



MINITUBE CaniPRO™ EXTENDERS FOR DOG SEMEN:

COMPARATIVE STUDIES ON IN VITRO PERFORMANCE

Since 2007, Minitube CaniPRO™ media have been available for small animal veterinary practices and dog breeders. The media development was guided by Prof. John Verstegen, world known specialist in canine reproduction techniques. The following data give a scientific prove for the outstanding ability of CaniPRO™ to maintain dog semen viability and motility: they are considered to be the state of the art in dog semen preservation.

1. CaniPro™ Chill 5 and Chill 10

Motility evolution of Canine Spermatozoa diluted at a ratio of 1/3 in CaniPRO™ Chill 5 and CaniPRO™ Chill 10 after preservation at 4 °C

Figure 1: Motility evolution after chill preservation at 4°C

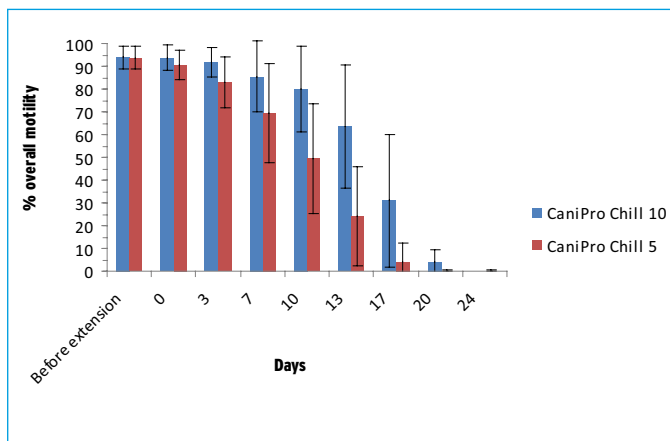
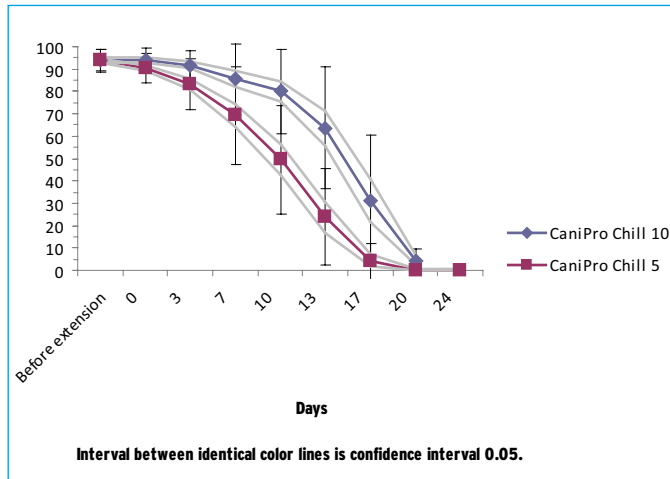


Figure 2: Motility evolution after chill preservation at 4°C



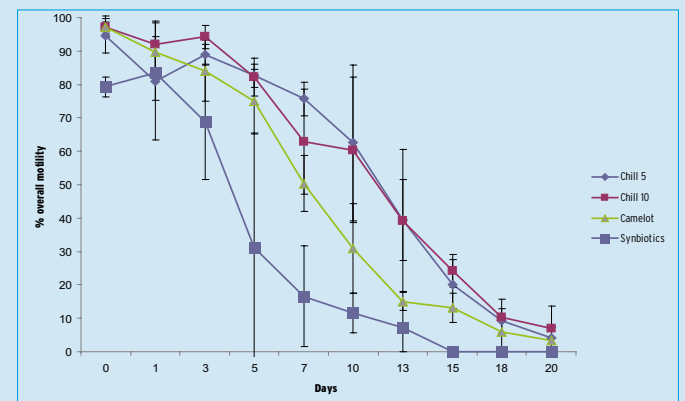
Figures 1 and 2 show:

- a) performance of CaniPRO™ Chill 5 and Chill 10: 70% of the initial motility is still observed at day 7 for the Chill 5 and up to days 10 - 13 for the Chill 10.
- b) the superiority of Chill 10 in long term preservation of canine chilled semen.

