Preclinical Research Center
Ben-Gurion University of the Negev

STANDARD OPERATING PROCEDURE 802

HUMANE INTERVENTION POINTS FOR AGING RODENTS

1. PURPOSE

This Standard Operating Procedure (SOP) describes humane intervention points for aging rodents.

2. RESPONSIBILITY

Principal investigator (PI) and their research staff.

3. CONSIDERATIONS

3.1 Rodents older than 18 months are considered aging animals.

3.2 Aging rodents will develop age-associated clinical manifestations that, although they may make the animal appear ill and cause some discomfort, are not life-threatening. These may include:

- 3.2.1 Skin lesions, alopecia, excessive barbering, ulcerative dermatitis, scarring.
- 3.2.2 Rectal prolapse of varying severity.
- 3.2.3 Ocular lesions include conjunctivitis, blepharitis, keratitis, opacity, retrobulbar masses, etc.
- 3.2.4 Palpable masses, subcutaneous or internal.

3.3 Aging rodents may suffer from a combination of subclinical diseases that can vary in their clinical presentations and should be considered part of the progressive decline in organ function that defines aging. These include, but are not limited to, neoplasia, heart lesions, renal disease, systemic inflammation, and degenerative joint and dental lesions.

3.4 Age-associated decline in physiologic reserve, i.e., the capacity to compensate and adapt to various stressors and loss of function across multiple organ systems, is clinically defined as frailty. Frailty leads to increased vulnerability, the risk for poor health outcomes, and higher mortality risk. The Frailty Index is a non-invasive method that uses clinical signs and observations to quantify frailty. A higher frailty index can be a significant predictor of mortality.

3.5 Furthermore, a distinction can be made between studies where aging animals are to be kept up to a predetermined age (e.g., 24 months) and studies where aging animals are to be maintained as long as possible until the end of their natural life.

3.6 The Ethical Committee in the Animal Protocol must provide justification for rodent colonies until the end of life.

4. PROCEDURES

4.1. Monitoring:

4.1.1. Intensive monitoring in studies involving aging rodents is critical to prevent undue suffering, distress, and loss of experimental data when animals are found dead.

4.1.2. Monitoring is the responsibility of the PI and research staff.

4.1.3. Cages of aging rodents should be labeled with the experimental endpoint (e.g., 24 months) or as near end of life (NEAL).

4.1.4. Animals showing clinical signs are reported to the veterinary care staff, who will determine follow-up actions such as frequency of monitoring and weighing, treatments and supportive therapy, or euthanasia.

4.1.5. Incorporate regular assessment of the Frailty Index as part of the monitoring.

4.1.6. Frequency of monitoring:

4.1.6.1. Daily observation by animal care staff

4.1.6.2. Weekly monitoring by research staff

4.1.6.3. Animals nearing the end of life, as determined by clinical signs, and up to 20% weight loss, be monitored twice per day, every day, including weekends and holidays.

4.2. Supportive Care:

4.2.1. Supportive care is essential in preventing a decline in the functional health of aging rodents and should be provided when clinical signs are noted.

4.2.2. Supportive care may include:

4.2.2.1. Food at the bottom of the cage

4.2.2.2. Water bottles with long sipper tubes to ease drinking

4.2.2.3. Administration of parenteral fluids.

4.3. Recommended intervention points for aging rodents in the near end of life studies:

4.3.1. To prevent unnecessary early euthanasia of animals with non-terminal diseases associated with old age, descriptive criteria for end-of-life and humane euthanasia have been determined. To be considered at the end of life, rodents must appear moribund and demonstrate clinical signs suggesting imminent death within 24 hours. These clinical signs may include:

4.3.1.1. No response to external stimuli

4.3.1.2. Cold body temperature to the touch

4.3.1.3. Slow or labored breathing

4.3.1.4. Hunched posture with a matted coat

4.3.1.5. Failure to eat and drink marked dehydration

4.3.1.6. Incoordination, paralysis

4.3.1.7. Body condition score less than 2

4.3.1.8. Rapid weight loss exceeding 10% reduction of recent bodyweight

4.3.1.9. Pale eyes and extremities (rodents) or mucous membranes

4.3.1.10. Mass that is ulcerated, necrotic, or impairing normal function (e.g., eating, drinking) or exceeding acceptable size endpoints:

4.3.1.10.1. Mice: 1.5cm^{3}or 10% of the baseline body weight

4.3.1.10.2. Rats: 3.5cm^3 or 5% of the baseline body weight

4. FRAILTY INDEX

5.1. Frailty can be quantified by performing a non-invasive clinical observation that evaluates eight organ systems or parameters:

