Simposio Sexo & Ictus

V Congreso Annual de Ictus-RICORS 2026

Modelos Animales de Ictus y sexo: Cómo llevar a la práctica los criterios STAIR.

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Modelling Preclinical Animal Studies in Stroke

Experimental Design should consider:

- Type of stroke
- Method of stroke induction
- Animal specie & strain
- Sex, age, potential comorbidities
- Reperfusion time, if ischemic
- Anesthesia, analgesia, euthanasia
- Administration route
- Dose and controls
- Sample size
- Neurological assessment
- Efficacy endpoints
- Safety endpoints
- Follow-up period
- Randomization & allocation
- Blinding
- Animal housing, habituation & post-stroke care
- …

Guidelines & Recommendations to consider/follow:

- **STAIR** (Stroke Therapy Academic Industry Roundtable): helping to advance the development of acute stroke therapies NIH-NNDS Initiative.

- **ARRIVE** (Animal Research: Reporting of In Vivo Experiments): full and transparent reporting of research involving animals. NC3Rs initiative.

- **STEPS** (stem cell therapies as an emerging paradigm in stroke): parallel the STAIR committee by documenting recommendation for developing stem cell therapy for stroke.

- **RIGOR:** a set of rigorous preclinical standards designed to prevent translational failures and reduce bias in animal models of stroke. NIH initiative

- **IMPROVE** (Ischaemia Models: Procedural Refinements Of in Vivo Experiments): animal welfare. NC3Rs initiative.

The STAIR CONFERENCES & CRITERIA

STAIR XIII was March 27-28 2025 Click Here for Meeting Results

STAIR XIV Coming March 2027! Stay Tuned for More Information

Click Here to See All STAIR Publications 1999-2025

STAIR® Consensus Conferences

Stroke Treatment Academic Industry Roundtable

First Meeting in 1998

https://www.thestair.com/

Aim: to bring new solutions to stroke treatment field which was precedded by relentless clinical trials failures of promising stroke treatments previously tested in preclinical studies.

Objective: to develop a new approach that would identify barriers impeding successful trials and then establish multi-disciplinary, cross-cutting collaborations of public and private sector experts to share knowledge and achieve consensus on strategies intended to overcome impediments limiting positive stroke research outcomes.

STAIR includes the major "stakeholder" sectors in stroke research:
Academia Industry Government/Policy Makers

Delivery of consensus reports published at Stroke Journal covering different topics/recommendations:

STAIR I- Preclinical Standards (1999)
STAIR II-Clinical Trials Evaluation
STAIR III-Development of Stroke Treatment
…

STAIR I: "Recommendations for Standards Regarding Preclinical Neuroprotective and Restorative Drug Development". Stroke 1999.

The paper addresses the problem that many neuroprotective drugs showed benefit in animals but failed in clinical trials. To improve translation, the authors propose a set of standards and recommendations for preclinical stroke research.

The main recommendations include

Use of appropriate animal models: Standardized, well-described models of focal cerebral. Clear reporting of model, species, strain, and surgical methods.

Include both sexes and age considerations. Emphasis that preclinical studies should consider **sex and age** as biological variables, not just young male animals.

Dose-ranging and timing: Test multiple doses, test treatment at different time points relative to stroke onset. Include both acute and delayed treatment windows to assess translational potential.

Outcome measures: Require both **functional outcomes** (behavioral tests) and **histological outcomes** (infarct volume). Use validated, standardized behavioral tests with clear, predefined endpoints.

Blinding and randomization: Randomize animals to treatment groups. Blind investigators to treatment during outcome assessment,

Replication and independent validation: Results should be replicated in independent laboratories before moving to clinical trials.

Clear reporting: Full description of methods, including anesthesia, temperature control, blood gases, and other physiological parameters that can affect stroke outcomes.

STAIR recommendations on preclinical models-10 Years Update:
"Update of the stroke therapy academic industry roundtable preclinical recommendations". Stroke. 2009.

Reproducibility

The positive results obtained in 1 laboratory need to be replicated in at least 1 independent laboratory before advancing to clinical studies.

Several additional areas are now proposed:

1. The fundamentals of good scientific inquiry should be satisfied by implementing **randomization** and eliminating outcome assessment bias, **defining inclusion/exclusion criteria** a priori, and reporting the reasons for excluding animals from the final data analysis, performing appropriate **power and sample size calculations**, and disclosure of relevant conflicts of interest.