The grey lines of the second graph represent the confidence interval for semen preserved in either Chill 5 or 10. They indicate that 95 % of the samples will be within this limit. However, some exceptions are always to be expected, this is the reason why the standard deviation is higher with long term preservation.

Comparative study of CaniPRO™ Chill 5 and Chill 10 vs Synbiotics and Camelot

by Phillips T. & Verstegen J., Theriogenology 2010, submitted



The graph above shows the effects on overall motility after dilution with CaniPRO™ Chill 5, CaniPRO™ Chill 10, Camelot and Synbiotics extenders. 70% of the initial motility is obtained at day 3 for Synbiotics, day 5-7 for Camelot, 7-10 for Chill 5 / 10.

50% of the initial motility is obtained at day 3, day 7 and day 10-13 for Synbiotics, Camelot and Chill 5 or 10 respectively. Motility is lower than 10 % at day 10, 15 and 18-20 for Synbiotics, Camelot and Chill 5 / 10 respectively. These differences are statistically significant: Chill 5 is superior to Synbiotics and Camelot and Chill 10 is superior to Chill 5.

The study repeated 3 times involved only 5 male dogs explaining the high standard deviations

Statistical comparison between Chill 5 and Chill 10

The following data provide the statistical analysis and confirm the above data.

Two-way ANOVA

Source of Variation	% of total variation	P value
Interaction	2,57	< 0.0001
Extenders	2,76	< 0.0001
Time	69,66	< 0.0001

Source of Variation	P value summary	Significant?
Interaction	***	Yes
Extenders	***	Yes
Time	***	Yes

Source of Variation	Df	Sum-of-squares	Mean square	F
Interaction	8	22150	2769	12
Extenders	1	23822	23822	107
Time	8	601287	75161	339
Residual	974	215909	222	-

Does "Interaction" have the same effect at all values of time?

Interaction accounts for approximately 2.57 % of the total variance.

F = 12.49. DFn=8 DFd=974

The P value is < 0.0001

If there is no interaction overall, there is less than 0.01 % chance of randomly observing so much interaction in an experiment of this size. The interaction is considered to be extremely significant.

Does "Extenders" affect the result?

Extenders accounts for approximately 2.76 % of the total variance.

F = 107.47. DFn=1 DFd=974

The P value is < 0.0001

If Extenders has no effect overall, there is less than a 0.01 % chance of randomly observing an effect this big (or bigger) in an experiment of this size. The effect is considered to be extremely significant.

Does "Time" affect the result?

Time accounts for approximately 69.66 % of the total variance.

F = 339.06. DFn=8 DFd=974

The P value is < 0.0001

If Time has no effect overall, there is less than 0.01 % chance of randomly observing an effect this big (or bigger) in an experiment of this size. The effect is considered to be extremely significant.

Bonferroni posttests

Canipro™ Chill 5 vs Canipro™ Chill 10

Time	Canipro™ Chill 5	Canipro™ Chill 10	Difference	95% CI of diff.
Before extension	94	94	0,12	-6.2 to 6.4
0	90	94	3,4	-2.9 to 9.7
3	83	92	8,8	2.4 to 15
7	69	86	16	9.7 to 23
10	49	80	31	23 to 38
13	24	64	40	31 to 49
17	4,4	31	27	17 to 37
20	0,2	4,4	4,2	-12 to 20
24	0,2	0	-0,2	-24 to 23

Time	Difference	t	P value	Summary
Before extension	0,12	0,052	P > 0.05	ns
0	3,4	1,5	P > 0.05	ns
3	8,8	3,8	P < 0.01	**
7	16	6,8	P < 0.001	***
10	31	11	P < 0.001	***
13	40	12	P < 0.001	***
17	27	7,3	P < 0.001	***
20	4,2	0,74	P > 0.05	ns
24	-0,2	0,024	P > 0.05	ns

Source of Variation	Degrees of Freedom	Sum of Squares	Mean square
Extenders	1	23822	23822
Time	8	601287	75161
Interaction	8	22150	2769
Residual (error)	974	215909	222
Total	991		



2. CaniPRO™ Freeze

Motility evolution of Canine Spermatozoa before and after extension and freezing in CaniPRO™ Freeze A and B.

