



Operativna nega miši pri embriotransferju in vazektomiji

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CRISPR Cas9

- Mikroinjiciranje, embriotransfer in vazektomija potekajo v okviru metode CRISPR Cas9
- Razvoj transgenih linij miši

MIKROINJICIRANJE 1. - 4. dan

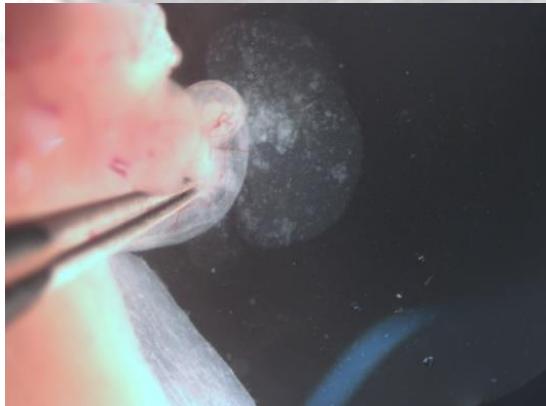
- Donor zigot so F1 samice (B6D2F1)
- Superovulacija
- Pregled samic na prisotnost vaginalnega čepa
- Izolacija jajčnikov pri samicah s čepom

ČEP



MIKROINJICIRANJE - 4. dan

- Sprostitev kumulusa z zigotami iz ampule jajcevoda v medij M2 (hialuronidaza)
- Spiranje zigot in shranitev v M2, pokrito z oljem za embrije (Zenith)
- Mikroinjiciranje, inkubacija preko noči v M16



VAZEKTOMIJA

- Samci HSD:ICR (CD-1®)
- Vsaj 2 meseca pred MI/ET
- Pri starosti 8-12 tednov
- Preverjanje plodnost in vaginalnega čepa po OP
- Paritev s samicami - nadomestnimi materami za ET

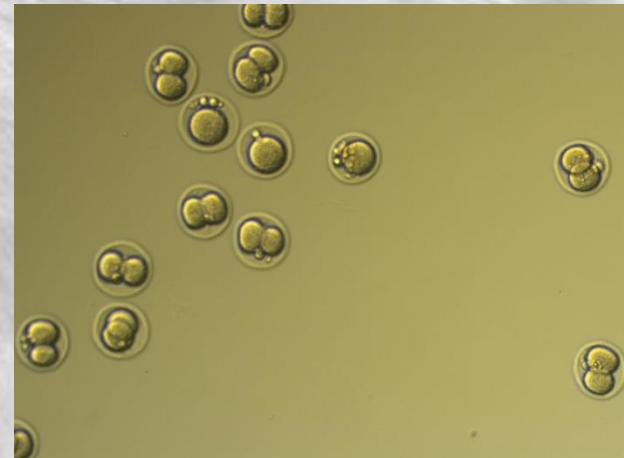
EMBRIOTRANSFER - 4. dan

- Nadomestne matere samice HSD:ICR (CD-1[®]) → 15 - 20 samic
- Parimo z vazektomiranimi samci HSD:ICR (CD-1[®])
- Razmerje: 1 samec in 2 samici



EMBRIOTRANSFER - 5. dan

- Pregled HSD:ICR (CD-1[®]) samic na prisotnost vaginalnega čepa
- Priprava HSD:ICR (CD-1[®]) samic
- Izbor dvoceličnih zigot
- Pripravimo zigote v ustno pipeto

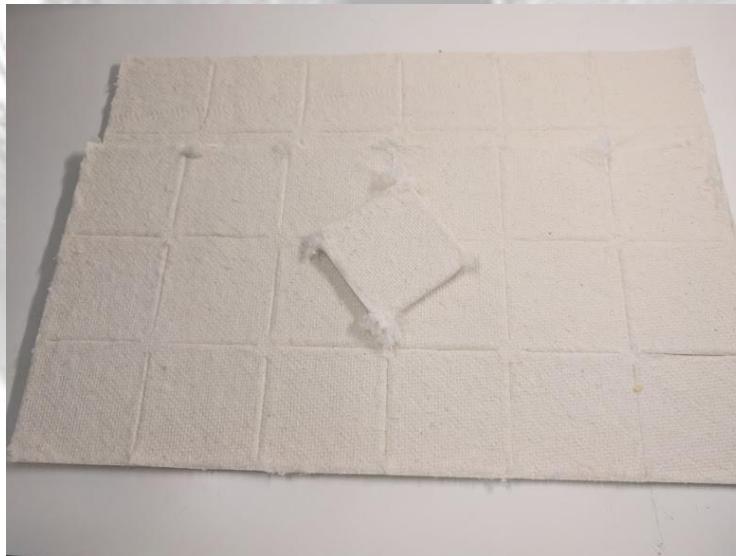


TRAJANJE POSEGA

- Povprečno 20 minut (14 - 34 minut)
- Trajanje narkoze povprečno 52 minut
- Ocena težavnosti poskusa:
Moderate/Zmeren