5.1.1. The integument

5.1.2. The musculoskeletal system

5.1.3. The vestibulocochlear/auditory and neurological systems

5.1.4. The ocular and nasal systems

5.1.5. The digestive system

5.1.6. The urogenital system

5.1.7. The respiratory system

5.1.8. Signs of pain or discomfort

5.1.9. Body weight (g)

5.1.10. Body surface temperature (°C).

5.2. Each component is rated using a simple scale:

5.2.1. A score of 0: no sign of a deficit

5.2.2. A score of 0.5: mild deficit

5.2.3. A score of 1: severe deficit

5.3. The Frailty Index, a value between 0 and 1, is calculated by adding the scores for each component and dividing the total by the maximum possible score. When not all components are assessed, the total is divided by the number of components evaluated.

5.4. The veterinarian must be notified when an animal reaches a Frailty Index of 0.75.

5.5. Clinical assessment of Frailty Index in mice:

SYSTEM/PARAMETER	POTENTIAL DEFICITS	SCORING
INTEGUMENT		
Alopecia	Hair loss due to age-related balding and barbering	0 = average fur density 0.5 = < 25% fur loss 1 = > 25% fur loss
Loss of fur color	Change in fur color from back to grey or brown	0 = standard fur color 0.5 = focal color changes 1 = grey/brown fur throughout the body
Dermatitis	Inflammation, overgrooming, barbering, or scratching causing skin erosion/ulceration	0 = absent 0.5 = focal lesions 1 = widespread or multifocal lesions
Loss of whiskers	Loss of vibrissae (whiskers) due to aging and whisker trimming	0 = no loss 0.5 = reduced number of whiskers 1 = absence of whiskers

Coat condition	Ruffled fur and/or matted fur. Ungroomed appearance. The coat does not look smooth, sleek, and shiny	0 = smooth, shiny coat 0.5 = coat slightly ruffled 1 = unkempt, ungroomed, matted coat
PHYSICAL/MUSCULOSKELETAL		
Tumors	Development of visible or palpable tumors or masses anywhere on the body	0 = absent 0.5 = < 1cc 1 = > 1cc
Distended Abdomen	Enlarged abdomen. This may be due to tumor growth, organ enlargement, or intraperitoneal fluid accumulation	0 = absent 0.5 = slight bulge 1 = abdomen distended
Kyphosis	Exaggerated outward curvature of the lower cervical/thoracic vertebral column. Hunched back or posture	0 = absent 0.5 = mild 1 = clear hunched posture
Tail stiffening	The tail appears stiff, even when the animal moves in the cage. The tail does not wrap freely when stroked	0 = no stiffening 0.5 = tail responsive but does not curl 1 = tail completely unresponsive
Gait disorders	Lack of coordination in the movement, including hopping, wobbling, or uncoordinated gait. Wide stance. Circling or weakness	0 = no abnormality 0.5 = abnormal gait, but the animal can walk 1 = impaired ability to move
Tremor	Involuntary shaking at rest or during movement	0 = no tremor 0.5 = slight tremor 1 = marked tremor; animal cannot climb
Forelimb grip strength	A decline in forelimb grip strength. Assessed allowing the animal to grip the bars of the cage and lifting the animal.	0 = sustained grip 0.5 = reduction in grip strength 1 = no grip strength, no resistance
Body Condition Score	Visual signs of muscle wasting or obesity based on the amount of flesh covering bony protuberances	0 = BCS of 3 or 4 0.5 = BCS of 2 or 5 1 = BCS <2
VESTIBULOCOCHLEAR/AUDITORY/NEUROLOGICAL		
Vestibular disturbance	Disruption in the ability to perceive motion and gravity. Reflected in problems with balance, orientation, and acceleration. Seen as head tilt, spinning, circling, head tuck, or trunk curling.	0 = absent 0.5 = mild head tilt, a slight spin 1 = severe disequilibrium
Hearing loss	Failure to respond to sudden sound (e.g., clicker) indicative of hearing loss or impairment	0 = always reacts, 3/3 times 0.5 = reacts 1/3 or 2/3 times 1 = unresponsive, 0/3 times
OCULAR/NASAL		
Cataracts	Clouding of the lens of the eye. An opaque spot in the center of the eye	0 = no cataract 0.5 = small opaque spot 1 = opaque lens
Corneal opacity	Development of white spots on the cornea. Cloudy cornea	0 = normal 0.5 = minimal changes in the cornea 1 = marked clouding/spotting of the cornea
Eye discharge/swelling	Eyes are swollen or bulging (exophthalmia). They may exhibit abnormal secretions and crusting	0 = normal 0.5 = slight swelling and secretions 1 = marked swelling and secretions
Microphthalmia	Eyes are small and sunken. It may involve one or both eyes	0 = normal size 0.5 = one or both eyes slightly small or sunken 1 = one or both eyes are very small

