Prevention of neural tube defects through maternal supplementation

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Introduction

Neurulation and neural tube defects (NTDs)

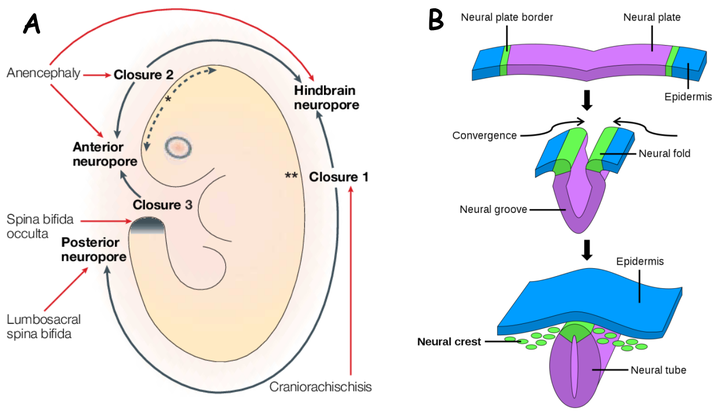


Figure 1. A) Neural tube closure points. **B)** Neurulation process (**ref 1**)

Neurulation is a crucial step in embryonic development that leads to the formation of the neural tube, a structure that ends up shaping the central nervous system. Neural tube defects (NTDs) appear when neurulation fails and the neural tube does not close completely.

Mouse model of NTDs: *Loop-tail*

Loop-tail is a murine mutant strain for the *Vangl2* gene, a member of the non-canonical Wnt-PCP pathway for planar cell polarity. During neurulation, this pathway regulates convergent extension movements that occur during morphogenesis and neural plate closure. Thus, the *Vangl2* mutation causes defects in the closure of the neural tube.

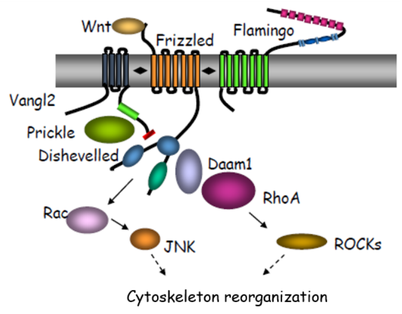
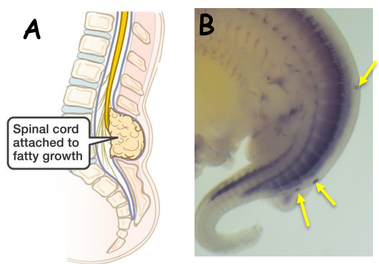


Figure 2. Intracellular signaling of the Wnt-PCP pathway.

Caudal neural tube cellular aggregates

In heterozygosity, *Loop-tail* mice present a low incidence of spina bifida aperta, however in our lab we observed a high incidence of spina bifida occulta. This is detected by the presence of cellular aggregates covering a failure of dorsal fusion in the neural tube that resembles the most common form of spina bifida occulta in humans. Cells that form these aggregates come from the neural crest cells (NCCs, *Sox10* positive).

Figure 3.
A) Lipomyelomeningocele, the most common type of spina bífida occulta
B) Detection of *Sox10* mRNA expression by *in situ* hybridation. Caudal cellular aggregates in *Vangl2*$^{+/Lp}$ embryos, marked with yellow arrows.

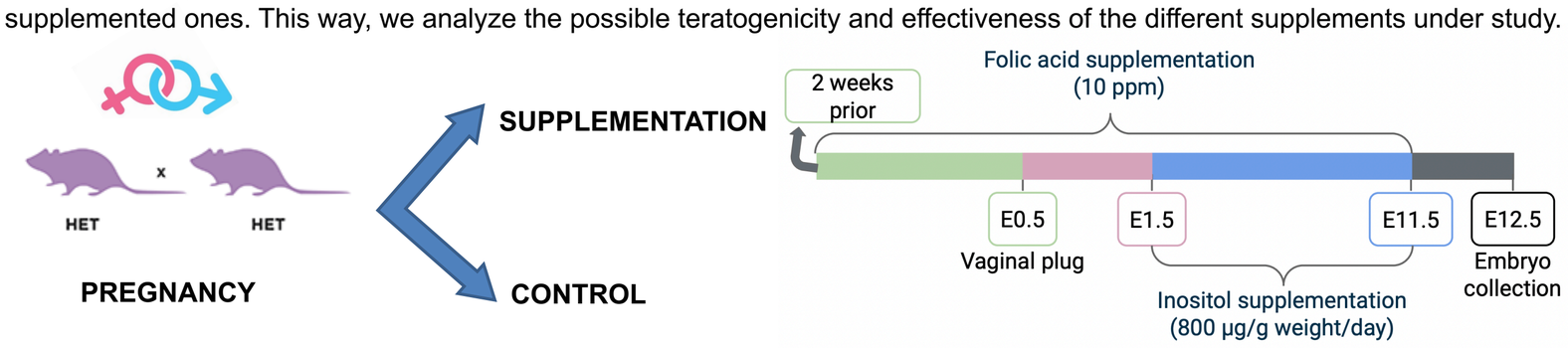


Maternal supplementation

Currently, 70% of NTDs are preventable by maternal supplementation with folic acid. Inositol is an organic compound that has already shown promising results in preliminary trials. Our group is testing inositol in combination with folic acid for the prevention of the remaining 30% folate-resistant NTDs.