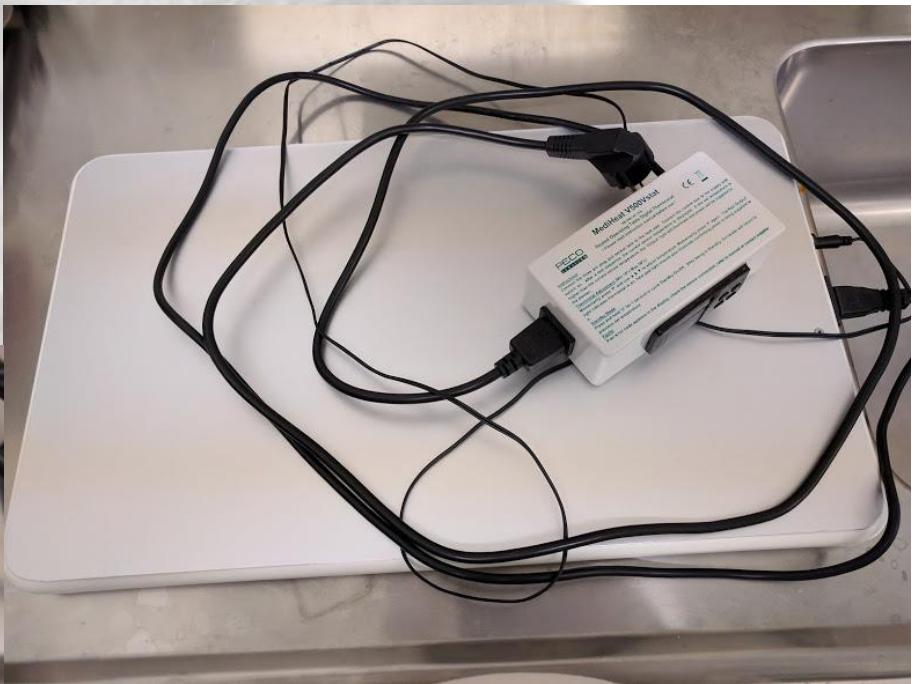
PERIOPERATIVNA NEGA

- Obogatitev - Nestlets, samica si pripravi gnezdo prej (Nestlest)
- 3 - 4 ure pred OP
- Hrana v kletko



TERMOREGULACIJA

■ Peco Services



PERIOPERATIVNA NEGA

- Termoregulacija v kletki (38°C)



PERIOPERATIVNA NEGA

- Anestetika:
 - Ketamin (100mg/ml)
 - $0,5 \mu\text{l}/1\text{g}$
 - Xylazin (20mg/ml)
 - $0,75/1\text{g}$
 - V PBS
 - $3,75 \mu\text{l}/1\text{g}$
 - $150-200 \mu\text{l}/30-35 \text{ g miš}$



