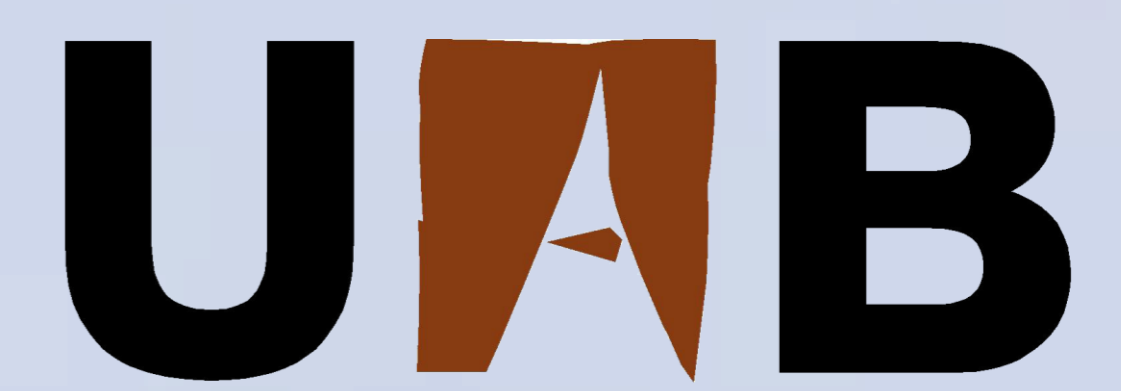


# Improving results of freezing equine semen

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## INTRODUCTION

The necessity to breed with the top horses of the world has increased the use of frozen semen in artificial insemination.<sup>1,2</sup> Cryopreservation reduces the fertility and the survival of spermatozoa. Furthermore, some stallions do not respond as well as others to cryopreservation.<sup>1</sup>

## OBJECTIVE

The aim of this study was to determinate which glutathione's concentration (2mM Vs. 4mM) gives the best results after thawing the equine spermatozoa.

## OXIDATIVE STRESS

Oxidative stress is an excessive production of reactive oxygen species as  $O_2^-$ ,  $\cdot OH$  and  $H_2O_2$ . ROS is produced by spermatozoa's metabolism, oxidative burst of neutrophils, abnormal spermatozoa and cryopreservation. We have to consider that ROS cause cellular damage. Semen is able to fight against oxidative stress by using antioxidants and enzyme scavengers which are in spermatozoa and principally in seminal plasma.<sup>2,3,4</sup> When we cryopreserve semen we remove about 95% of seminal plasma, so we need to be conscious about antioxidants.<sup>5</sup>

## RESULTS

STALLIONS "Bad freezers"	1	2	3	4	5	Normal values	Interval
Volume (mL)	35	65	35	30	20	70	40-80
Spz/mL (x10 <sup>6</sup> )	623,4	87,5	295,31	120	90	400	150-700
Spz total (x10 <sup>6</sup> )	21619	5687,5	10335,9	3600	1800	15000	5000-25000
Viability (%)	63,7	50,93	84,6	52	53,1	>75	
Abnormalities (%)	66,7	36,51	28,28	42,5	63,5	<25	

Table 1. Shows the fresh semen analyses of stallions . All considered bad freezers. Spz: Spermatozoa

