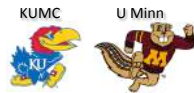


A SINGLE SUBCUTANEOUS DOSE OF KU-AS-272* ELICITS COMPLETE LOSS OF SPERMATOGENIC SUPPORT IN ADULT MALE RATS

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*US Patents: 7,514,463; 8,377,958; and others are pending

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Outline

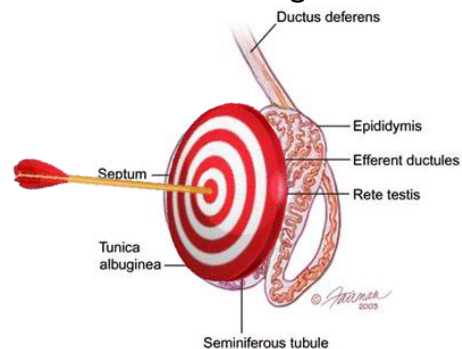
- KU-AS-272 mechanism of action
- Single subcutaneous dose finding in adult male rats
 - Study design
 - Side effects
 - Testicular effects
 - Hormone effects
 - Epididymal clearance
- Ongoing studies
- Conclusions
- Acknowledgements

2

KU-AS-272 Mechanism of Action

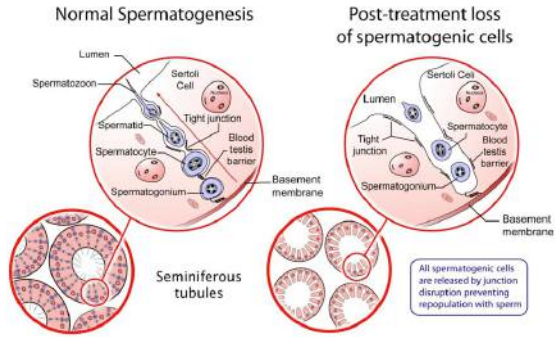
3

A Non-hormonal Approach to Sterilization: Target the Testis

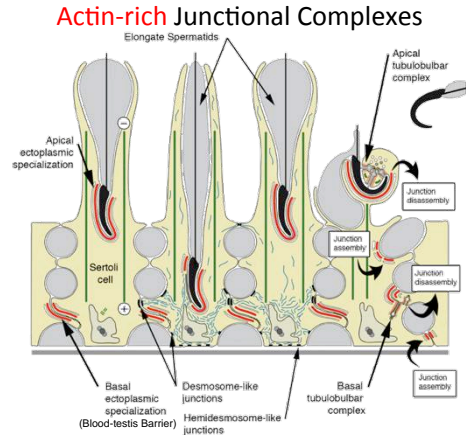


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Male Sterilization by Disruption of Sertoli cell-Spermatogenic cell Junctions



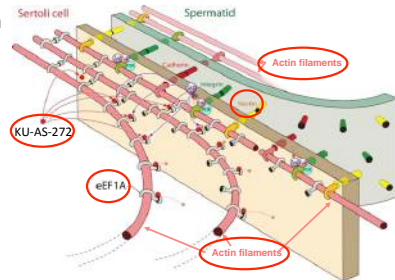
Maintenance of Spermatogenesis Requires Actin-rich Junctional Complexes



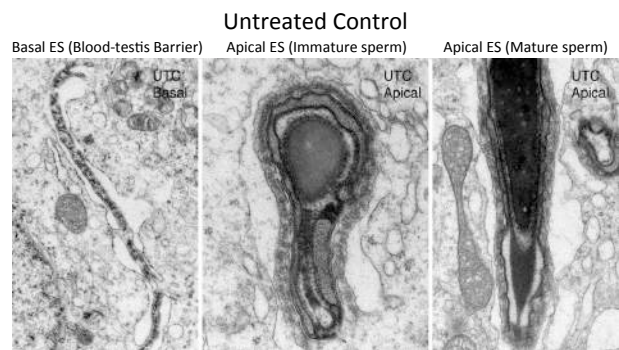
KU-AS-272 Mechanism of Action: Unbundling of Actin that Stabilizes apical ES and BTB

F-actin filaments which form a vital structural component of the aES are bundled by eEF1A-1.

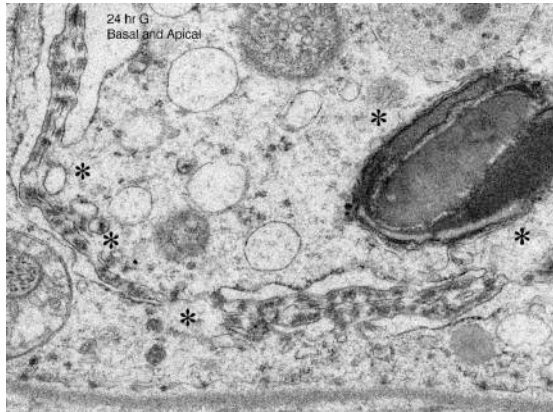
KU-AS-272 binds to eEF1A-1 causing unbundling and instability of the apical and basal ES, causing release of spermatogenic cells.