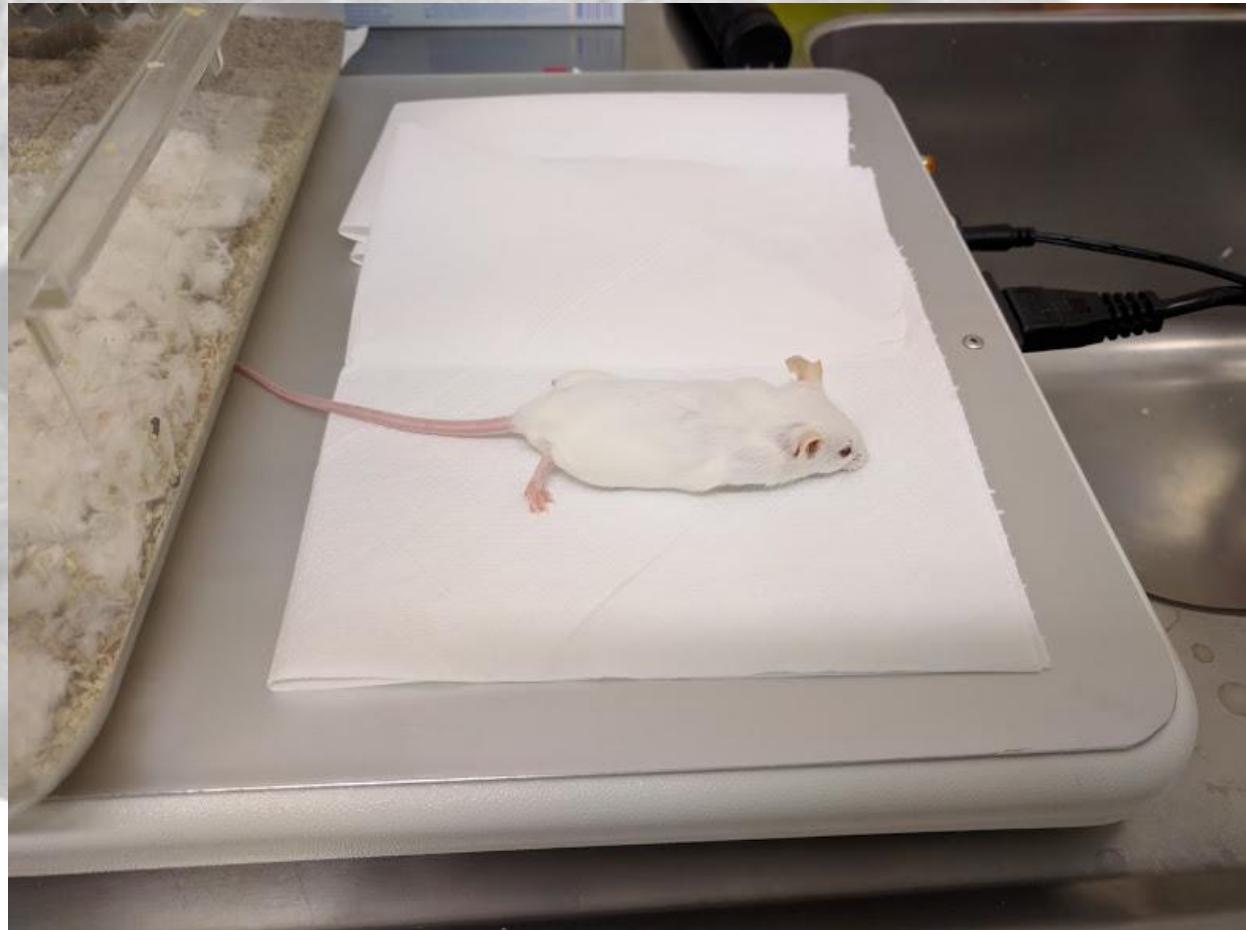
PERIOPERATIVNA NEGA

- 2 minuti po i/p aplikaciji anestetika



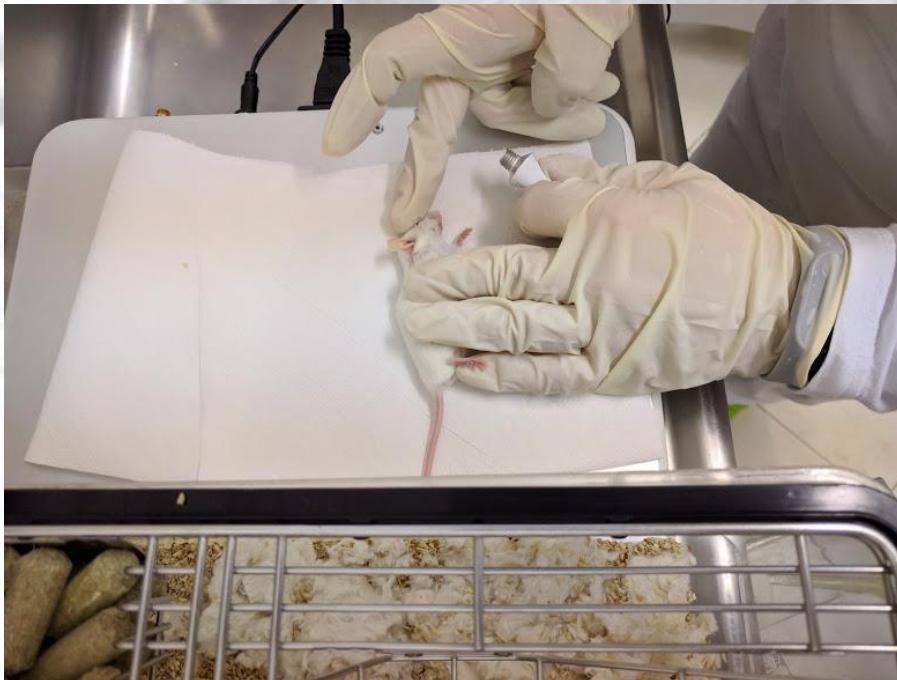
PERIOPERATIVNA NEGA

- 3 minute po aplikaciji anestetika



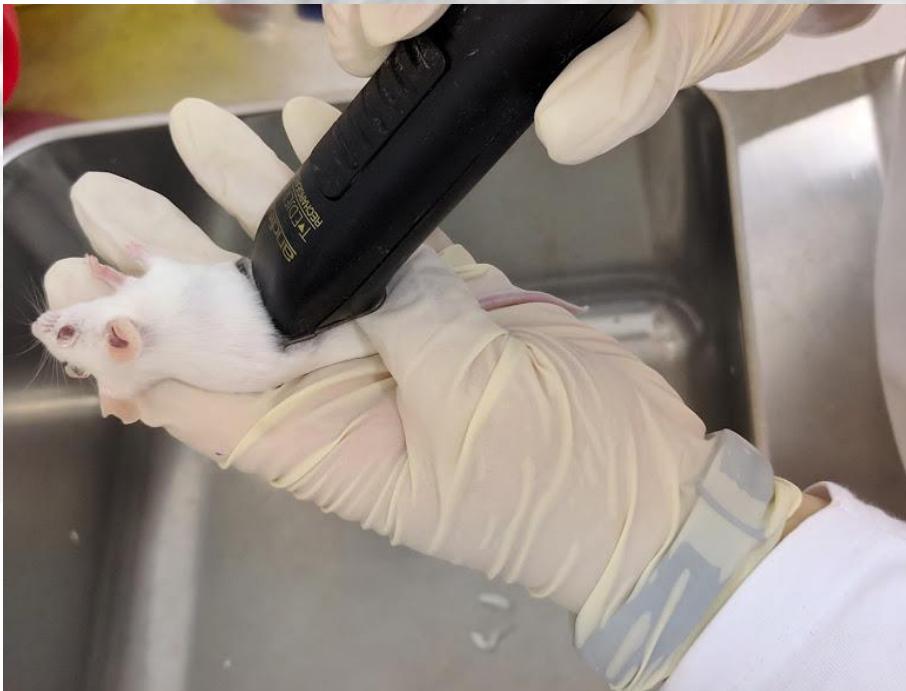
PERIOPERATIVNA NEGA

- Zaščita oči
- Normgel - 0,9 % NaCl v obliki gela



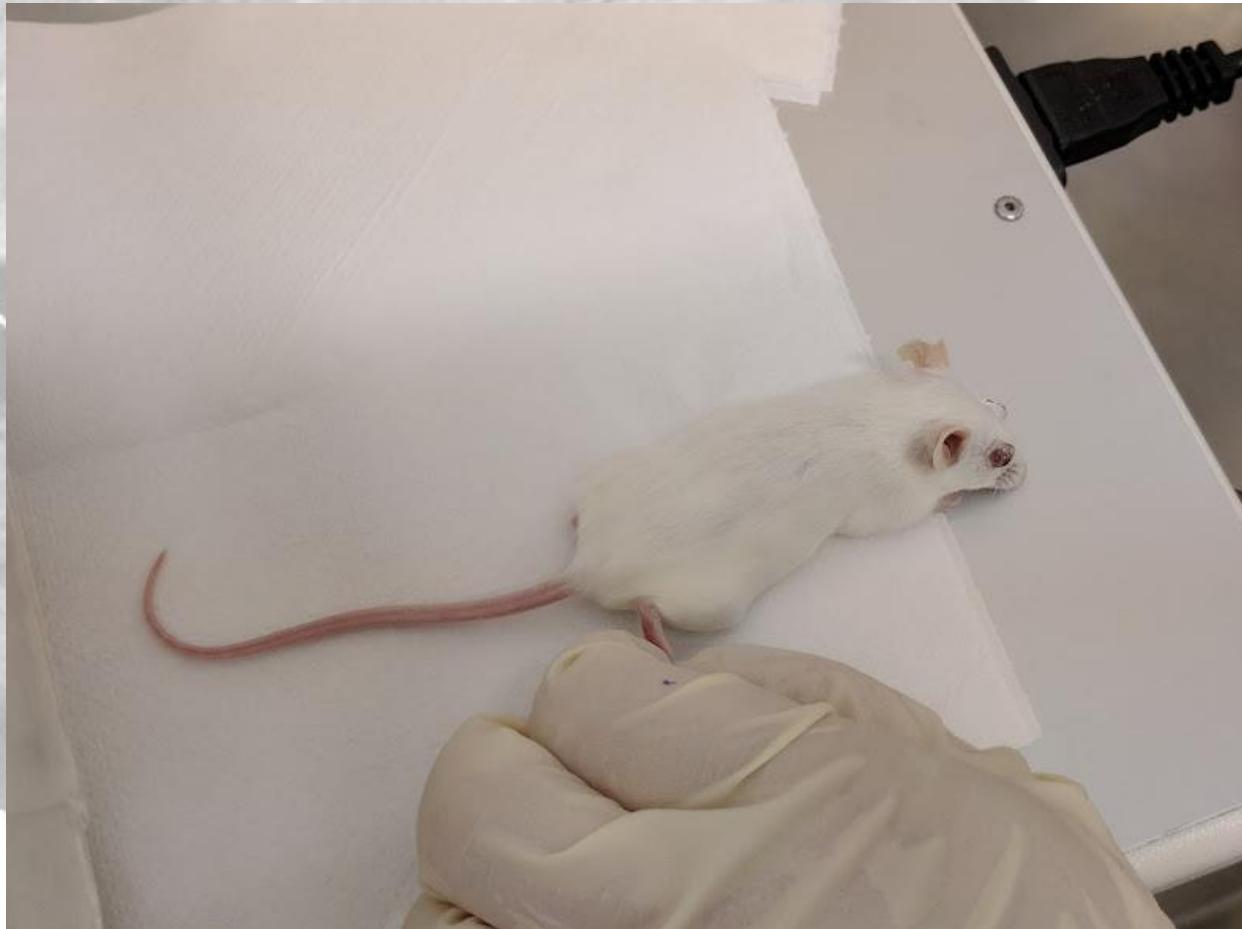
PERIOPERATIVNA NEGA

- Britje - 4 minute po aplikaciji anestetika
- Razkuževanje



MEDOPERATIVNA NEGA

- Odsotnost prstnega refleksa

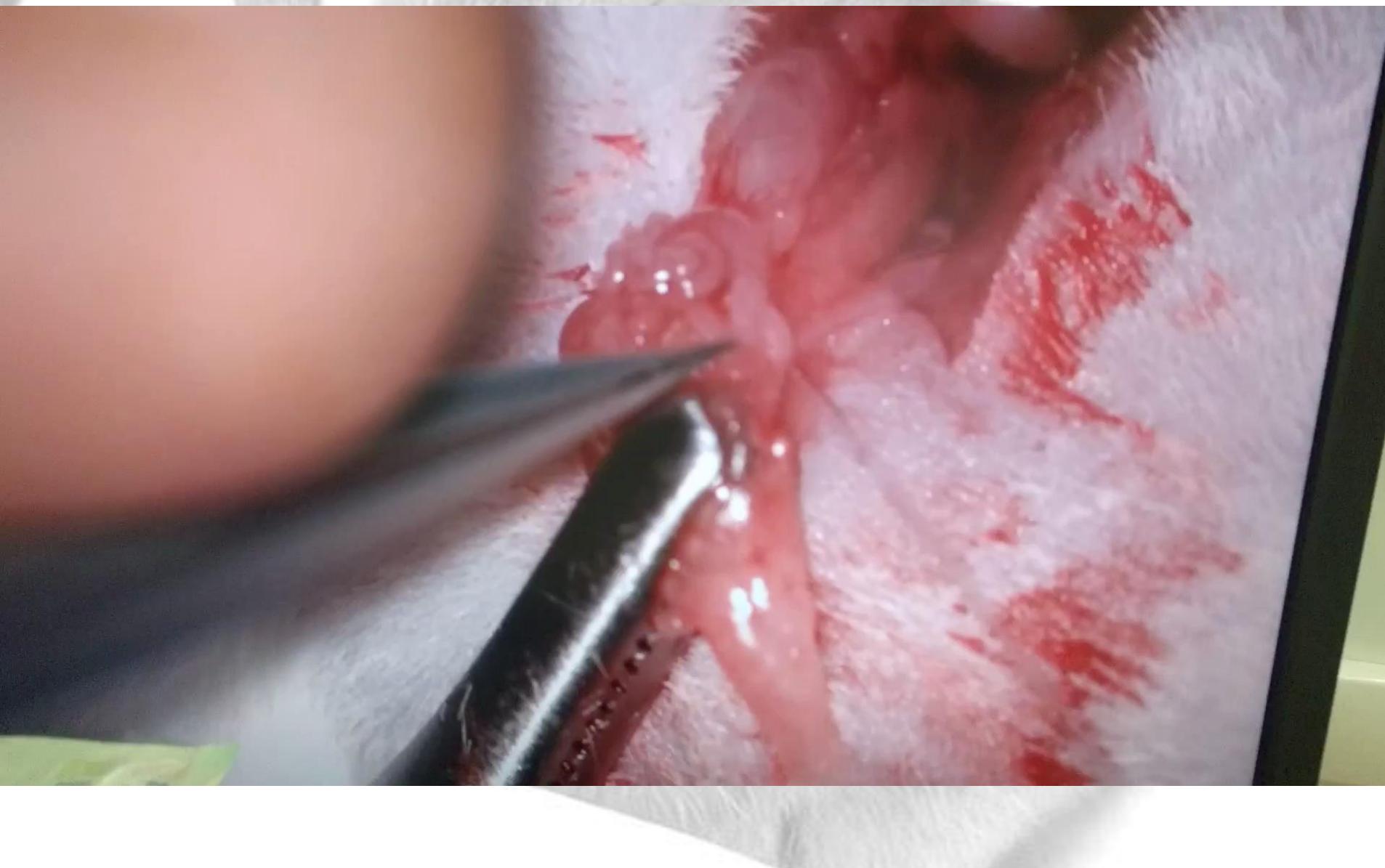


MEDOPERATIVNA NEGA

- Žival pripravljena za poseg
