

## Target: The testes

Inactivation &/or destruction  
of gonadal stem cells

Session Chair: Michelle Anne Kutzler, DVM, PhD, DACT

Presenters: Dr. Kuladip Jana, Dr. Joseph Tash & Dr. Raphaël  
Vanderstichel



## Chemical Castration

- Non-surgical approach to male contraception
- These techniques are not technically challenging & are suitable for large-scale sterilization programs particularly in remote locations lacking sophisticated clinical facilities or skilled surgeons & staff
- Unlike surgical castration, chemical castration does not completely eliminate gonadal sources of testosterone



## Chemical Castration

- Chemical agents injected into the testis, epididymis or vas deferens cause infertility by means of causing azoospermia
- Used as a method for inducing male contraception for >5 decades



## Intratesticular Injections

- Freund's complete adjuvant (FCA)
- Bacillus Calmette Guerin (BCG)
- Methallibure
- Dexamethasone
- Metopiron
- Niridazol
- $\alpha$ -chlorohydrin
- Danazol



## Intratesticular Injections

- Zinc gluconate
  - Zinc is considered to be nonmutagenic, noncarcinogenic & nonteratogenic
  - Proper injection technique is critical when zinc gluconate is administered intratesticularly because leakage or injection into non-target tissues can result in severe tissue damage



## Intratesticular Injections

- In 2003, the FDA approved the 1<sup>st</sup> product labeled for chemical castration via intratesticular injection in the male dog
  - Neutersol™, Esterisol™, Zeuterin™
  - Labeled for use in prepubertal dogs (3-10 months old) with testes measuring 10-27 mm in width



## Intratesticular Injections

- Calcium chloride
  - 20% CaCl<sub>2</sub> in ethanol
  - Not FDA approved



## Intraepididymal Injections

- Dogs
  - Zinc arginine
  - 3.5% formalin solution in PBS
  - 1.5% chlorhexidine gluconate in 50% DMSO
- Cats
  - Chlorhexidine digluconate (4.5%)
  - Requires general anesthesia



## Intra Vas Deferens Injections

- Sclerosing agents
  - 10% silver nitrate
  - 3.6% formaldehyde in ethanol
  - 5% potassium permagnate
  - 100% ethanol
  - 3.6% formaldehyde



## Intra Vas Deferens Injections

- Occlusive agents
  - Tested in cats as an alternative to surgical vasectomy
  - A clear polymer gel made of styrene maleic anhydride mixed with DMSO is injected into the vas deferens & then solidifies



## Reproductive Toxins

- Parenteral administration of a chemical agent that targets the seminiferous epithelium
  - Ketoconazole
  - Embelin (benzoquinone)
  - $\alpha$ -chlorohydrin
  - Bisdiamine compounds (amebicidal drugs)
  - Prolactin



## Featured Speakers

- Dr. Kuladip Jana
  - Intratesticular injection of calcium chloride
- Dr. Joseph Tash
  - Parenteral administration of KU-AS-272
- Dr. Raphaël Vanderstichel
  - Intratesticular injection of zinc gluconate (Zeuterin/Esterisol)



## Chemical Castration

- Ketoconazole
  - Inhibits cellular division & has been shown to exert spermatostatic effects in several species including the dog, rabbit, monkey & man
  - Within 4-24 hours of oral administration of ketoconazole (50-246 mg/kg) to male dogs, the motility of ejaculated sperm decline at an accelerated rate compared with control samples from the same animals



## Chemical Castration

- Ketoconazole
  - Serum testosterone concentrations were profoundly suppressed following oral ketoconazole treatment
    - Poorly tolerated by the gastrointestinal tract & can cause hepatotoxicity at high dosages
  - Similar spermatostatic effects occur following treatment with other orally administered 1-substituted imidazole compounds in dogs without gastrointestinal & hepatic side effects



## Chemical Castration

- Embelin (*Embelia ribes*)
  - Indigenous benzoquinone plant used in Asia for the prevention of pregnancy
  - Oral treatment of embelin (80 mg/kg every other day for 100 days) in male dogs causes a significant decrease in testicular weight & variable degrees of spermatogenic arrest mainly at the spermatocyte state (absence of post-meiotic cells)