## Increased parameters

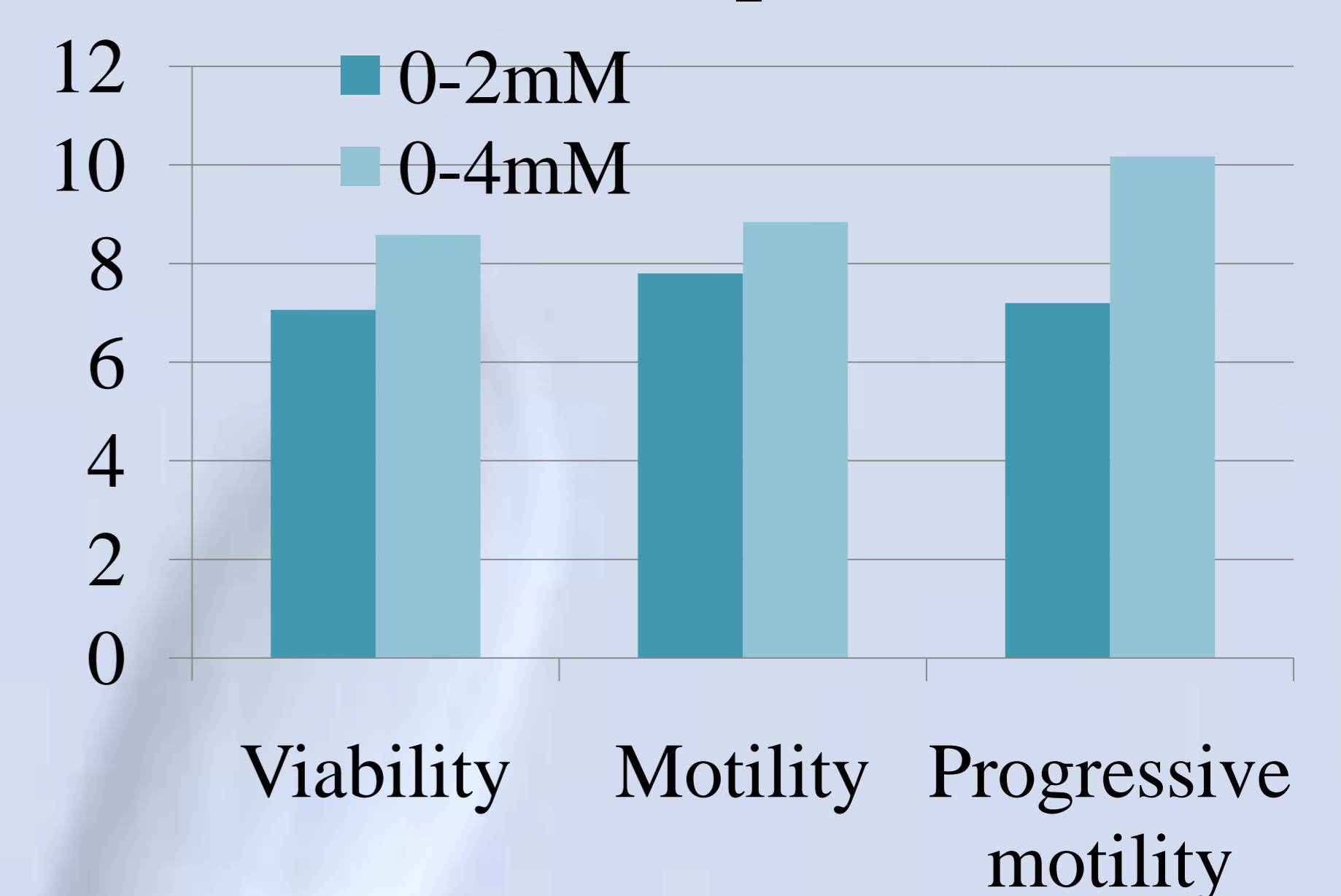


Figure 2. 4mM of glutathione gives better increased parameters.

