Male Reproduction

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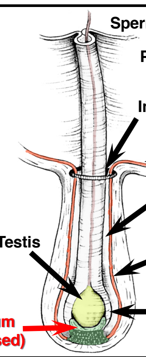
Continued regression of Gubernaculum

Testis pulled deeper into Scrotum

Vaginal Process attaches to Scrotum

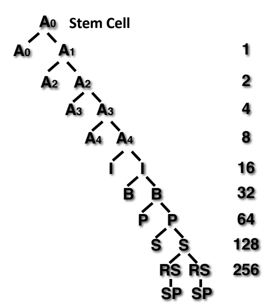
Space between Visceral and Parietal T.V. is continuous with Peritoneum



Testicular Descent

- Cryptorchid - failure of descent
- Controlling mechanisms
  - Androgen dependent
  - DHT supported
  - Estrogen inhibits
- Gene Expression
  - INSL3 - insulin like growth factor 3
    - From leydig cells
  - Great/LGR8 - receptor for INSL3
    - In gubernaculum

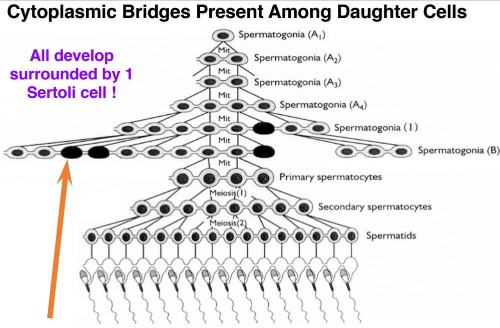
Potential Production of Germ Cells in Boar



França LR, Avelar GF, Almeida FFL. 2005. Theriogenology 63:300–318.

Cytoplasmic Bridges Present Among Daughter Cells

All develop surrounded by 1 Sertoli cell !



Degenerating Spermatogonia

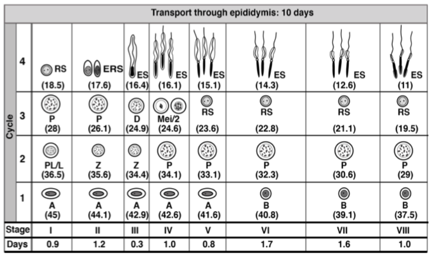
Losses During Spermatogenesis

Citation	Round Spermatids/ Sertoli Cell	% Loss
Theoretical	256	0
Franca et al., 2005	68	73.4%
Costa et al., 2013	21.5	91.6%
Parrish unpublished	30.4	88.1%

Most losses (approx. 90%) occur during Mitotic processes

- A, I, B or Spermatogonia or Primary Spermatocyte

Boar Spermatogenesis

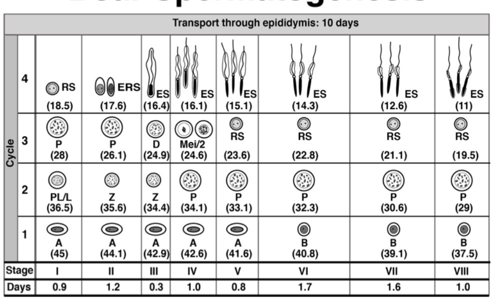


Durations and description of stages were from Swierstra (1968); specific cellular associations are from Frankenhuis et al. (1982) and Costa et al. (2013). 2, secondary spermatocyte; A, spermatogonium type A; B, spermatogonium type B; D, diplotene primary spermatocyte; ERS, elongating round spermatid; ES, elongating spermatid; L, leptotene primary spermatocyte; Z, zygotene primary spermatocyte; P, pachytene primary spermatocyte; PL, preleptotene primary spermatocyte; RS, round spermatid; ( ), estimated days to ejaculation (assuming an epididymal transit time of 10 days).

