Supplementary Methods and Results

Inhibition of maternal-to-fetal transfer of IgG antibodies by FcRn blockade in a mouse model of arthrogryposis multiplex congenita

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Supplementary methods

Preparation of purified human IgG

Purification of human IgG was done using the ammonium sulphate precipitation method (M. Page 1996), to avoid damage to the Fc region, as it is likely to occur after using the acidic elution buffers required for column purification. Saturated ammonium sulphate solution was added to the human plasma to produce a 45% final saturation and left to stir at 4°C overnight. The following day, this solution was centrifuged at 12000 rpm (16000g) for 2 hours. The supernatant was discarded and the precipitate was re-suspended in 10% of the original volume in Hartmann's solution (Viaflo, FKE 232Y). After homogenization, the solution was dialysed against a minimum of three changes of Hartmann's buffer (2 L per change). IgG concentration was determined using a commercially available human IgG radial immunodiffusion kit (BINDARID human IgG NL RID kit; Binding site, RIN055).

Neonatal testing

Testing was done daily, from P1 to P21, in the light phase (lights on at 7pm). Pups were separated from the dam and placed in a petri dish with home cage nesting material at the beginning of testing. All testing was done with the pups placed in a heating pad and, for the first five postnatal days, under a lamp with a 60 watt bulb. Pups were returned to the dam after testing. Care was taken to extensively rub the examiners hands and the pups in the home cage shavings in order to avoid maternal rejection. All material was disposed of or washed with tepid water between litters.

Modified battery of Fox

The modified battery of Fox (Hill J. Neuromethods, vol 39) (Figure 2-3) allows a comprehensive evaluation of neonatal developmental milestones. The battery includes maturation readouts describing physical development (individual weight, pinna detachment and eye opening), neurodevelopmental measures based on neurological reflexes (surface righting, negative geotaxis, cliff aversion, rooting, ear twitch and air righting) and tests of neuromotor coordination (forelimb grasping and open field traversal). Tests were repeated daily until the pup met the test criterion for 2 consecutive days. If a pup did not reach criterion by the end of the specified testing days, the day following the last test day was used for statistical analysis.

Weight measurements

Weight was measured daily, from P1 to P21, for each individual pup, prior to the testing.

Eye opening and pinna detachment

The mouse pup was examined daily, from P1 to P21, to determine the first day when both eyes were opened or both ears detached from the skull.

Surface righting

The mouse pup was held on its back, supported on both sides of the head and on the hind quarters, and released onto a smooth surface. The time for the pup to flip to its abdomen and touch the surface with all four paws was measured in seconds. If the pup could not reach that position within 30 seconds, the test was terminated. The test was performed from P1 to P13 or until the pup could reach that position in less than 1 second for two consecutive days.

Negative geotaxis

The pup was placed in a screen at a 45º angle with its head facing down. The time for the pup to turn 180º, so the head faced up, was measured in seconds. If the pup fell, the test was repeated once. If the pup could not reach that position within 30 seconds, the test was terminated. The test was performed from P1 to P14 or until the pup could reach that position in less than 30 seconds for two consecutive days.

Cliff aversion

The mouse pup was positioned on the top of a box with the forepaws and snout placed on the edge of the box. Time for the pup to crawl away from the edge of the box was measured in seconds. If the pup fell, the test was repeated once. If the pup could not reach that position within 30 seconds, the test was terminated. The test was performed from P1 to P14 or until the pup could reach that position in less than 30 seconds for two consecutive days.

Rooting

A cotton filament was used to stroke three times the pup's head, laterally, from front to back. A rooting response was considered to occur if the pup moved the head towards the filament. If no response occurred, the other side of the head was stroked in the same way. The test was performed from P1 to P12 or until a response was elicited for two consecutive days.

Ear twitch

A cotton filament was used to stroke three times the pup's ear tip. A response was considered to occur if the mouse would flatten the ear against the side of the head. If no response occurred, the other ear was stroked in the same way. The test was performed from P7 to P15 or until a response was elicited for two consecutive days.

Air righting

The mouse pup was held on its back, supported on both sides of the head and on the hind quarters, and released approximately 10cm above a clean cage padded with nesting material. The day in which the pup would flip to its abdomen and land on the surface with all four paws was recorded. The test was performed from P8 to P21 or until the pup could reach that position for two consecutive days.