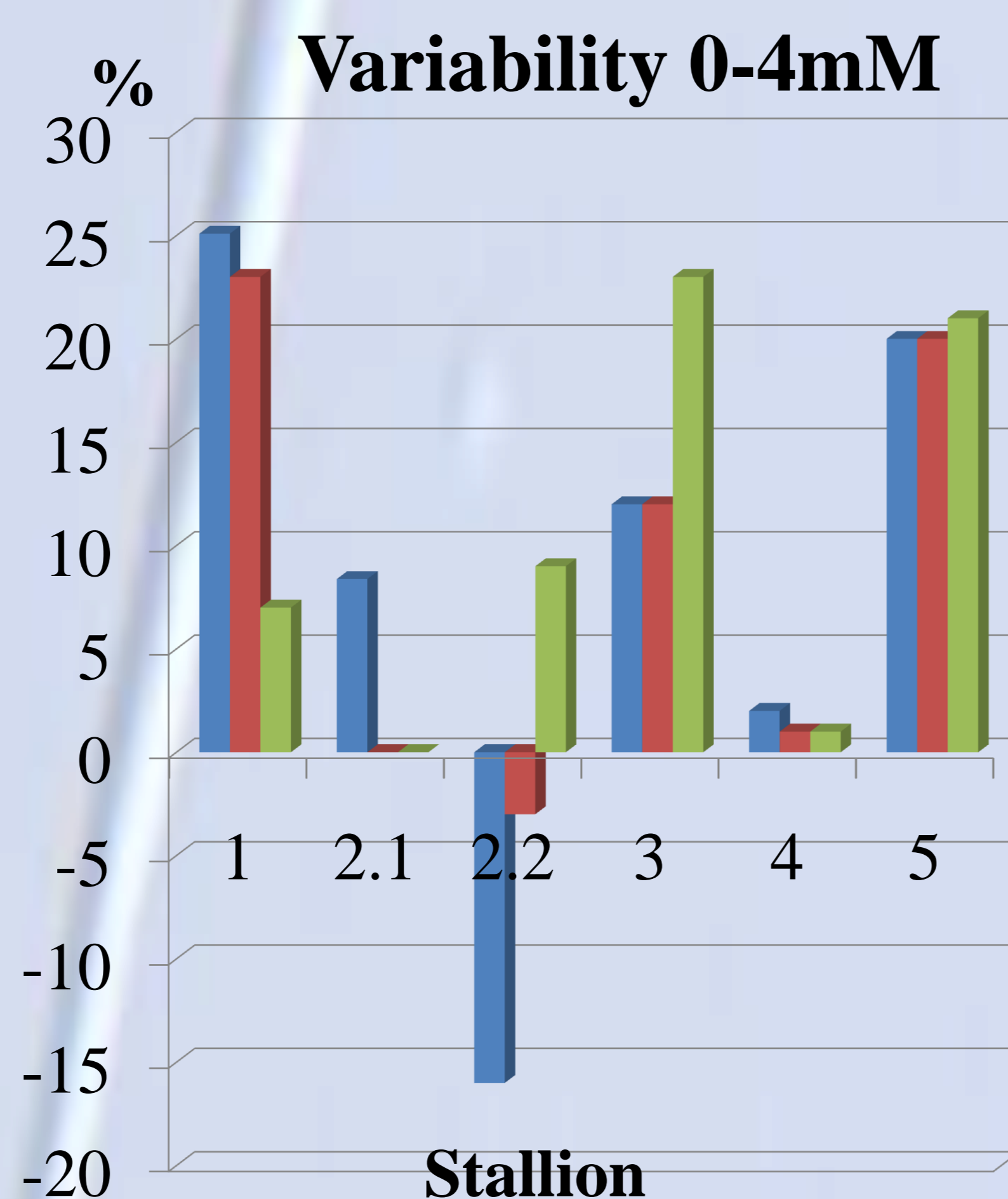
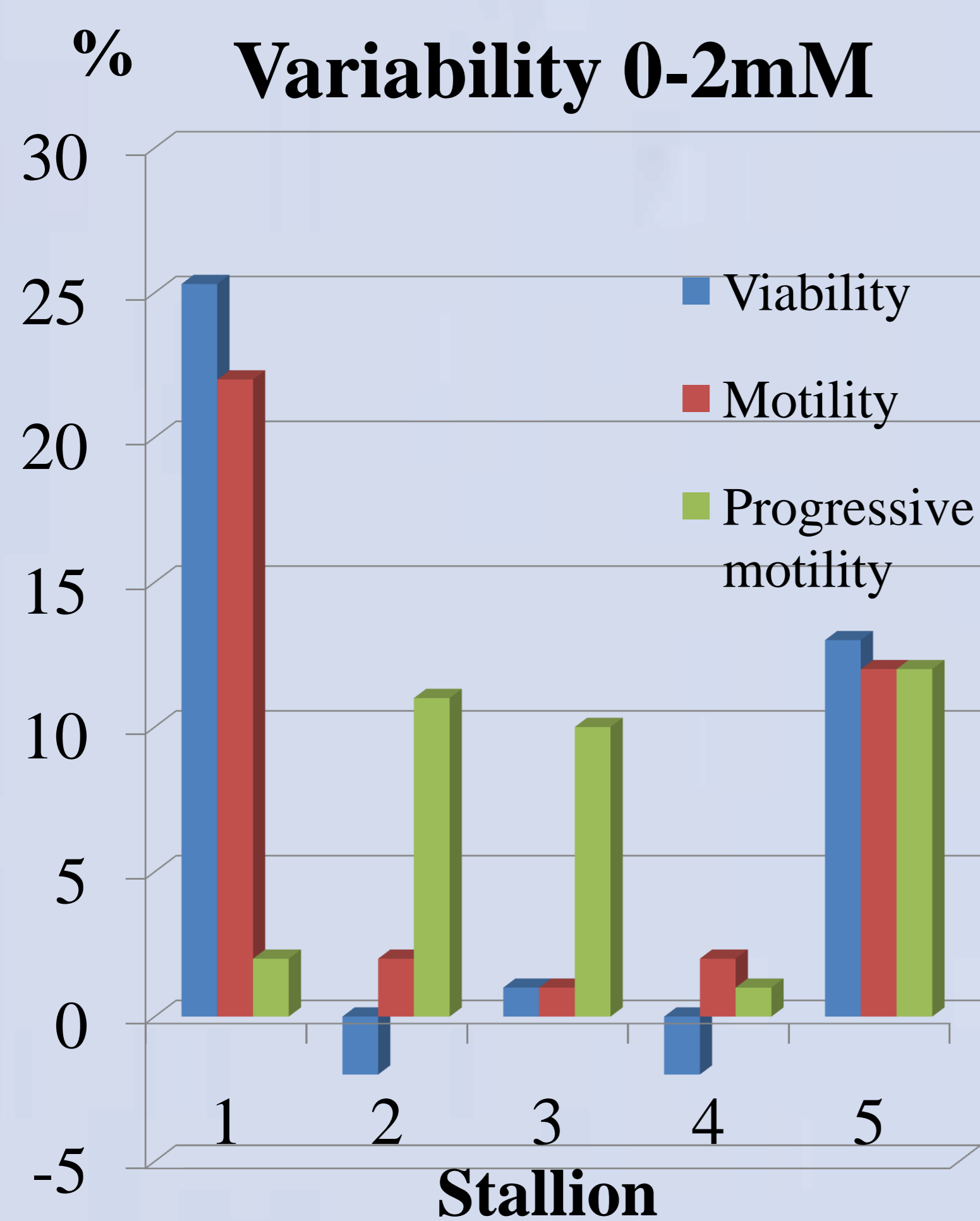