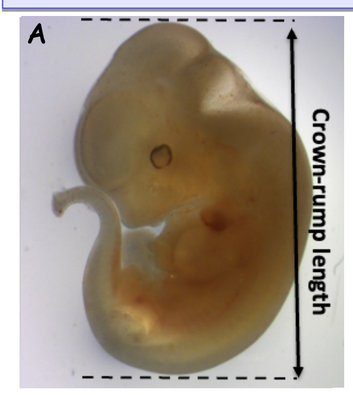
Methodology

RNA *in situ* hybridization technique in mid-developed embryos allows us to compare the number, intensity and size of cellular aggregates between embryos from untreated females and those from supplemented ones. This way, we analyze the possible teratogenicity and effectiveness of the different supplements under study.

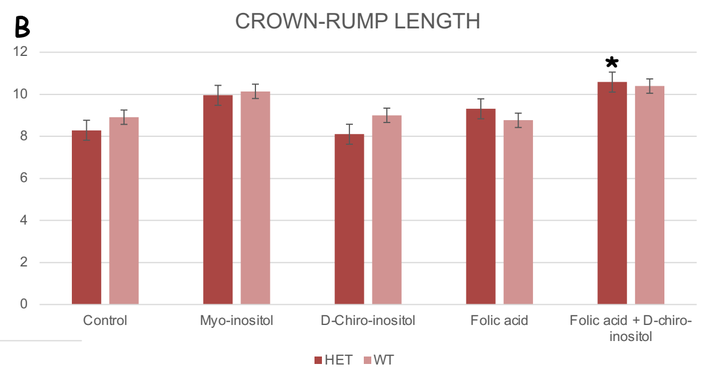


Results

Embryonic development: % resorptions and crown-rump length



Graphic representations of the effect of different maternal supplementations on embryonic development. Comparing crown-rump length as well as the percentage of resorptions allow us to test potential toxicity.



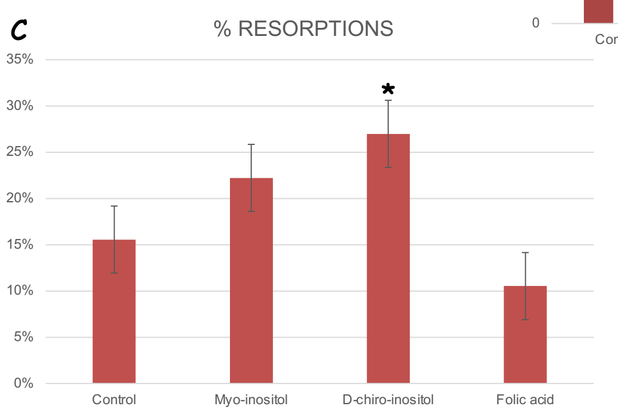
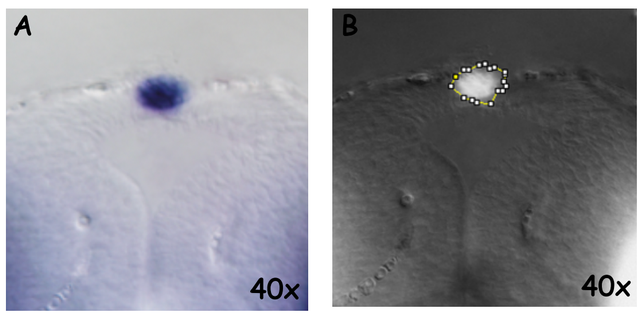
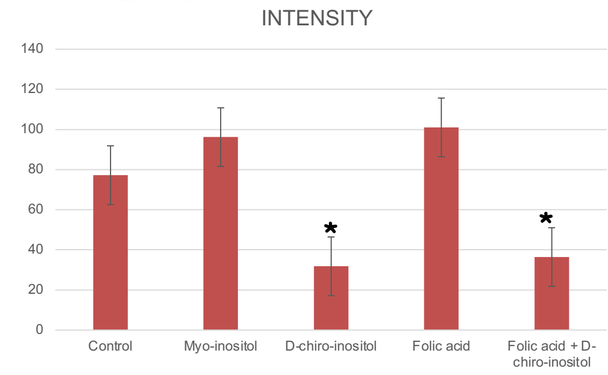


Figure 4. A) Method used to obtain the crown-rump length. **B)** Embryos from supplementation combining folic acid and *D-chiro*-inositol show a significally larger Crown-rump length ($p < 0.05$) **C)** D-*chiro*-inositol on its own triggers a higher percentage of resorptions ($p<0.05$).