2. After initial studies demonstrate positive effects in younger healthy animals, additional studies in **aged animals and animals with comorbidities** such as hypertension, diabetes, and hypercholesterolemia should be performed if that is the population for clinical trials.

3. Efficacy studies should be performed in both male and female animals.

4. Interaction studies with medications commonly used in stroke patients should be performed for advanced preclinical drug development.

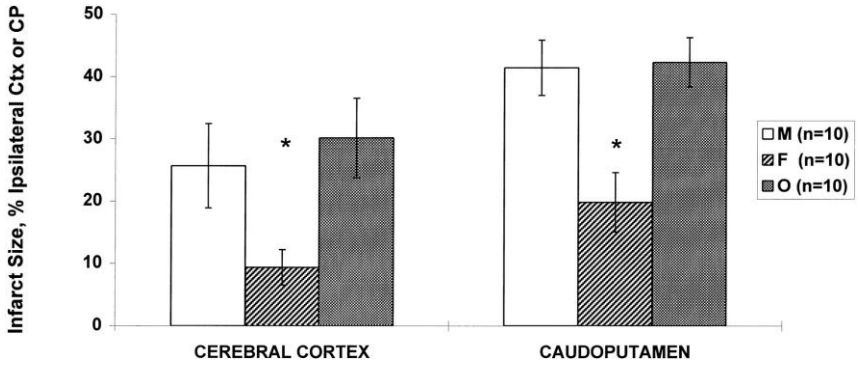
5. Relevant **biomarker endpoints** such as diffusion/perfusion MRI and serum markers of tissue injury should be include.

STAIR Guidelines & Sex Requirements

Equal Inclusion: strongly recommend that both male and female animals are used in preclinical designs.
Evaluating Biological Variables: Researchers must assess whether sex significantly alters the efficacy of a drug or intervention.
Preventing Translational Failure: Evaluating treatments in both sexes accounts for genetic and hormonal differences, which historically contributed to promising animal treatments failing in human trials.

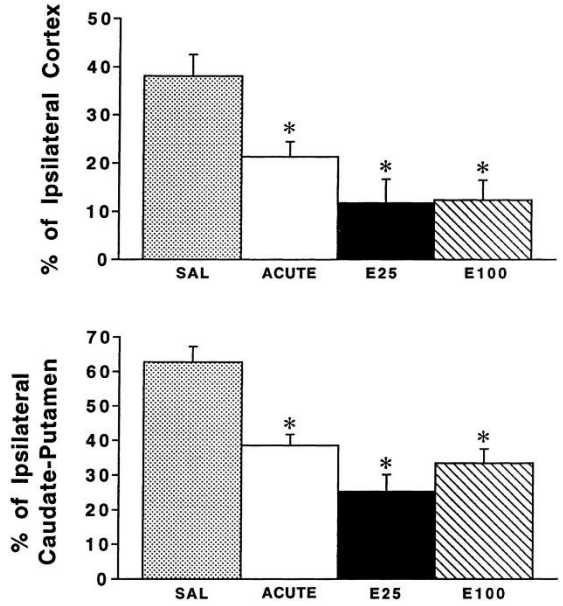
STAIR criterio motivation for using both males & females in preclinical stroke experimental designs specifically mentions the influence of hormones.

- At the same time (late ‘90s), it was proposed that preclinical animal models conconsistently demonstrate that young female rodents experience significantly smaller brain infarcts compared to age-matched males.
- That these disparities were largely attributed to the neuroprotective effects of female sex hormones (mainly estradiol protection at young ages).
- In this regard, **studies in rodents suggest that estrogen has a neuroprotective effect in stroke, with females showing smaller infarcts and better functional recovery. This benefit is lost after ovariectomy, but restored with estrogen supplementation in both sexes, indicating a protective effect.**



Alkayed et al. 1998. Plasma estradiol concentrations: 7.36±1.11 (F), 3.95±1.46 (M), and 4.11±1.58 pg/mL (O), and plasma progesterone concentrations were 27±2.8 (F), 5.9±0.8 (M), and 16±3.3 ng/mL (O groups).