Electron Microscopy of Junctional Targets of KU-AS-272 in Testis



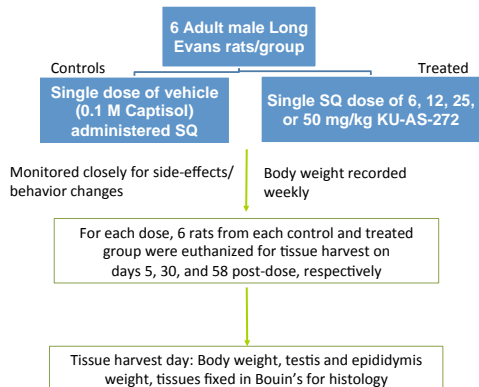
Rapid KU-AS-272 Elicited Loss of Actin



Single SQ Dose Finding Study Design

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Efficacy and Safety Methodology



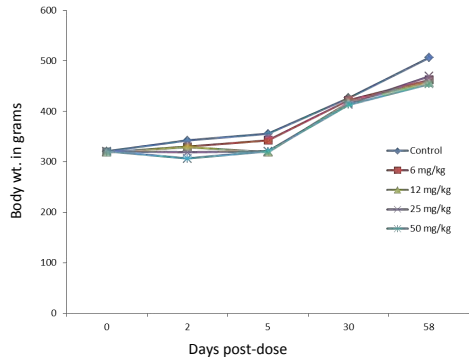
11

No Side Effects at 6 and 25 mg/kg, Minor Transient Effects Observed at 25 and 50 mg/kg

- Vehicle control, **6mg/kg** and **12 mg/kg** KU-AS-272 appeared normal after dosing.
- **25 mg/kg**: slight lethargy onset at 20 min ~12 hrs, total recovery by 24 hr.
- **50 mg/kg**: slight lethargy and wobbly onset within 15-20 min appeared. By next morning, all rats showed good mobility, though slight tremor and imbalance was visible. On day 2 all 50 mg/kg treated rat behavior was comparable to control.

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Body Weight Was Not Significantly Different Between Control and All Treatment Groups

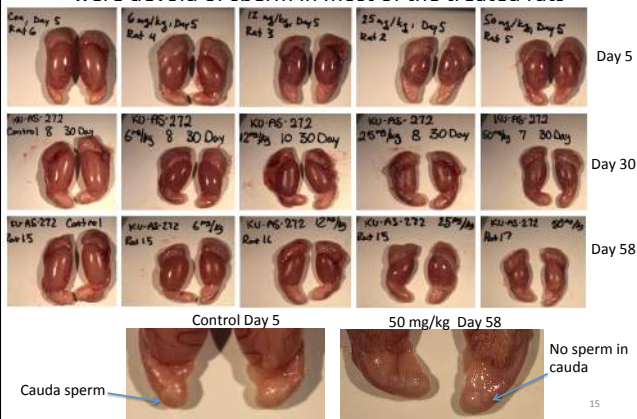


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Testicular Effects

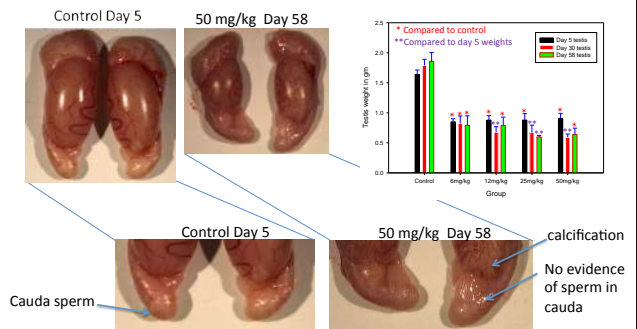
14

Testis were smaller in all treated rats and cauda epididymis were devoid of sperm in most of the treated rats



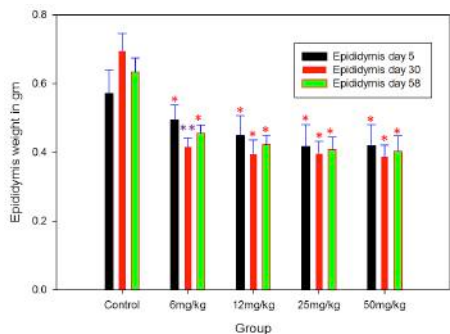
15

Testis Were Smaller In All Treated Rats And Cauda Epididymis Were Vacating Sperm (all pictures at same magnification)