Figure 1. All stallions respond different. Some respond better with 2mM of glutathione and other with 4mM . We can see the variability between ejaculates, too (Stallion 2)

Post Thaw	Viability (%)	Motility (%)	Progressive motility (%)
0mM	43,07 ± 20,56	16,57 ± 11,36	8,17 ± 10,03
2mM	45,66 ± 21,51	20,8 ± 13,85	15,6 ± 15,14
4mM	51,64 ± 17,05	24 ± 16,84	18,34 ± 17,36

Table 2. Mean with SD of studied parameters. The table shows that 2mM and 4mM have increased the studied parameters, but with a big deviation in each one.

## REFERENCES

- Oliveira RA, Piersanti RL, Wolf CA, Viu MAO, Gambarini ML. Glutathione for the freezing of cooled equine semen, using different protocols. *Anim Reprod.* 2014;11(2):104–9.
- Baumber J, Ball BA, Linfor JJ. Assessment of the cryopreservation of equine spermatozoa in the presence of enzyme scavengers and antioxidants. *Am J Vet Res.* 2005;66(5):772–9.
- Ball BA. Oxidative stress, osmotic stress and apoptosis: Impacts on sperm function and preservation in the horse. *Anim Reprod Sci.* 2008;107(3-4):257–67.
- Ball BA. Oxidative stress in sperm. In: McKinnon AO, Squires EL, Vaala WE, Varner DD, editors. *Equine Reproduction.* 2nd ed. Vol. 1. Chichester (UK): Wiley-Blackwell; 2011. p. 991–5.
- Kareskoski M, Katila T. Components of stallion seminal plasma and the effects of seminal plasma on sperm longevity. *Anim Reprod Sci.* 2008;107(3-4):249–56.

## CONCLUSIONS

The quality of the spermatozoa is very individual. The addition of 2mM and 4mM of glutathione improves the quality spermatozoa after thawing. But viability, motility and progressive motility increase more when we add 4mM than 2mM. Not all horses respond identically to a glutathione concentration; there are stallions that respond better to 2mM, although the best results are with 4mM; and others that do not respond to antioxidants because their spermatozoa are more sensitive to cryodamage, dehydration or osmotic stress.