Figure 3: Effects of freezing on overall motility

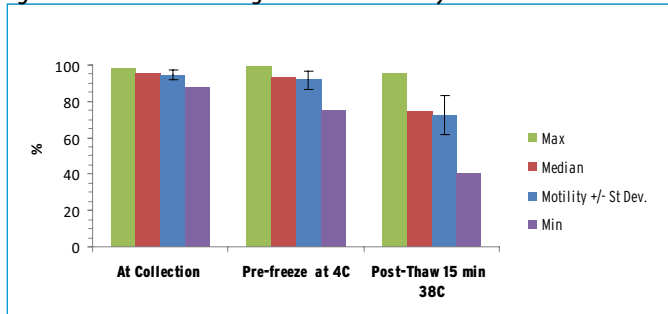
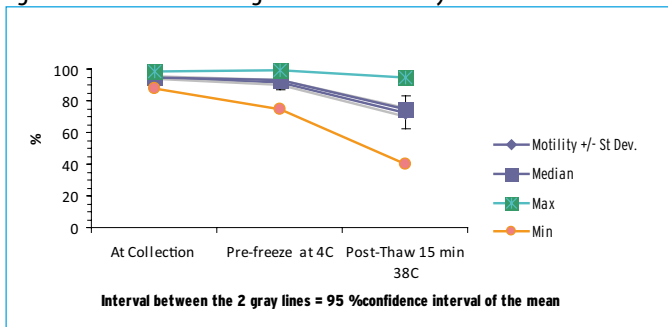
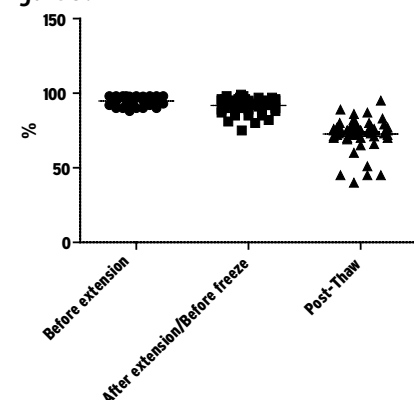


Figure 4: Effects of freezing on overall motility



Figures 3 and 4 display the motility evaluation of canine semen extended for freezing with CaniPRO™ freeze A and B. The first data represent semen characteristics before dilution. The second group shows data after extension, chilling at 4 °C and before freezing, demonstrating a total absence of toxicity of the extender on semen. The last group (Post-Thaw) represents the motility after semen freezing with the CaniPRO™ freeze extender and thawing. The average post-thaw motility is 72% while the initial motility was 94%, which is very good. The confidence interval 95% is close to the means and the standard deviation is more than acceptable (2 to 10 % depending on the values) This deviation is essentially due to a few samples (Figure 5) which are not freezing as well but are still over 50% of initial motility.

Figure 5:



Statistical analysis

The following results demonstrate the presence of differences between the 3 groups due to changes in some samples:

Repeated Measures ANOVA	
P value	< 0.0001
P value summary	***
Are means signif. different? (P < 0.05)	Yes
Number of groups	3
F	217
R square	0,8

Was the pairing significantly effective?	
R square	0,16
F	2
P value	0,0015
P value summary	**
Is there significant matching? (P < 0.05)	Yes

Bonferroni's Multiple Comparison Test	Mean Diff.	t	Significant? P < 0.05?	Summary	95% CI of diff
Before extension vs After extension/Before freezing	3,1	2,6	Yes	*	0.25 to 5.9
Before extension vs Post-Thaw	22	19	Yes	***	19 to 25
After extension/Before freezing vs Post-Thaw	19	17	Yes	***	16 to 22