Stages

- Specific cellular associations within a small segment of a seminiferous tubule
- stages are not the same length in time

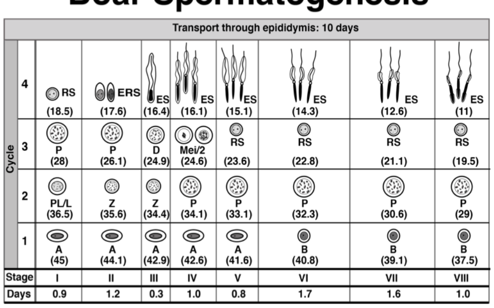
Boar Spermatogenesis



Cycle

- progression through sequence of all stages
- Approx. 4 - 4.5 cycles to form spermatozoa
  - ➢some species variation

Boar Spermatogenesis



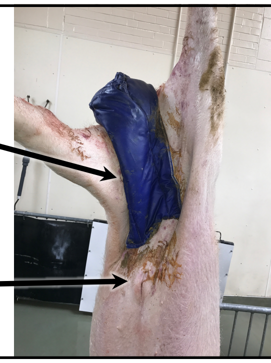
Duration of Spermatogenesis

	Bull	Ram	Boar	Stallion	Man
cycle (days)	13.5	10.4	8.5	12.2	16
Spermatogenesis	61	47	34	57	75

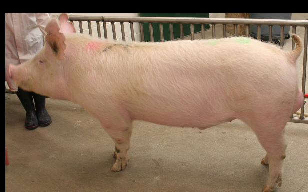
For Ejaculation - Add 9 – 11 days for epididymal transit

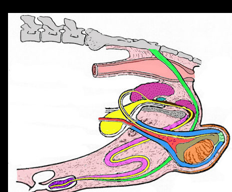
Insulator covers vascular cone of pampiniform plexus.

Prepuce



Boar Anatomy

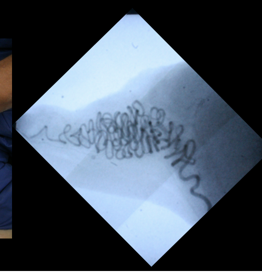




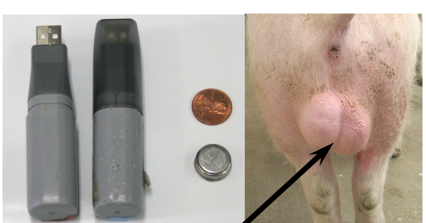
Pampiniform Plexus

Ultrasound

Angiography



Temperature Monitors



Location of Temperature Monitor on/in Scrotum

Effect of Insulation on Scrotal Temperatures

	Control[b]	Full Insulation
Surface Measurement[a]	32.1 ± 0.4°C N=5	34.0 ± 0.3°C N=5
Below Scrotal Skin[a]	34.9 ± 0.3°C N=6	38.0 ± 0.1°C N=3

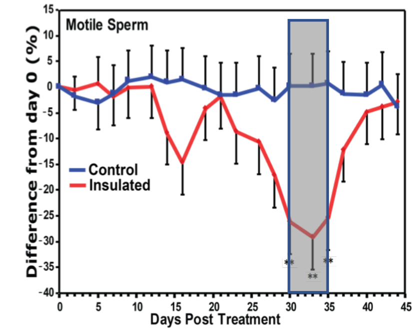
[a]Surface measurement with old style USB device; Below skin with new iButton device

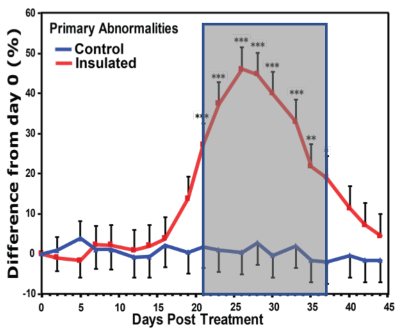
[b]Control for surface used sham sack with no insulation; Control for below skin had not sack present.

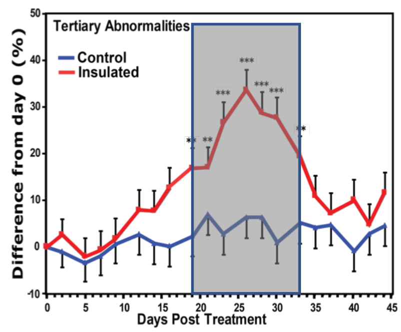
Core body temp = 38.5°C

Experimental Procedures

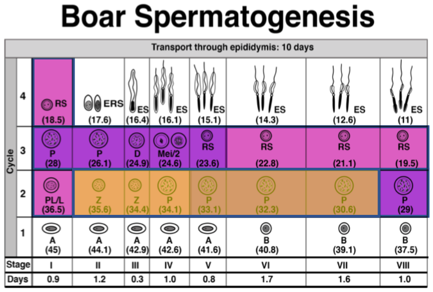
- 5 control (sham), 5 insulated boars
- 48 hr insulation
- Semen collected MWF 2 weeks pre- through 6 weeks post-insulation
- Semen evaluation
  - Motility
  - Morphology
  - Sperm Nuclear Shape (Fourier Harmonic Analysis)