Young rats, proximal MCAO (filament)



In males

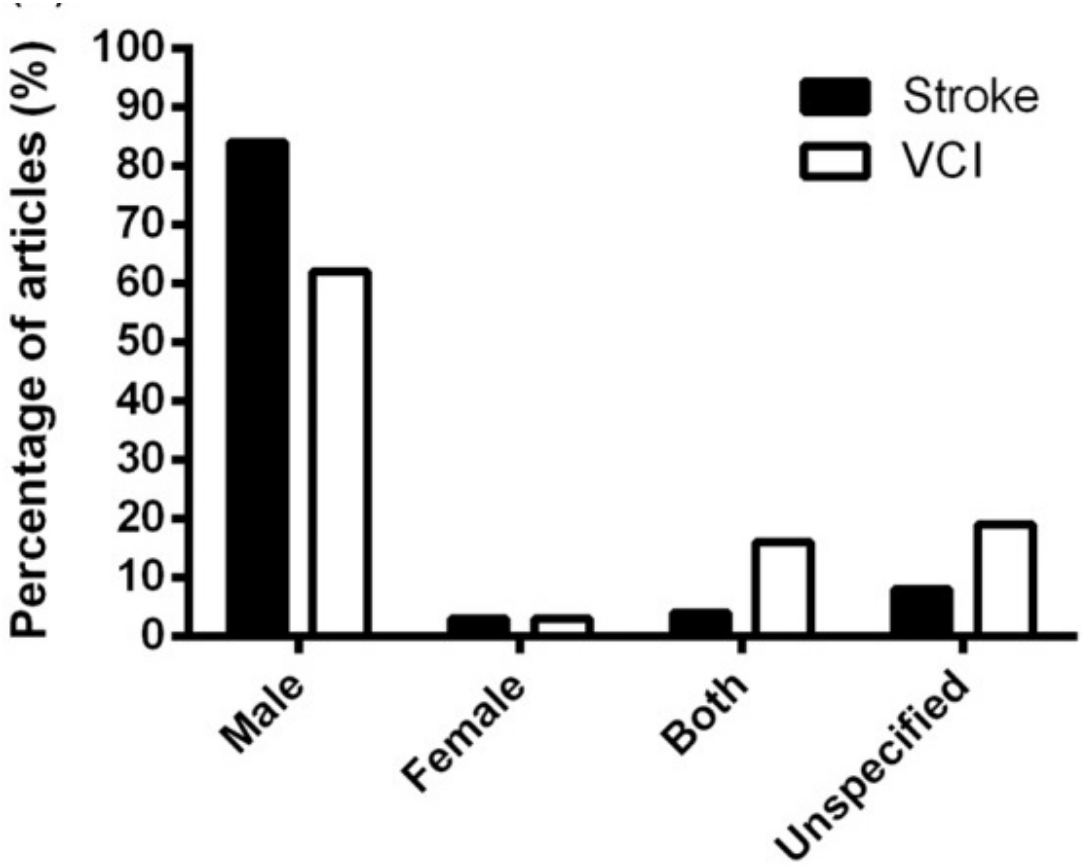
Toung et al. 1998

These observations lead to the massive use of young male animals in preclincial stroke studies considering potential differences in stroke injury and outcome in control conditions if using both sexes.

Reality on the use of both male & female animals in preclinical stroke research

Variability of functional outcome measures used in animal models of stroke and vascular cognitive impairment – a review of contemporary studies. J Cereb Blood Flow Metab. 2018.

Systematic Review: the search was limited to original papers published January 2005–December 2015 **limited to 14 journals, representing a broad field of translational cerebrovascular research and based on impact factor, reputation within the field**.
Stroke n=636; VCI n=37.



For stroke papers: 84% used males, 3% females, 4% both male and female and 8% sex unspecified.

For VCI studies: 62% sued males, 3% females, 6% both male and female and 19% did not specify sex.

Use of Females or both sexes in <10% studies

Recent PUBMED Search (not a proper Systematic Review):

(stroke OR ischemic stroke OR ischaemic stroke OR cerebral ischemia OR cerebral ischaemia OR hemorrahge OR hemorrhagic stroke OR haemorrhagic stroke OR haemorrhage) AND (animal model OR animal models OR experimental stroke OR preclinical OR rodent OR rodents OR mouse OR mice OR rat OR rats) AND ("**2020/01/01**"[Date - Publication] : "2026/05/31"[Date - Publication])

(stroke OR ischemic stroke OR ischaemic stroke OR cerebral ischemia OR cerebral ischaemia OR hemorrahge OR hemorrhagic stroke OR haemorrhagic stroke OR haemorrhage) AND (animal model OR animal models OR experimental stroke OR preclinical OR rodent OR rodents OR mouse OR mice OR rat OR rats) AND ("**2020/01/01**"[Date - Publication] : "2026/05/31"[Date - Publication]) **AND (female)**