## Chemical Castration

- Embelin (*Embelia ribes*)
  - Within 8 months following embelin ingestion, normal spermatogenesis returns
  - Embelin treatment does not result in any adverse effects as noted on serum biochemistry & liver histology



## Chemical Castration

- $\alpha$ -chlorohydrin
  - Alkylating agent that causes depletion of spermatogenic elements from the seminiferous tubules
  - A single high-dose (70 mg/kg) of  $\alpha$ -chlorohydrin or chronic administration (8 mg/kg body wt for 30 days) inhibits spermatogenesis within 33 days in dogs
  - These effects are reversible within 100 days following treatment



## Chemical Castration

- Bisdiamine compounds (amebicidal drugs)
  - Target the male germinal epithelium & appear to have no harmful systemic effects
  - A safety & efficacy trial in male cats demonstrated that 150 mg/kg of the bisdiamine WIN 18,446 (Fertilysin<sup>®</sup>, SAF Bulk Chemicals, Milwaukee, WI, USA) administered daily in food causes complete & reversible spermatogenic arrest in all treated animals without damage to spermatogonia



## Chemical Castration

- Bisdiamine compounds (amebicidal drugs)
  - Although bisdiamines appear safe & effective for male cats, they can induce serious teratogenic effects in pregnant females
    - Administration must be done with care to assure only males receive the drug



## Chemical Castration

- Prolactin
  - Protein hormone secreted by the anterior pituitary gland
  - In men, hyperprolactinemia resulting from pituitary adenoma results in oligo- or azoospermia



## Chemical Castration

- Prolactin
  - Intramuscular administration of exogenous prolactin (600 µg/kg of body weight weekly for six months) to male dogs results in severe asthenozoospermia, teratozoospermia & oligospermia or azoospermia within six weeks of treatment
    - Serum testosterone, LH and FSH concentrations were not significantly changed from prolactin treatment



## Chemical Castration

- Prolactin
  - At the end of 3 months of treatment in dogs, degenerative changes within the seminiferous tubules were evident on testicular biopsy
  - Within 3 months of drug withdrawal, the spermatozoal count normalized, dog mating produced pregnancy & offspring exhibited no developmental abnormalities



## Owner Acceptance

- Many dog owners oppose surgical castration & other non-surgical methods of castration because of anthropomorphic empathy regarding emasculation & a fear that desired behaviors (e.g. protection & hunting instinct) will be decreased following androgen withdrawal
- Preservation of testicular tissue & testosterone production in conjunction with sterilization can be obtained using chemical castration, which increases the cultural acceptance



## Minimal Pain

- Studies using these models of male contraception report no or minimal signs of discomfort observed following injection, with variation depending on the route of administration & agent injected
- Afferent nerve endings associated with pain sensation are located on the scrotal skin & in the capsule of the testis rather than within the testicular & epididymal parenchyma



## Noninvasive Mechanical Sterilization

- Ultrasound is a form of acoustic vibration with frequencies higher than the auditory range. Ultrasound has been used to suppress spermatogenesis through a combined effect of heat and mechanics. The testes of male dogs and cats were treated with a high-intensity focused ultrasound manufactured by Whitewater Electronics. Each treatment consisted of 1-2 watts/cm<sup>2</sup> for 10-15 minutes administered 1-3 times at 2-7 day intervals. Ultrasound treatment significantly suppressed spermatogenesis without affecting testosterone concentrations. In separate studies, a burst (20-120 seconds) of ultrasound energy (3-19 W) was focused onto the vas deferens or epididymides of anesthetized dogs. The ultrasound induced thermal coagulative necrosis of subsurface structures resulting in luminal occlusion within two weeks after treatment. However, skin burns occurred in approximately 20% of cases).