		or sunken
Vision loss	Vision loss, indicated by failure to reach toward the ground when lowered by the tail	0 = reaches >8cm above the surface 0.5 = reaches 2-8cm above the surface 1 = reaches <2cm above the surface
Menace reflex	Rapid eye blink and closure of the palpebral fissure in response to a non-tactile visual treatment to the eye. Measures the integrity of the entire visual pathway including cortical components	0 = always responds 0.5 = no response to 1-2 approaches 1 = no response to 3 approaches
Nasal discharge	Signs of abnormal discharge from the nares	0 = no discharge 0.5 = small amount of discharge 1 = apparent discharge, both nares
DIGESTIVE/UROGENITAL		
Malocclusions	Incisor teeth are uneven or overgrown. Top teeth grow back into the roof of the mouth, or bottom teeth are long and easily seen	0 = mandibular longer than maxillary incisors 0.5 = teeth slightly uneven 1 = teeth very uneven and overgrown
Rectal/vaginal/uterine/penile prolapse	Protrusion of the rectum just below the tail. The vagina or uterus protrudes through the vagina and vulva. The penis cannot reenter the penile sheath	0 = no prolapse 0.5 = mild prolapse 1 = marked prolapse
Diarrhea	Feces on the walls of the home cage. Bedding adheres to feces in the cage. Feces, blood, or bedding around the rectum	0 = normal stools 0.5 = some feces or bedding near the rectum 1 = marked soft stools or bloody stools
RESPIRATORY		
Breathing rate/depth	Difficulty breathing (dyspnea), pulmonary congestion (rales), and rapid breathing (tachypnea)	0 = normal 0.5 = slight change in breathing rate/depth 1 = marked changes in breathing rate/depth
PAIN/DISCOMFORT		
Mouse Grimace Scale	A measure of pain/discomfort based on facial expression. Assessment of five facial features: orbital tightening, nose bulge, cheek bulge, ear position (drawn back), or whisker change (either backward or forward)	0 = no signs present 0.5 = 1-2 signs present 1 = 3 or more signs present
Piloerection	Involuntary bristling of the fur due to sympathetic nervous system activation, particularly on the back of the neck	0 = no piloerection 0.5 = piloerection at the base of the neck only 1 = generalized piloerection
OTHER		
Body Temperature	Increase or decrease body temperature as measured with an infrared thermometer directed at the abdomen (3 measures). Compare with reference values from sex-matched adults.	0 = <1 SD from reference values 0.25 = differs by 1 SD 0.5 = differs by 2 SD 0.75 = differs by 3 SD 1 = differs by >3 SD
Body Weight	Increase or decrease in body weight. Compare with reference values from sex-matched adult animals of	0 = <1 SD from reference values 0.25 = differs by 1 SD 0.5 = differs by 2 SD