Total publicacions **since 2010**: 540K AND “female”: 136K **(25%)**
Total publicacions **since 2020**: 214K AND “female”: 47K **(22%)**

AND (neuroprotection OR neuroprotectant) AND (female)

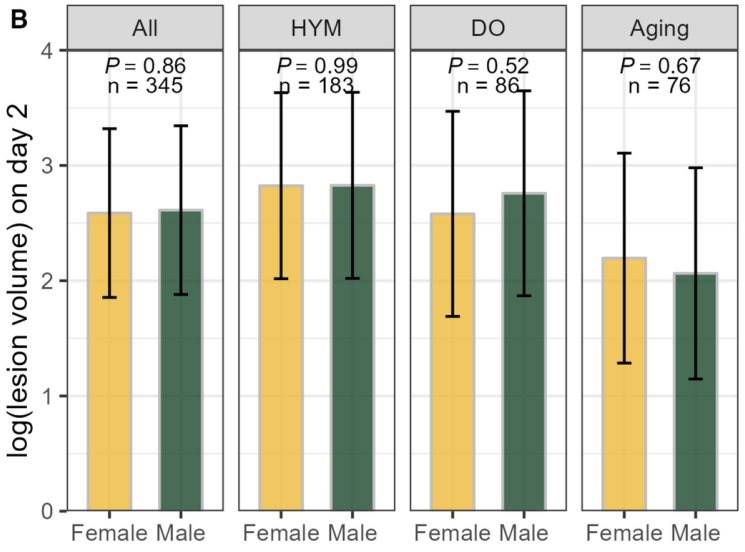
Total publicacions **since 2010**: 25K AND “neuroprotection” AND “female”: 2,9K **(11%)**
Total publicacions **since 2020**: 11K AND “neuroprotection” AND “female”: 984 **(9%)**

Recent data from the large multi-center SPAN study suggests no differences in brain injury and neurological outcome between sexes at short/long term and without the presence of comorbid conditions (lesion size, brain atrophy and corner test).

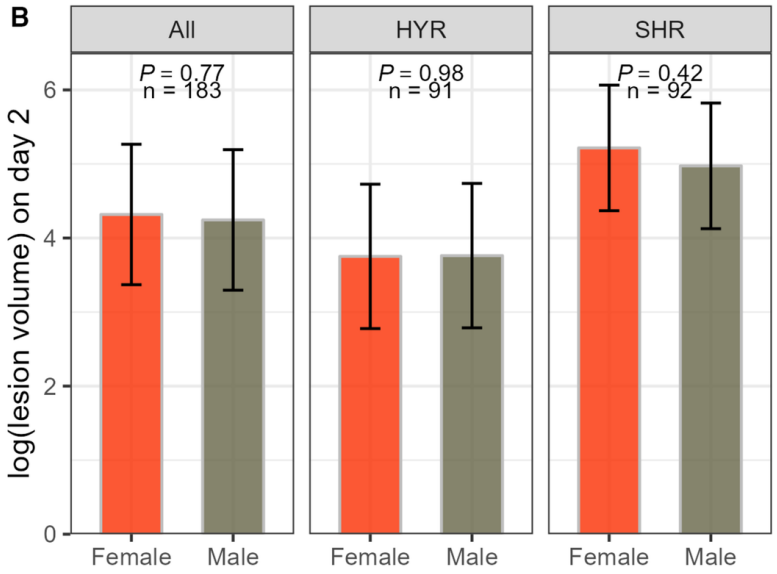
The Stroke Preclinical Assessment Network: Rationale, Design, Feasibility, and Stage 1 Results. Stroke. 2022.
Exploring Sex Differences in Stroke Outcomes: A Comprehensive Analysis From the SPAN 1 Trial. J Am Heart Assoc. 2026

All SPAN Labs: Filament MCAo in young male and female C57BL6/J, 60 min occlusion and MRI-imaging at 48h and 30 days. The size of the Doccol suture followed the manufacturer's recommendation on the basis of mice weight.

ANALYSIS: large cohort from SPAN trial including mice & rats that served as saline-treated stroke controls.