NTDs prevention: intensity of cell aggregates and No of aggregates per

In order to assess the preventive character of the supplementation, the size and number of cellular aggregates in our embryos were measured under different conditions.





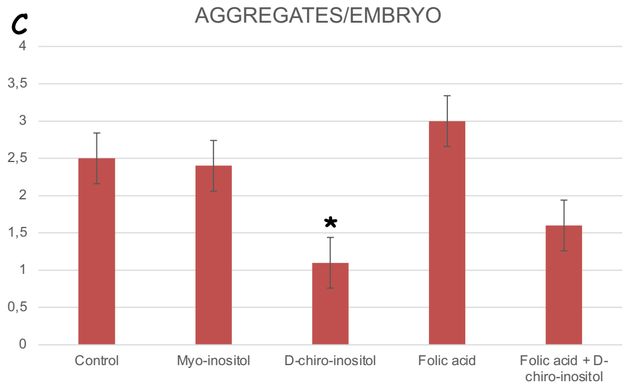


Figure 5. A) Method used to calculate the intensity of cell aggregates using ImageJ program. The picture is inverted and converted to 8-bit format and the aggregate is analised via histogram. The background is also analised and subtracted. **B)** D-*chiro*-inositol on its own and combined with folic acid has shown a significant reduction in the intensity of cellular agregates. **C)** D-*chiro*-inositol has shown a significant reduction ($p<0.05$) in the number of cellular agregates per embryo.

D-*chiro*-inositol combined with folic acid reduces cellular aggregates in *Loop-tail* mutants

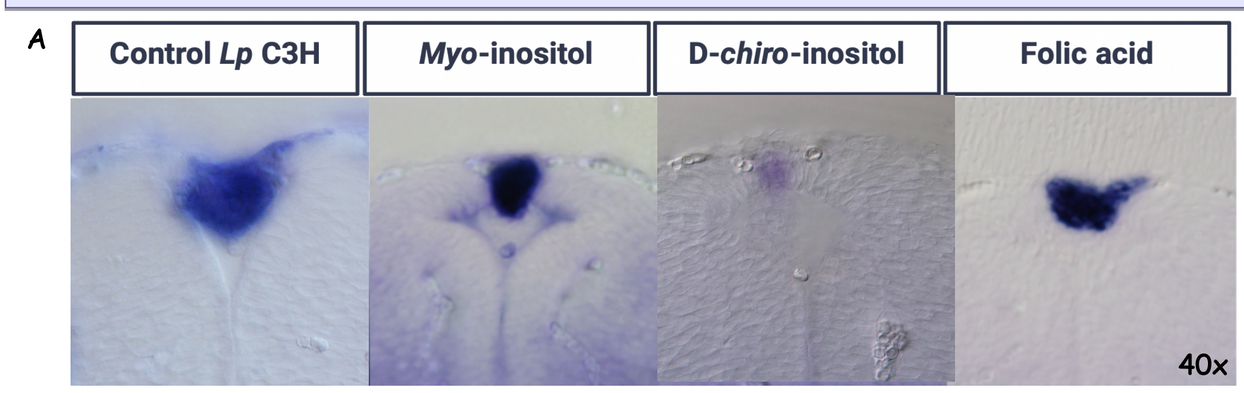


Figure 6. A) Only embryos treated with D-*chiro*-inositol show a significant reduction in size of cellular aggregates compared to the control embryos. **B)** Combination of Folic acid and D-*chiro*-inositol is also able to significantly reduce cellular aggregates in size ($p<0.05$). *In situ* hybridization, Nomarski microscopy. Pictures were taken in a direct optical microscope, at 40x dry lens.

Conclusions

The only supplementation with significant results in the prevention of aggregates in our *Vangl2*$^{+/Lp}$ embryos has been D-*chiro*-inositol. This treatment is truly effective reducing the incidence of spina bifida occulta. D-*chiro*-inositol supplementation produces a certain degree of embryotoxicity but this effect is reversed when combined with folic acid, its efficacy against NTDs is maintained but its potential toxicity not. Thus, combining both supplements, folic acid and D-*chiro*-inositol, the best outcome is obtained, preventing more effectively the development of spina bifida occult in cases of NTD variants resistant to the action of folic acid alone.

Bibliography

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