- → 5 minut po aplikaciji anestetika



EMBRIOTRANSFER - 5. dan



POSTOPERATIVNA NEGA

- Ogrevanje na plošči do prebujanja
- Cca 1 ura po aplikaciji
- $T = 39,5 \text{ } ^\circ\text{C}$
- Zaščita oči



POSTOPERATIVNA NEGA

- Ogrevanje v kletki na plošči 3 - 4 ure
- Monitoring



POSTOPERATIVNA NEGA

- Monitoring naslednje dni



POSTOPERATIVNA NEGA

- Obogatitev okolja - Nestlets
- Mouse house (hiška)
- Mezzanine (balkon)



POSTOPERATIVNA NEGA

- Mouse grimase scale
- Ocena
 - bolečine
 - stresa,
 - nelagodja ...

**National Centres
for the Replacement,
Refinement & Reduction
of Animals in Research**

The Mouse Grimace Scale

Research has demonstrated that changes in facial expression provide a means of assessing pain in mice.

The specific facial action units shown below have been used to generate the Mouse Grimace Scale. These action units increase in intensity in response to post-procedural pain and can be used as part of a clinical assessment.

The action units should only be used in *awake* animals. Each animal should be observed for a short period of time to assess scoring brief changes in facial expression that are unrelated to the animal's welfare.

	Not present "0"	Moderately present "1"	Obviously present "2"
Orbital tightening • Closing of the eyelid (narrowing of orbital area) • A wrinkle may be visible around the eye			
Nose bulge • Bulging on the bridge of the nose • Vertical wrinkles on the sides of the nose			
Cheek bulge • Bulging of the cheeks			
Ear position • Ears rotate outwards and/or backwards, away from the face • Ears may fold to form a 'pocket' shape • Space between the ears increases			
Whisker change • Whiskers are either pulled back against the cheek, or pulled forward to 'stand on end' • Whiskers may clasp together • Whiskers lose their natural 'downward' curve			

