	3	1	181	1008.0	85.0	75.0
17	Lab	4.56	551.0	86.0	95.0	36.06	63.65	8.42	3	2	139,165	832.5	89.0	67.5
18	Lab	5.60	629.0	70.0	85.0	50.57	66.48	6.15	3	2	152,173	1234.5	64.5	70.0
19	Lab	6.01	1662.5	75.0	90.0	53.25	57.76	5.52	4	2	145,243	1949.0	87.5	72.5
20	GRet	2.36	1020.0	72.0	90.0	50.84	66.85	5.62	3	3	167,177,184	2060.0	80.0	75.0
21	Lab	2.14	762.5	82.0	90.0	50.70	68.34	6.40	3	3	143,150,241	1182.3	80.7	71.7
22	GRet	7.68	390.0	80.0	90.0	49.58	62.44	5.29	3	4	169,175,176, 183	1917.5	74.5	63.8
23	Lab	5.16	990.0	76.0	90.0	49.17	54.65	6.43	3	5	99,137,144, 226,227	875.0	70.8	64.0
24	GRet	2.53	342.0	70.0	80.0	42.32	64.46	4.55	5	7	155,172,173, 182,183,190, 191	1583.1	86.4	73.6

Table 2. The results of subjective classification of the ultrasonographic appearance of the right and left testes of 24 fertile dogs with normal semen quality and the number of dogs with each overall score based on their highest (worst) score.

Classification	Description	Number of right testes scored	Number of left testes scored	Number of dogs with overall score
1	Hypoechoic	0	0	0
2	Normal echogenicity with hypoechoic/ anechoic cysts	2	2	2
3	Normal echogenicity	18	16	16
4	Normal echogenicity with echogenic stippling	4	4	4
5	Hyperechoic parenchyma	0	1	1
6	Multiple changes	0	1	1
Total		24	24	24

Table 3. The data for the 31 individual dogs showing each dog's age, breed, initial semen collection results (total sperm output [TSO], total live normal sperm [TLNS], normal forward progressive motility [NFPM]), testicular artery resistance index (RI) and pulsatility index (PI), number of subsequent semen collections and subsequent semen collection results (shown as a mean value where there was more than one subsequent collection in the six-month period). Four dogs were semen quality declined to a poor value are highlighted in bold.

Dog		Initial semen collection						Subsequent semen collections				
Dog no.	Breed	Age	TSO (x 10^6)	TLNS (%)	NFPM (%)	RI	PI	N	Days from initial collection	TSO (x 10^6)	TLNS (%)	NFPM (%)
1	Lab	6.94	660.0	88.0	75.0	0.32	0.39	1	123	385.0	61.0	65.0
2	GRet	4.04	1600.0	91.0	80.0	0.46	0.69	1	245	768.0	74.0	70.0
3	GRet	4.04	424.0	87.0	70.0	0.30	0.41	1	161	570.0	72.0	75.0
4	Lab	3.04	330.0	89.0	80.0	0.32	0.40	1	216	802.0	93.0	70.0
5	Lab	5.69	865.0	87.0	85.0	0.36	0.52	1	156	1060.0	80.0	80.0
6	GRet	4.18	525.0	88.0	80.0	0.58	1.02	1	225	642.0	91.0	80.0
7	Lab	3.45	504.0	88.0	75.0	0.31	0.45	1	196	488.0	84.0	75.0
8	Lab	1.65	1230.0	87.0	85.0	0.38	0.51	1	207	180.0	77.0	80.0
9	Lab	6.07	2166.0	86.0	80.0	0.45	0.66	1	173	760.0	86.0	80.0
10	GRet	1.55	570.0	70.0	75.0	0.57	0.95	1	188	122.5	79.0	75.0
11	Lab	3.92	487.5	89.0	85.0	0.44	0.62	1	232	475.0	86.0	65.0
12	**GRet**	**4.14**	**1000.0**	**62.0**	**70.0**	**0.36**	**0.52**	**1**	**233**	**357.5**	**22.0**	**20.0**
13	Lab	4.25	1370.0	92.0	90.0	0.38	0.48	1	190	940.0	79.0	70.0
14	Lab	6.73	215.0	84.0	75.0	0.37	0.48	1	219	220.0	73.0	80.0
15	GRet	3.43	950.0	83.0	65.0	0.41	0.59	1	62	862.5	73.0	70.0
16	Lab	4.64	1515.0	85.0	80.0	0.55	0.81	1	217	941.0	89.0	85.0
17	Lab	4.49	1400.0	80.0	80.0	0.58	0.93	1	196	872.0	66.0	65.0
18	GRet	3.26	900.0	91.0	90.0	0.43	0.59	1	192	2750.0	81.0	85.0
19	Lab	2.24	1360.0	83.0	90.0	0.37	0.52	1	177	500.0	72.0	80.0
20	Lab	4.32	820.0	95.0	80.0	0.26	0.31	1	181	1100.0	91.0	80.0
21	Lab	4.71	2448.0	90.0	90.0	0.57	0.96	1	239	980.0	86.0	80.0
22	Lab	4.31	775.0	86.0	85.0	0.39	0.54	1	198	1025.0	68.0	70.0
23	**GRet**	**5.53**	**650.0**	**62.0**	**65.0**	**0.42**	**0.61**	**2**	**152,203**	**740.0**	**37.0**	**35.0**
24	GRet	4.47	200.0	84.0	75.0	0.39	0.53	2	133,235	1307.5	77.0	72.5
25	Lab	2.84	1295.0	86.0	85.0	0.17	0.19	2	78,221	1488.5	76.0	77.5
26	**Lab**	**1.68**	**1455.0**	**85.0**	**85.0**	**0.26**	**0.31**	**2**	**133,195**	**710.0**	**39.0**	**67.5**
27	Lab	4.13	985.0	80.0	90.0	0.47	0.72	2	65,102	1073.8	75.5	77.5
28	Lab	5.71	1297.5	74.0	70.0	0.36	0.50	2	83,211	804.0	81.5	70.0
29	Lab	4.08	1115.0	81.0	80.0	0.35	0.49	2	85,244	1270.5	81.5	82.5
30	Lab	5.92	875.5	82.0	80.0	0.48	0.73	3	131,159,182	1261.7	61.0	75.0
31	**GRet**	**6.30**	**600.0**	**78.0**	**60.0**	**0.46**	**0.67**	**4**	**112,140,189, 228**	**869.1**	**15.5**	**42.5**