## Chemical Castration

- Dogs
  - Sedation is typically used for this procedure
- Cats
  - General anesthesia may be necessary
  - The tail of the epididymis in cats is smaller & more difficult to locate compared to the dog



## Chemical Castration

- Preparation
  - Scrotal area is clipped & disinfected
- Injection
  - 22 gauge (dogs) or 27 gauge (cats) ½ inch needle is inserted percutaneously into the structure of interest (testis, epididymis or vas deferens)



## Intratesticular Injections

- Procedure involves inserting the needle from the caudal pole of the testis & gently pushing it towards the other pole, depositing the injection homogenously as far as possible through the tissue
- Induces aspermatogenic orchitis





## Intratesticular Injections

- Injection of an adjuvant agent (e.g. Freund's complete adjuvant (FCA) or Bacillus Calmette Guerin (BCG)) directly into the testis incites a local inflammatory response that enables lymphoid cells to gain access to testicular tissue, resulting in autoimmune response



## Intratesticular Injections

- A single intratesticular injection of FCA or BCG (10-25 units) results in severe oligospermia or azoospermia without granuloma formation or the development of circulating anti-sperm antibodies
  - Few spermatozoa that may be present are immotile
  - Infertility occurs within 6 weeks & lasts for several months
- A single intratesticular injection of higher doses (>75 units) of FCA or BCG results in a severe granulomatous reaction



## Intratesticular Injections

- In addition to using bacterial cell wall products, reproductive toxins can be directly injected into the testis
  - Intratesticular administration of a 100 mg solution (0.5 ml total volume per testis) of methallibure, dexamethasone, metopiron, niridazol,  $\alpha$ -chlorohydrin or danazol causes testicular & epididymal atrophy & azoospermia in dogs



## Intratesticular Injections

- The procedure involves injecting a predetermined amount of zinc solution based on scrotal width into each testis
  - The needle should be changed following filling of the syringe & then inserted into the dorsocranial portion of the testis beside the head of the epididymis
  - The center of the testis is the indicated target for zinc gluconate deposition





## Neutersol Results

- Histopathologic findings within 2.5 months of injection demonstrate almost complete fibrosis of the seminiferous tubules & Leydig cells
  - Intratesticular injection of a 70% glycerol solution does not result in azoospermia & sterility in dogs
- Scrotal swelling & tenderness are common in the 1<sup>st</sup> few days following injection & usually resolve without treatment
  - However, scrotal ulcers or draining tracts in the scrotum or preputial area can occur 4-6 days after treatment



imagine...  
preventing unwanted litters without surgery

## Neutersol Side Effects

- In one study, necrotizing zinc-gluconate injection site reactions occurred in 3.9% (4/103) of research dogs following intratesticular injection of the zinc gluconate solution
  - One of these dogs chewed away a portion of his scrotum & testis
  - All four dogs with severe reactions were large mature dogs that had received a dose of the drug at the upper end of the label range (3 dogs received 0.8 mL/testis & 1 dog received 1.0 mL/testis)



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## Intraepididymal Injections

- Zinc arginine injection into the tail of the epididymis
  - Intraepididymal injection of 50 mg of zinc arginine (0.5 ml per testis) results in azoospermia within ninety days following injection
  - Histologic examination of the testes reveals normal seminiferous tubules with atrophy of the rete testes, an absence of spermatozoa within the epididymis & ductus deferens & no sperm granuloma formation
  - Intraepididymal saline injection does not induce azoospermia



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## Intraepididymal Injections

- Injections of sclerosing agents (3.5% formalin solution in phosphate buffered saline or 1.5% chlorhexidine gluconate in 50% DMSO) into the tail of both epididymides in dogs results in irreversible azoospermia via chemical occlusion with secondary testicular atrophy
  - However, intraepididymal treatment with formalin alone induces only temporary azoospermia or oligospermia in treated dogs



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## Intraepididymal Injections

- A single bilateral intraepididymal injection of chlorhexidine in DMSO to male dogs resulted in the development of azoospermia by 91 days after treatment
  - 2 bilateral intraepididymal injections of chlorhexidine in DMSO resulted in the development of azoospermia within 35 days after treatment



## Intraepididymal Injections

- Tom cats
  - Injection of a chlorhexidine digluconate (4.5%) solution into the tail of both epididymides results in azoospermia or severe oligospermia
  - Unlike the dog, sperm granulomas & spermatocoeles are consistently observed in cats following intraepididymal injections
  - In addition, transient scrotal swelling & pain as may persist for up to two weeks following intraepididymal injection in cats



## Intra Vas Deferens Injections

- A single injection directly into the vas deferens with sclerosing chemical agents (10% silver nitrate, 3.6% formaldehyde in ethanol, 5% potassium permagnate, 100% ethanol, or 3.6% formaldehyde) results in irreversible infertility similar to intraepididymal injections with the same agents