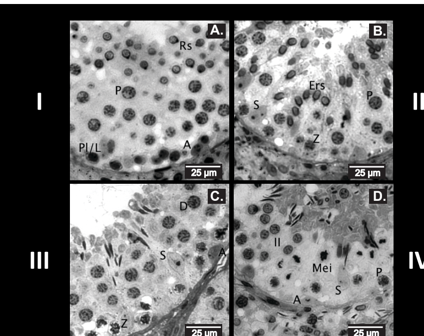


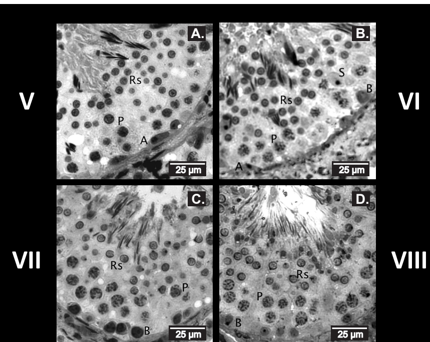


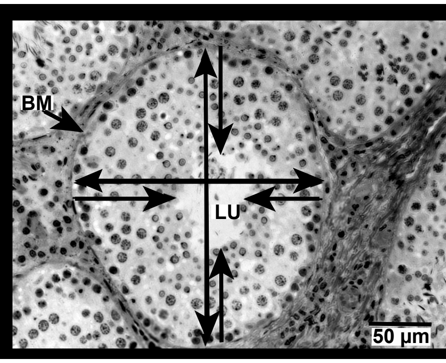
Boar Spermatogenesis



Motility

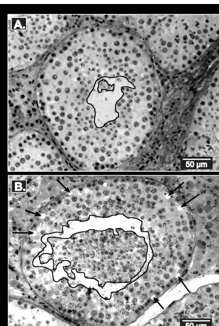






Control

Insulated



The effect of scrotal insulation on scrotal and testicular parameters.[a]

Measurement	Control	Insulated	Pooled SE
Scrotal temperature (°C)	31.1	33.9*	0.6
Height of seminal epithelium (µm)	64.4	55.1*	1.22
Amount of debris in the lumen (%)	26.0	45.5*	2.8

[a]Data are the mean ± sem for 5 control (sham insulated) and 5 insulated boars.

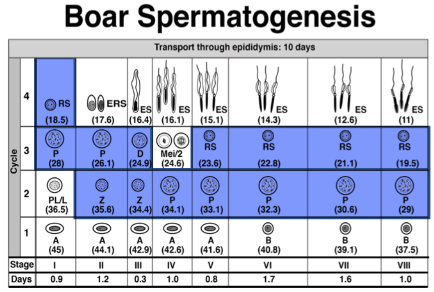
* Indicates a difference within the row, p< 0.05.

The effect of scrotal insulation on cells within the seminiferous tubules.[a]

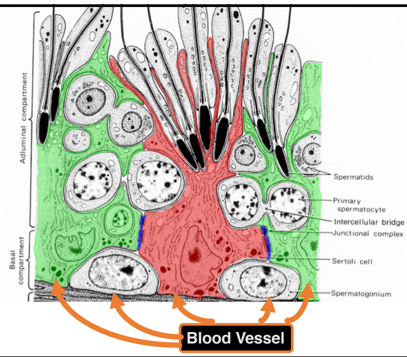
Cell Type	Control	Insulated	Pooled SE
Sertoli cells	0.9	1.0[nd]	0.1
A spermatogonia	2.2	1.9[nd]	0.1
B spermatogonia	5.7	5.7[nd]	0.4
Prelep./lep. 1° spermatocytes	11.2	9.0[nd]	0.8
Zygotene 1° spermatocytes	13.2	10.7 *	0.5
Pachytene 1° spermatocytes	13.6	10.4 *	0.4
Diplotene 1° spermatocytes	14.2	10.6 *	0.9
Meiotic bodies	5.2	4.8[nd]	0.7
2° spermatocytes	14.3	10.3[nd]	1.7
Round spermatids	33.0	28.0 *	1.4
Elongating round spermatids	29.1	22.3[nd]	2.7

