# **Protocol: Blastocyst Injection (usually on Mondays)**

#### I. Superovulation of C57BL/6 blastocyst donors:

Light-dark-cycle mouse house:	winter	6am – 6 pm
	summer	7am – 7 pm

Intergonan für Tiere, Intervet (=PMSG, pregnant mares serum gonadotropin)

5 x 1000 I.E. (lyophilized powder) stored at 4°C, in mouse house OP

To prepare the <u>500 U/ml stock solution (10x)</u> resolve 1 aliquot of 1000 I.E (lyophilized powder!) in 2 ml 0.9% sodium chloride solution.

### Make 120 ul aliquots and store at –80°C: Tower 9, Box 3.

Before use, prepare the <u>50 U/ml working solution</u>: Add to one 120 ul aliquot (500 U/ml stock) 1080 ul 0.9% sodium chloride solution (= 1:10 dilution).

I.p. injection of 100 ul per 3-5 week old C57BL6 female (= 5 IU in 100 ul) at 13.30 (Tues).

Thaw and use the hormone aliquot(s) for the injection only once; disgard the rest.

#### Predalon (für Tiere), Organon (=CG, Choriongonadotropin)

3 x 5000 I.E. (lyophilized powder) stored at 4°C, in mouse house OP

# Needs to be prepared once per month!! Once the hormones are resolved, they are not longer stable for a longer period of time.

To prepare the <u>500 U/ml stock solution (10x)</u> resolve 1 aliquot of 5000 I.E. (lyophilized powder!) in 10 ml 0.9% sodium chloride solution.

## Make 120 ul aliquots and store at –80 °C: Tower 9, Box 4.

Before use, prepare the <u>50 U/ml working solution</u>: Add to one 120 ul\_aliquot (500 U/ml stock) 1080 ul 0.9% sodium chloride solution (= 1:10 dilution).

I.p. injection of 100 ul per 3-5 week old C57BL6 female (= 5 IU in 100 ul) at 12.30 (Thur).

Thaw and use the hormone aliquot(s) for the injection only once; disgard the rest.

#### II. Mating of blastocyst donors:

On Thursday afternoon (not before 15:00) 20 superovulated C57BL/6 females are mated to 20 C57BL/6 males (max. 1 year old); one female per male in individual cages. Check for vaginal plugs next morning and separate the plugged females from the unplugged ones and put them into two individual cages.

#### III. Mating of fosters:

On Friday afternoon (not before 15:00) 20 NMRI females (between 2 and 5 months) are mated to 10 sterile (vasectomized) NMRI males (max. 1 year old); two females per male in individual cages.

Check for vaginal plugs next morning and separate the plugged females from the unplugged ones and put them into two individual cages.

#### IV. Preparation of blastocysts for microinjection on Mondays (day 3.5):

Blastocyst donors are sacrificed in the morning at 8:00 and embryos are collected from the uteri.

Wash the blastocysts after isolation in M2 media and store them in M16 in a CO2 incubator until microinjection.

#### V. Blastocyst injection:

15 – 25 (max.) ES cells are microinjected into a host blastocyst.

#### VI. Embryo transfer into day 2.5 foster females:

Transfer 12-16 injected blastocysts into one foster female (6-8 embryos per side).