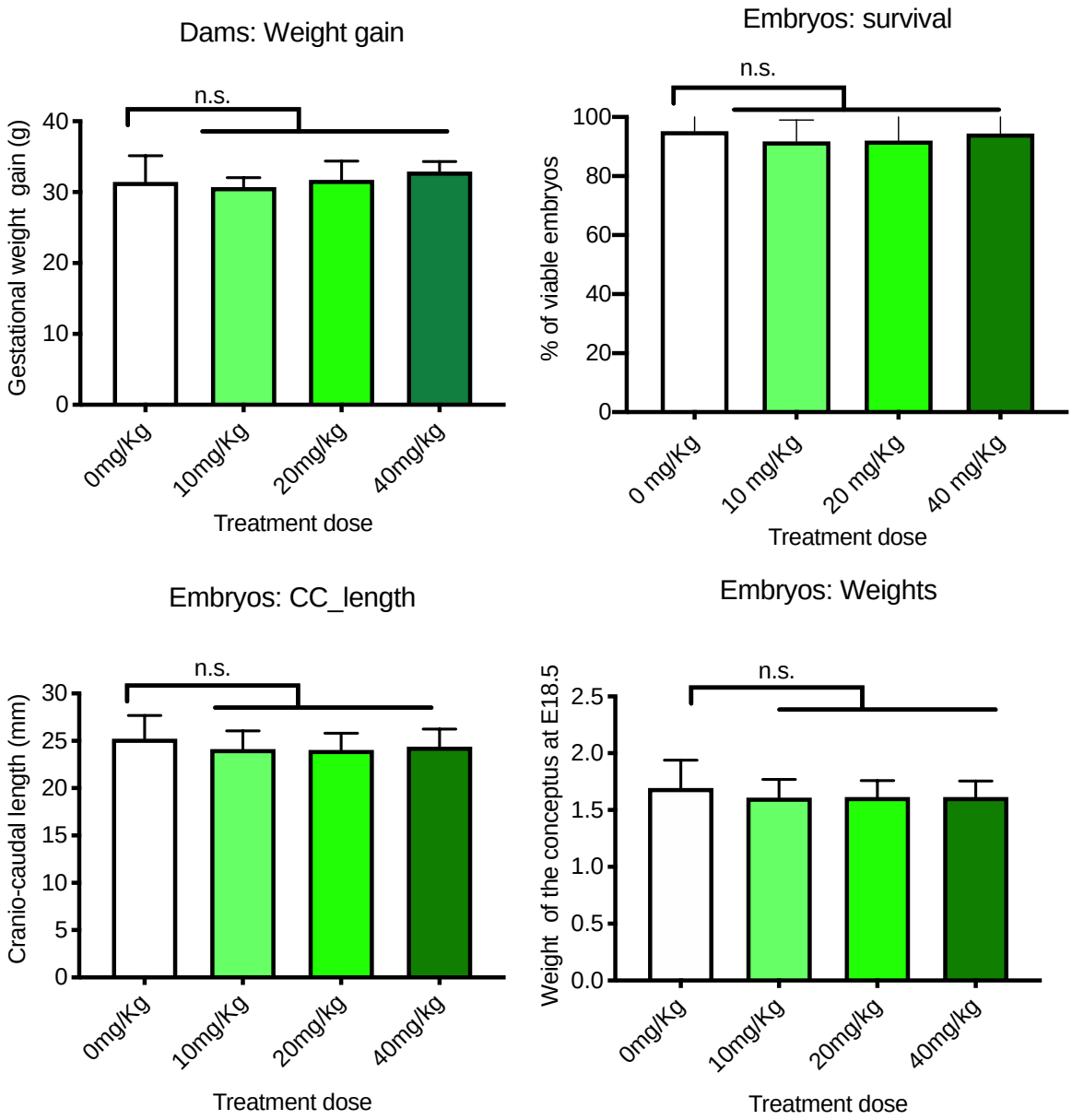
Forelimb grasping

The pup was held supported on both sides of its trunk and the forepaws placed on a small rod (approximately 5mm in diameter) suspended over a clean cage padded with nesting material. The pup was then released and the amount of time the mouse remained gripping the rod was measured. If the mouse fell immediately after release, the test was repeated once. The test was performed from P4 to P14 or until the mouse could hold the grip for 1 second or more for two consecutive days.

Open field traversal

The pup was placed in the centre of a circle with a 13cm diameter printed on a sheet of paper. The amount of time the mouse would take to move outside the circle with all four paws was recorded. If the mouse failed to leave the circle in 30 seconds, the test was terminated. The test was performed from P8 to P21 or until the mouse would reach criterion in less than 30 seconds in two consecutive days.

Supplemental Figure 1: Effects of treatment with 4470 mAb on general parameters of wellbeing: gestational weight, embryonic survival, embryonic cranio-caudal length and embryonic weight.



Supplemental Figure 2: Neonatal development using modified battery of Fox (male and female mice pooled)