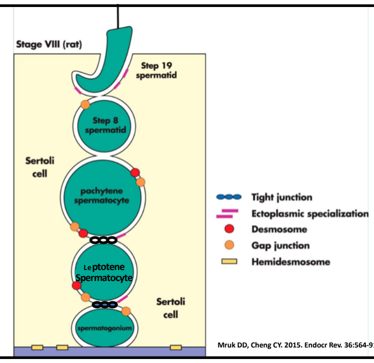
[a]Data are the mean ± sem for 5 control (sham insulated) and 5 insulated boars.
* Indicates a difference within the row, p< 0.05.
[nd]Indicates there was no difference within the row, p>0.05.

Boar Spermatogenesis

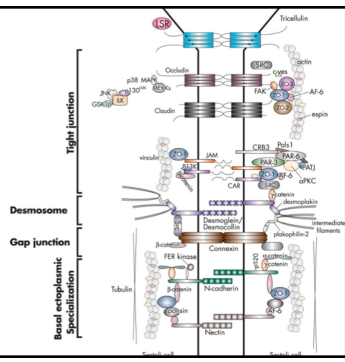


Decreases in Germ Cell Types Due to Scrotal Insulation





Mruk DD, Cheng CY. 2015. Endocr Rev. 36:564-91.



Mruk DD, Cheng CY. 2015. Endocr Rev. 36:564-91.

Mechanisms of Heat Stress Damage to Spermatogenesis

- **Dysregulation of BTB junctional complexes** (Cai et al., 2011)
  - **Observed in Mouse**
  - **Normal sequence**
    - **Breakdown of tight junctions and ectoplasmic specializations**
    - **Reassembly basal to primary spermatocyte with recycled proteins**
    - **Suggest relocalizing BTB components disrupted**
      - **BTB must recover as sperm are produced**
      - **Mice – BTB is damaged but recovers and involves Androgen signaling**
      - **Data in boar lacking**

Mechanisms of Heat Stress Damage to Spermatogenesis

- **Apoptosis** (Durairajanayagam et al., 2015)
  - **Rats – germ cells undergo apoptosis in response to heat stress**
    - **Consistent with gene expression and proteins in sperm**
    - **DNA repair occurs following DNA damage of apoptosis**
      - **Only partial however**
- **Stress Pathways** (Durairajanayagam et al., 2015)
  - **Increased lipid peroxidation, reduced superoxide dismutase and catalase activity.**
  - **Increased ROS**
- **DNA repair reduced** (Tramontano et al., 2000)
- **Intrinsic apoptosis – Mitochondria**
- **Extrinsic apoptosis – Fas/FAS-ligand signalling**

Mechanisms of Heat Stress Damage to Spermatogenesis

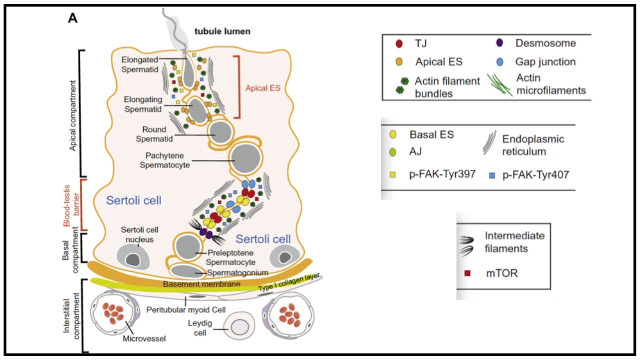
- **Autophagy** (Durairajanayagam et al., 2015)
  - **Flag cells for ubiquitin conjugation**
- **Cytoplasmic droplet retention** (Cooper, 2011)
  - **Involved in sperm volume regulation upon ejaculation**
  - **Retained droplet can not function and sperm compromised**