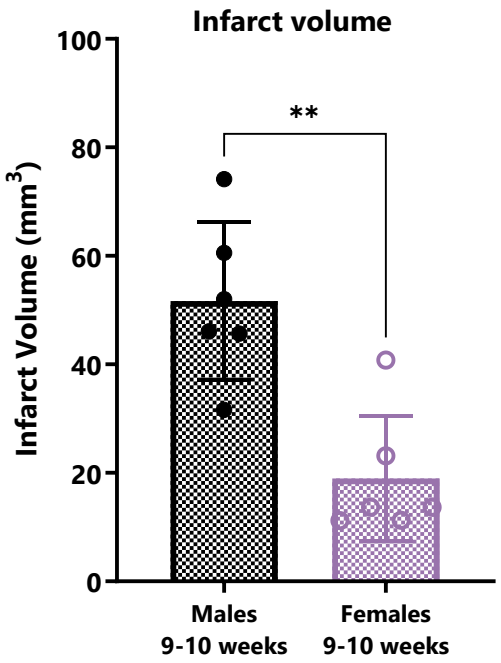
No differences on infarct lesion



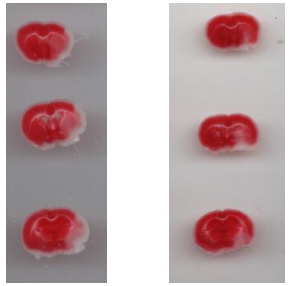
Only, sex differences were seen in the spontaneously hypertensive rats cohort where female rats exhibited a higher corner test index on day 30 than males, indicating more severe sensorimotor injury.

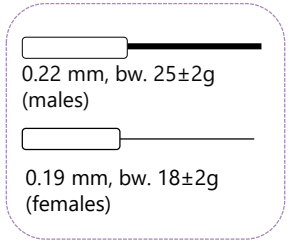
VHIR DATA (unpublished)- Transient proximal model, 75 minutes MCA Occlusion with docool filament

The same filament for both sexes

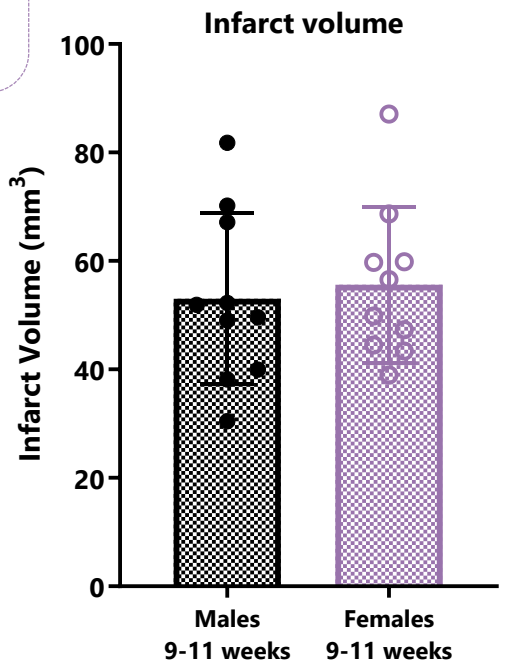


Unpaired t-test, p=0.0015



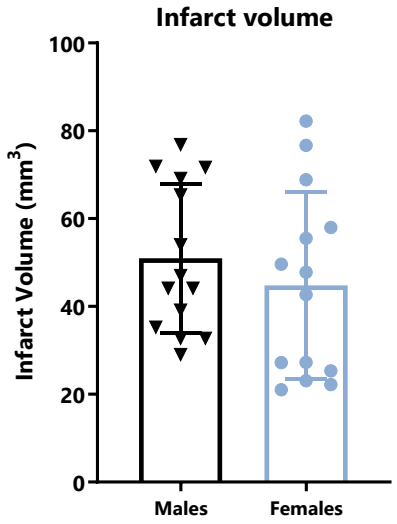


After filament adjusted to weight



Unpaired t-test, p=0.71

Confirmation in other studies conducted 1 year later (Control group)



Unpaired t-test, p=0.41

Also, no differences in inrfact extension in permanent models: distal MCA electrocoagulation and Collagenase-induced ICH.

Sesión de Póster
Viernes, 12 de junio de 2026 - 11:30 – 13:30 h.
MARTA KLIMCZAK. OBJETIVO 3.
Sex Comparability in Permanent and Transient Mouse Stroke Models: Strategies for Optimization and Limitations.

Conclusions

- Strong recommendations for almost 3 decades in conducting animal stroke models in both male & females (specially in drug-efficacy-studies).
- Less than 10% of the published studies consider both sexes.
- Multiples studies show reduced infarcts in females attributed to hormone protection.
- However, recent large multi-center studies in rodents do not show differences between sexes on lesion/outcome at short-long-term.
- Time to start using both males and females?

Neurovascular Research Lab-VHIR

Marta Klimczak, Kerrie Adrian, Anna Penalba