Read the original paper:
Langford DJ, Bailey AL, Chevalier ML, Choleris E, Cromhead TE, Dolezal S, Glotin S, Ingraham J, Jansson-Rosen T, LaCoste-Pallos M, Mervinayati, Ranga HS, Brackbill RS, Tolosa JA, Wang D, van den Maagdenberg AMJM, Ferrell MD, Collie KG, Argil AG. 2010. Coding of facial expression of pain in the laboratory mouse. *Histochemistry and Cell Biology* 135: 487–498. doi:10.1007/s00412-010-0689-6

For guidance on using the Mouse Grimace Scale, research papers that underpin this technique and for grimace scales in other species, visit www.nc3rs.org.uk/mgscale. To request copies of this poster, please email: enquiries@nc3rs.org.uk. The NC3Rs provides a range of free resources at www.nc3rs.org.uk/resources.

Images kindly provided by Dr Jeffrey Hagg, MRCI University.

KOMPLIKACIJE

- ET - brez post OP zapletov, ni poginov
- Vazektomije - 2 pogina od 12 OP v 2017

UČENJE IZ LASTNIH NAPAK

- Uporaba mazila za oči
- Čas ogrevanja post OP
- Sponk ne odstranjujemo



KASNEJŠA NEGA

- Rokovanje z živalmi za čim manj stresa



KASNEJŠA NEGA

- The National Centre for the Replacement, Refinement & Reduction of Animals in Research (NC3Rs)
- Dve novi metodi
 - tunnel handling
 - cupping



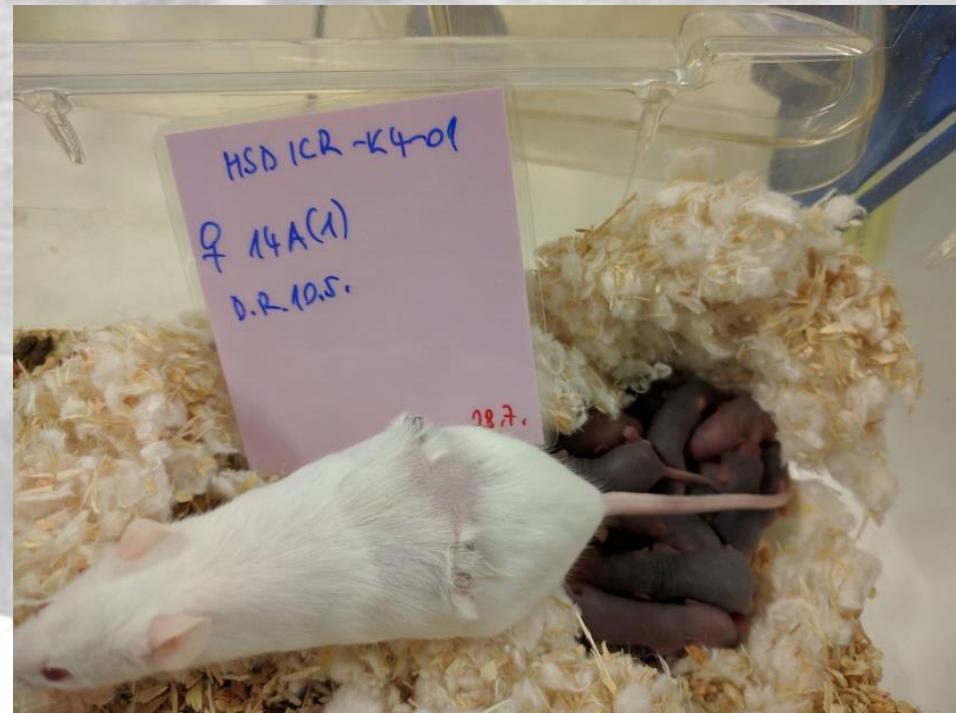
KASNEJŠA NEGA

- Dnevni monitoring (3. dan po OP)
- Naredile so gnezda
- Rane so suhe
- So živahne



USPEHI - ET 2016

- 28 operacij ET, kotilo 19 samic (62 %)
- Skupaj 135 mladičev, povprečno 8,4
Največ mladičev v gnezdu → 14 (tri gnezda)



USPEHI - ET 2017

- 13 operacij ET, kotilo 9 samic (69,2 %)
- Skupaj 72 mladičev, povprečno → 9



USPEHI - ET 2017

- 35% uspešnost → število kotenih mladičev/število vstavljenih zigot
- Največ mladičev v gnezdu → 16



HVALA