16

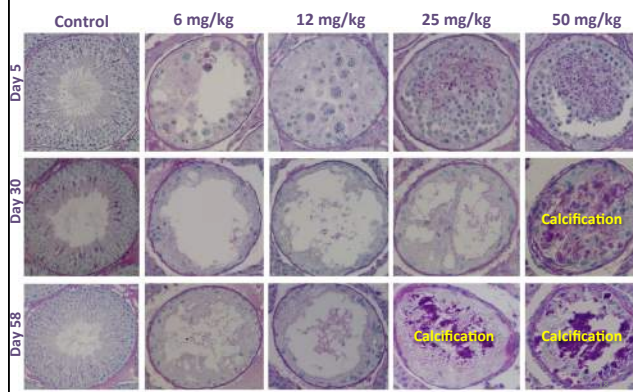
Significant Drop In Epididymal Weight Was Seen At All Doses of KU-AS-272 At All Time Points (P*/**=0.05)



* Compared to control
** Compared to day 5 weights

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Remarkable Efficacy at All Doses By Day 30 and Continued at Day 58 Post Dose



Spermatogenic Index At Day 5, 30 And 58 Post-Dose Indicated Irreversible Spermatogenic Arrest

- Based on Whitsett et al. (1984)
- 6 All cell types present and >75 late spermatids per tubule
 - 5 All cell types present and 50-75 late spermatids per tubule
 - 4 Spermatogonia, spermatocytes, and round spermatids present, and up to 25 late spermatids per tubule
 - 3 Spermatogonia, spermatocytes, and round spermatids present with less than 25 late spermatids per tubule
 - 2 Spermatogonia and spermatocytes present
 - 1 Only spermatogonia present
- Note: Categories based on observed KU-AS-272 effects
- 0 Sertoli cell only
 - 1 No Sertoli cells

Day 30

Group	SI	T-test
Control	5.46 ± 0.10	
6 mg/kg	0.00 ± 0.00	1.6E-17
12 mg/kg	0.00 ± 0.00	1.6E-17
25 mg/kg*	0.00 ± 0.00	1.6E-17
50 mg/kg	0.00 ± 0.00	1.6E-17

*n=5 for 25 mg/kg, one outlier had a partial response, but all animals at this dose had no spermatogenic cells at this dose at day 58

Day 5

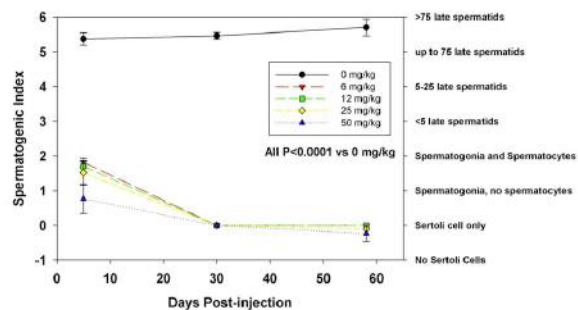
Group	SI	T-test
Control	5.37 ± 0.18	
6 mg/kg	1.82 ± 0.12	1.7E-12
12 mg/kg	1.70 ± 0.15	3.4E-12
25 mg/kg	1.52 ± 0.35	3.4E-10
50 mg/kg	0.77 ± 0.42	2.5E-10

Day 58

Group	SI	T-test
Control	5.70 ± 0.24	
6 mg/kg	0.00 ± 0.00	6.70E-14
12 mg/kg	0.00 ± 0.00	6.70E-14
25 mg/kg	-0.10 ± 0.00	7.76E-14
50 mg/kg	-0.23 ± 0.23	1.10E-12

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KU-AS-272 Has Very Rapid Effect After Single SQ Dose



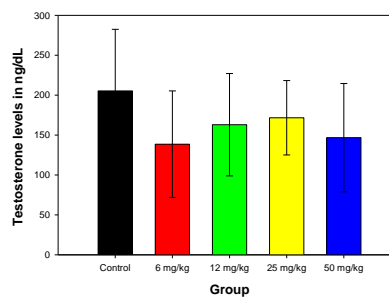
All P<0.0001 vs 0 mg/kg

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Hormone Effects

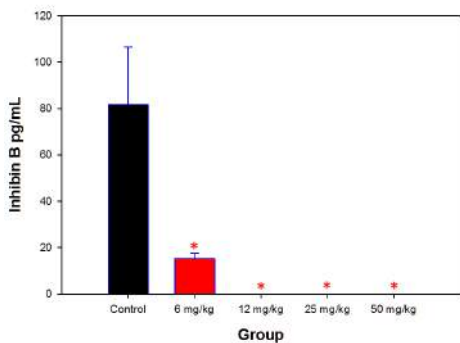
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Serum Testosterone Levels Measured at Day 58 Show "Normal" Levels