	The same background strain.	0.75 = differs by 3 SD 1 = differs by >3 SD

5.1. Clinical assessment of Frailty Index in rats:

SYSTEM/PARAMETER	POTENTIAL DEFICITS	SCORING
INTEGUMENT		
Alopecia	Acquired hair loss due to inflammation, an endocrine disorder, or idiopathic disease	0 = average fur density 0.5 = < 25% fur loss 1 = > 25% fur loss
Dermatitis	Excessive scratching, self-mutilation, or skin conditions leading to open sores on the body	0 = absent 0.5 = focal lesions 1 = widespread or multifocal lesions
Coat condition	Ungroomed appearance: fur appears ruffled and matted	0 = smooth, shiny coat 0.5 = coat slightly ruffled 1 = unkempt, ungroomed, matted appearance
PHYSICAL/MUSCULOSKELETAL		
Tumors	Development of visible or palpable tumors or masses anywhere on the body	0 = absent 0.5 = < 1cc 1 = > 1cc
Distended Abdomen	Enlarged abdomen. This may be due to tumor growth, organ enlargement, or intraperitoneal fluid accumulation	0 = absent 0.5 = slight bulge 1 = abdomen distended
Kyphosis	Exaggerated outward curvature of the lower cervical/thoracic vertebral column. Hunched back or posture	0 = absent 0.5 = mild 1 = clear hunched posture
Gait disorders	Abnormal locomotion: slow movement, lack of coordination, stumbling, falling, or limping	0 = no abnormality 0.5 = abnormal gait, but the animal can walk 1 = impaired ability to move
Tremor	Involuntary shaking at rest or during movement	0 = no tremor 0.5 = slight tremor 1 = marked tremor; animal cannot climb
Body Condition Score	Visual signs of emaciation or obesity. Based on the amount of flesh covering the vertebral column and dorsal pelvis.	0 = BCS of 3 or 4 0.5 = BCS of 2 or 5 1 = BCS <2
VESTIBULOCOCHLEAR/AUDITORY/NEUROLOGICAL		
Vestibular disturbance	Abnormal/asymmetric head position associated with a central nervous system disturbance	0 = absent 0.5 = mild head tilt, a slight spin 1 = severe disequilibrium
Hearing loss	Impaired acoustic startle reflex; associated with loss of hearing sensitivity	0 = always reacts, 3/3 times 0.5 = reacts 1/3 or 2/3 times 1 = unresponsive, 0/3 times
OCULAR/NASAL		
Cataracts	Clouding of the lens of the eye. An opaque spot in the center of the eye	0 = no cataract 0.5 = small opaque spot 1 = opaque lens
Chromo-da-cryorrhea	Porphyrin staining around the eyes/nose	0 = no staining 0.5 = minimal staining around eyes/nose 1 = marked staining around eyes/nose

Exophthalmos	Abnormal protrusion of the eye	0 = normal 0.5 = slight bulging 1 = marked bulging
Corneal opacity	The cornea appears white or clouded	0 = normal 0.5 = minimal changes in the cornea 1 = marked clouding/spotting of the cornea
Microphthalmos	Abnormally small eye. Sunken in appearance.	0 = normal size 0.5 = one/both eyes slightly small or sunken 1 = one or both eyes are very small or sunken
DIGESTIVE/UROGENITAL		
Malocclusions	Abnormal occlusion due to uneven or overgrown incisors	0 = mandibular longer than maxillary incisors 0.5 = teeth slightly uneven 1 = teeth very uneven and overgrown
Rectal/vaginal/uterine/penile prolapse	Protrusion of the rectum just below the tail. The vagina or uterus protrudes through the vagina and vulva. The penis cannot reenter the penile sheath	0 = no prolapse 0.5 = mild prolapse 1 = marked prolapse
Diarrhea	Increased frequency and decreased consistency of bowel movements. Fecal smearing in the cage	0 = normal stools 0.5 = some feces or bedding near the rectum 1 = marked soft stools or bloody stools
Jaundice	Yellowing of the feet, nose, ears, and tail associated with accumulation of bilirubin	0 = normal 0.5 = mild yellowing 1 = marked yellowing
RESPIRATORY		
Breathing rate/depth	Difficulty breathing (dyspnea), pulmonary congestion (rales), and rapid breathing (tachypnea)	0 = normal 0.5 = slight change in breathing rate/depth 1 = marked changes in breathing rate/depth
PAIN/DISCOMFORT		
Rat Grimace Scale	A measure of pain/discomfort based on facial expression. Assessment of five facial features: orbital tightening, nose/cheek flattening, ear position, or whisker change	0 = no signs present 0.5 = 1-2 signs present 1 = 3 or more signs present
Piloerection	Involuntary bristling of the fur due to sympathetic nervous system activation, particularly on the back of the neck	0 = no piloerection 0.5 = piloerection at the base of the neck only 1 = generalized piloerection
Vocalizations	Acute vocalization in response to touch	0 = no vocalizations 0.5 = mild vocalizations 1 = marked vocalizations
OTHER		
Body Temperature	Increase/decrease body temperature as measured with an infrared thermometer directed at the abdomen (3 measures). Compare with reference values from sex-matched adults.	0 = <1 SD from reference values 0.25 = differs by 1 SD 0.5 = differs by 2 SD 0.75 = differs by 3 SD 1 = differs by >3 SD
Body Weight	Increase or decrease in body weight. Compare with reference values from sex-matched adult animals of the same background strain.	0 = <1 SD from reference values 0.25 = differs by 1 SD 0.5 = differs by 2 SD 0.75 = differs by 3 SD 1 = differs by >3 SD

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