# **DOTTORATO DI RICERCA IN SCIENZE VETERINARIE**

## 33° CICLO

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# **EFFECT OF GLYPHOSATE AND ROUNDUP ON BOAR SPERM QUALITY PARAMETERS**

#### AIM

Lately, the wide use of glyphosate (GLY) based herbicides has become a controversial subject of discussion for its possible impact on population's health. The neurologic, reproductive, endocrine and gastroenteric toxicity of GLY due to chronic exposure is underestimate by official authorities, but from literature appears that commercial formulations used in the crops could have deleterious effects on human health, possibly being more toxic than GLY itself. Commercial formulations, the most famous one is Roundup® (R), contain a number of surfactants and adjuvants, most of which are patented and not publicly known, that can act differently from GLY alone and might potentiate its toxic effect. This could make GLY as pure active substance and Roundup not the same from a toxic standpoint.

The aim of this study was to investigate possible effects on boar sperm of different concentrations of pure GLY and of its formulation Roundup®.

M&M

Fresh boar semen diluited 30 x 10<sup>6</sup> in Androhep medium

### RESULTS

Our results demonstrate that the addiction of glyphosate, at the tested doses, to boar semen has no effects on the sperm quality parameters evaluated regardless of increasing concentrations and hours of incubation



Since tested doses of GLY had no effect on sperm quality, for Roundup® we chose to use concentrations starting with R 0,5µl/ml, which contains 180 µl/ml of glyphosate in its formulation.

Semen samples treated with Roundup 1 and 2 µl/ml induced a significant decrease of viable spermatozoa with active mitochondria compared to control (CTR) after 1h of incubation in a dose-dependent manner and after 3h with no difference between R1 and R2.

No alteration on acrosome integrity was found between CTR and Roundup samples.





ROUNDUP - VIABILITY				
SYBR+ JC1+	CTR	R 0,5 μL/ml	R1 μL/ml	R2 μL/ml
1h	77,3 ± 7,9ª	67,3 ± 3,8 <sup>ab</sup>	67,0 ± 11,5 <sup>b</sup>	59,4 ± 11,4°
3h	73,8 ± 6,7ª	$63,5 \pm 4,2^{ab}$	63,3 ± 12,4 <sup>b</sup>	59,1 ± 12,3 <sup>b</sup>

ROUNDUP - ACROSOME INTEGRITY					
PSA +	CTR	R0,5 μL/ml	R1 μL/ml	R2 μL/ml	
1h	93,8 ± 4,9	97,0 ± 1,3	94,3 ± 4,3	93,3 ± 5,4	
3h	94,0 ± 4,2	97,0 ± 1,7	95,1 ± 3,3	93,5 ± 5,3	

CASA system revealed a big drop in total and progressive motility in treated groups compared to CTR, regardless concentration and incubation time. Through microscope optical analysis, treated samples showed most sperm flocked in big agglomerate, whereas in CTR agglutination was occasional. After 1h, clusters in treated groups contained a higher rate of viable and motile cells compared to CTR, while after 3h the number of motile sperm in clusters decreased.

ROUNDUP – TOTAL AND PROGRESSIVE MOTILITY					
ТМ	CTR	R 0,5 μL/ml	R 1 μL/ml	R 2 μL/ml	
1h	$48,4\pm17,9^{\rm a}$	$19,7\pm8,0^{\mathrm{b}}$	$20,1\pm9,5^{\text{b}}$	$13,9\pm5,9^{\text{b}}$	
3h	$46,1\pm19,5^{\mathrm{a}}$	$\textbf{22,7} \pm \textbf{12,2^{b}}$	$12,9\pm12,5^{\text{b}}$	$7{,}9\pm5{,}1^{\text{b}}$	
PM					
1h	$\textbf{22,5} \pm \textbf{8,1^a}$	$8,7\pm6,7^{\text{b}}$	$6,4\pm3,8^{\text{b}}$	$4,6\pm3,5^{\rm b}$	
3h	$20,7 \pm 11,8^{a}$	$9,7\pm8,5^{\text{b}}$	$4,0\pm3,4^{\text{b}}$	$3,0\pm2,6^{b}$	

**ROUNDUP – AGGLUTINATION RATE** 

		CTR	R 0,5 μL/ml	R 1 μL/ml	R 2 μL/ml
1h	AGGLUTINATE	$10,2 \pm 4,8^{a}$	$56,7 \pm 12,0^{b}$	63,7 ± 11,6 <sup>b</sup>	60,9 ± 15,4 <sup>b</sup>
	AGG. MOTILE	32,7 ± 17,6 <sup>a</sup>	$78,8 \pm 8,1^{b}$	$80,1 \pm 7,4^{b}$	74,1 ± 11,3 <sup>b</sup>
3h	AGGLUTINATE	7,0 ± 5,1ª	47,9 ± 16,5 <sup>b</sup>	51,1 ± 17,5 <sup>b</sup>	54,7 ± 11,0 <sup>b</sup>
	AGG. MOTILE	37,4 ± 12,5 <sup>a</sup>	$41,5 \pm 24,8^{a^*}$	49,2 ± 17,5 <sup>a</sup>	53,3 ± 15,8ª

In all tables values are expressed as the mean ± SD. Different letters indicate significant difference for P < 0.05 between treatments; \* indicates significant difference in the same treatment between incubation times

#### CONCLUSIONS

While the active substance itself had no effect on semen quality parameters evaluated, Roundup® formulation induced a significative drop in sperm viability and motility since the first hour of incubation. The motility decrease recorded in Roundup® treated samples might be overestimated as CASA is set to analyzes only single cells and not agglutinates, where most viable and motile spermatozoa tended to be. Analysis on acrosome integrity, but also on tyrosine phosphorylation and hsp70 protein (data not shown), let us to suppose that the tendency to form head to head agglutinates in Roundup® treated samples is more probably a