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Serum Inhibin B Levels Were Undetectable at All Doses Above 6 mg/kg at Day 58

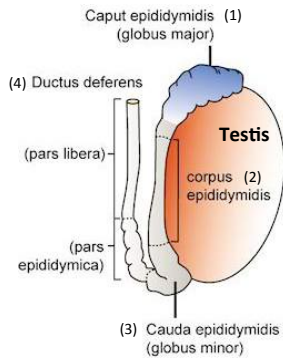


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Epididymal Clearance

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KU-AS-272 Elicits Complete Loss of All Spermatogenic Cells In Adult Male Rats

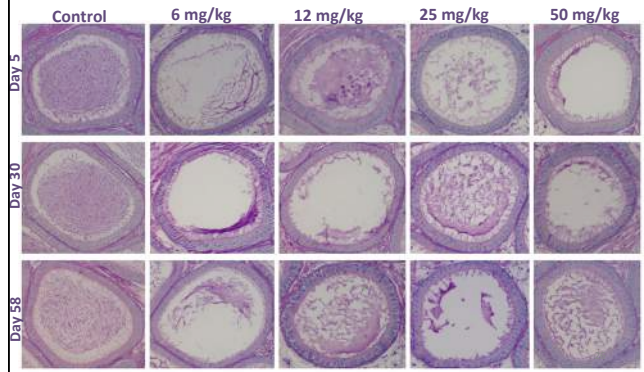


Testicular sperm must transit and mature through the ducts of the epididymis and enter the ductus deferens before they are capable of being ejaculated.

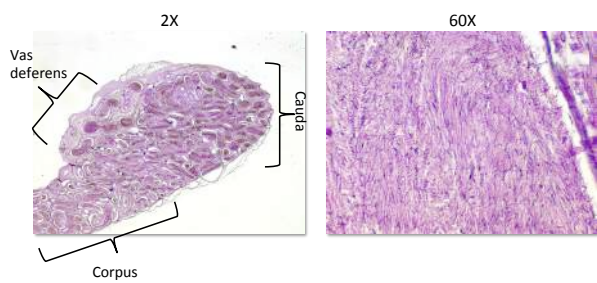
In the normal male this take 10-14 days, depending on species.

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Sperm Were Absent in the Caput Epididymis Ducts at All Doses Above 6 mg/kg at All Time Points

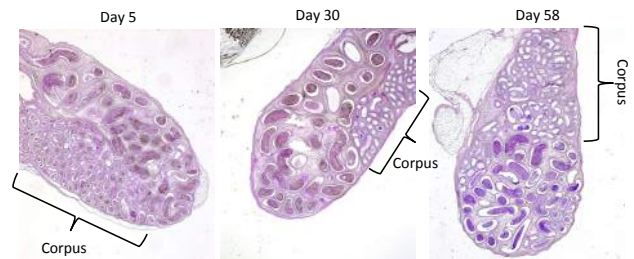


Normal Cauda Epididymal Ducts are Densely Packed with Mature Sperm



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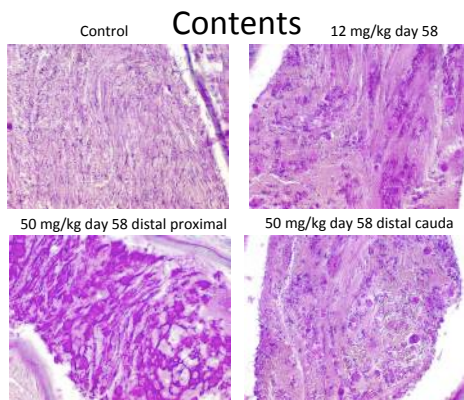
Epididymal Ducts Clear of Sperm after KU-AS-272 (12 mg/kg)



Time-dependent clearing of epididymal ducts is occurring, but slower than expected.

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Transition of Epididymal Duct



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Ongoing and Future Directions

- Are the retained epididymal sperm “fertile” even the testis is no longer producing flow
- Other active analogues have been identified
- Target species:
 - Complete adult male dog study
 - Other target species

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Conclusions

- Single SQ dose of KU-AS-272 at 12 mg/kg and higher achieved the desired total loss of all spermatogenic cells 58 days post single dose
- Testis histopathology suggests that these animals are sterile after a single SQ dose
- No adverse side effects at 6-12 mg/kg. Minor transient side-effects at 25-50 mg/kg
- There were no significant changes in body weight gain at any doses

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Conclusions – contd.

- Day 58 data identified no significant changes in circulating testosterone at all doses.
- Serum Inhibin B levels measured at day 58 were undetectable at all doses above 6 mg/kg, suggesting that sterilizing doses were achieved.
- Clearance of epididymal sperm appears to be slower than normal following “shutdown” of testis

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For Addition Details About KU-AS-272

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