ANOVA Table	SS	df	MS
Treatment (between columns)	15614	2	7807
Individual (between rows)	3767	53	71
Residual (random)	3816	106	36
Total	23198	161	

3. CaniPRO™ AI

Motility Evolution of Canine Spermatozoa before and after (up to 2 hours) dilution with CaniPRO™ AI

Figure 6: Effect of Extension in CaniPro AI on Motility (%)

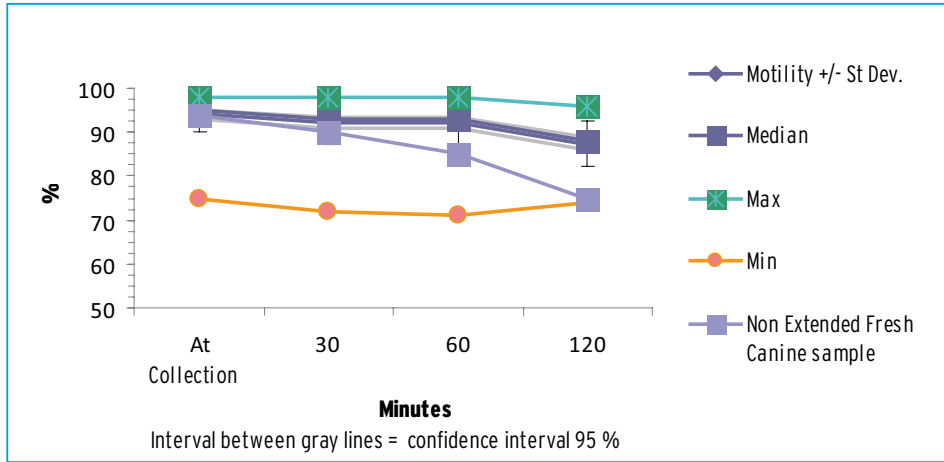
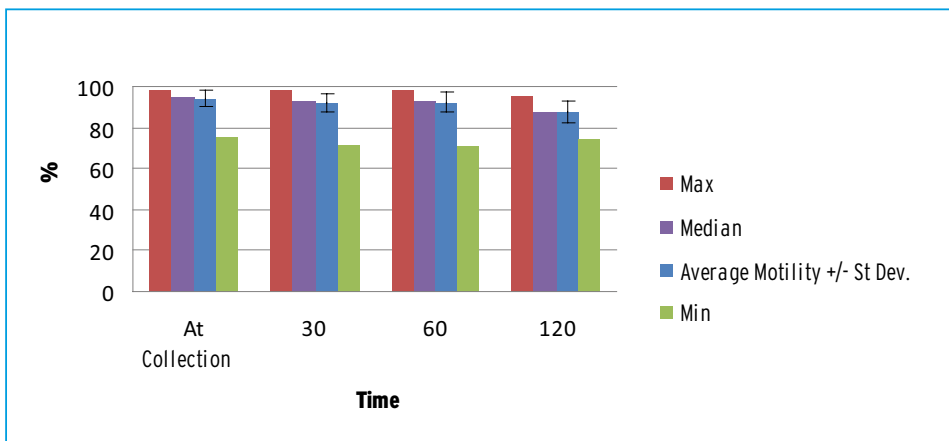


Figure 7: Effect of Extension in CaniPro AI on Motility (%)



Figures 6 and 7 show the motility after canine semen has been extended with CaniPRO™ AI.

The dilution with the AI extender is not affecting the overall motility of the semen preserved at 38 °C for at least 2 hours.

An increased dispersion of the values is observed after 2 hours. The minimum results are higher in this group compared to the others (Figure 8).

The light blue line represents the motility evolution of a fresh canine sample not extended with the CaniPRO™ AI and preserved in the same way at 38 °C.

A decline in the overall motility can be seen after 60 minutes.

Figure 8