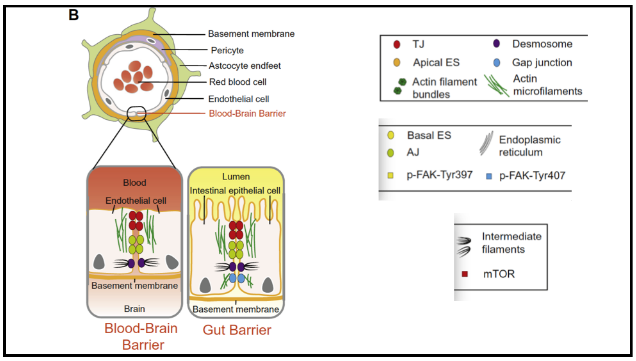
Preventing Heat Stress Damage

- Cooling
  - Cool-cell technology – reduces air temp. only 10°F
  - Air conditioning – cost expensive
  - Scrotal mists/drips – requires constant air flow
  - Humans – loose cloths, air conditioning
- Naturally resistant male
  - Livestock - How to identify?
- Impact BTB
  - Androgens, hCG, GnRH analogues
  - FSH, eCG
- Inhibit ROS and DNA damage
  - Vitamin E, catalase, antioxidants all given to boar

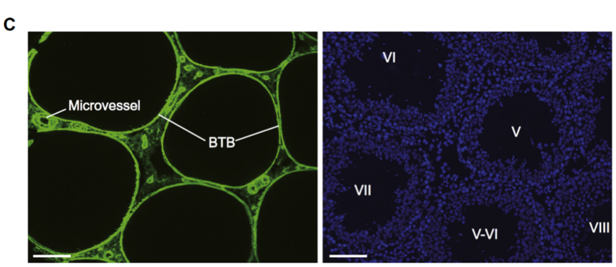
The Blood Testis Barrier

- What is it?
- What is its role?
- What are other barriers in the body similar to the BTB?
- As pre-leptotene primary spermatocyte moves across BTB, to form Leptotene primary spermatocyte what happens?
- What is the role of mTORC1 and TORC2?
- What is the role of p- FAK-Y407 and p-FAK-Y397?

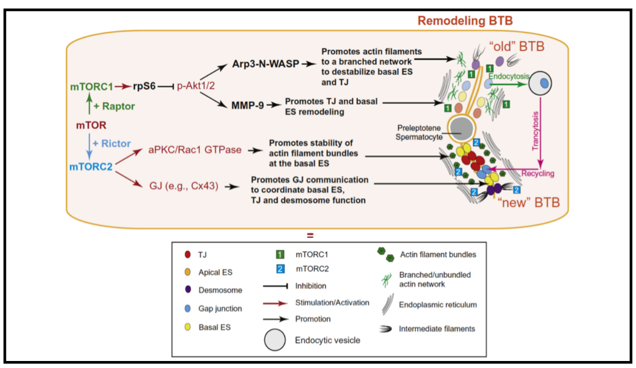


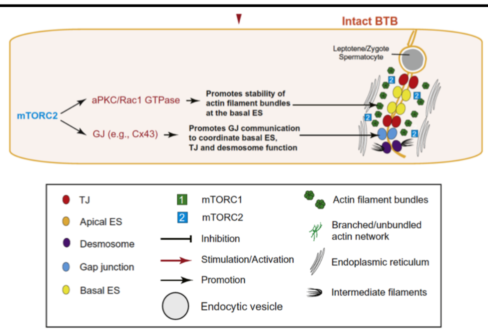


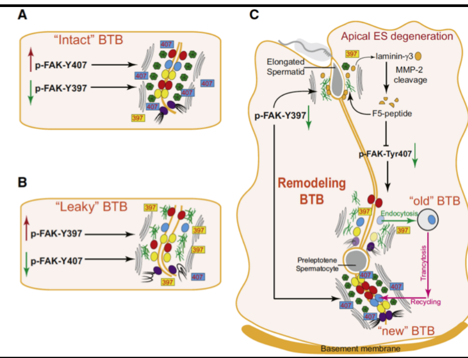
Does not become leaky as pre-leptotene to Leptotene movement.

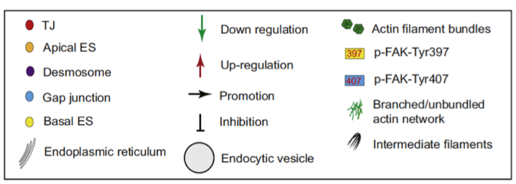


Biotin injected (not permeable thru BTB, avidin label









The Blood Testis Barrier and apical specializations are complex !!!