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In utero exposure to 5-azacytidine induces apoptosis in the fetal rat testis

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28th March 2008

Epigenetics in ART

I will talk about...

1. **DNA methylation in male germ line – testis development**
   - **5-azaC – basic facts**
   - **5-azaC – my research**
2. - **RESULTS: Histology and TEM**
   - **Cleaved caspase-3 immunolabelling**
3. - **Discussion**

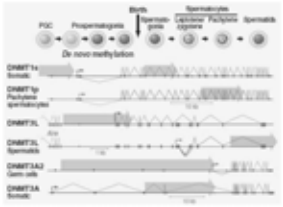
Male germ cell development

Species, Days of	First Detection of Primordial	Genital Ridge	Testicular Differentia-	Gonocyte Prolifer- ation	Mitotic	Mitosis Resump-
Mice, 19	7.5 dpc	11.5 dpc	12.5 dpc	13.5–16.5 dpc	16.5 dpc	1 dpp
Rat, 21	8 dpc	13.5 dpc	13.5 dpc	13–18 dpc	18 dpc	3–4 dpp

from: Olaso R *et al. J Androl*, 2000

Developmental timing and mechanisms underlying the acquisition of DNA methylation in germ cells

- **In male germ line – methylation of imprinted genes (*e.g. H19*) and repetitive elements occurs around 18.5-20.5dpc (rat) and completed by pachytene, persisting in spermatozoa**

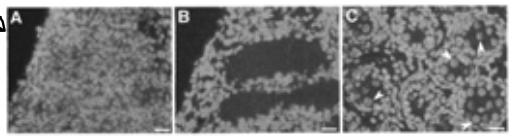


from: Bestor TH *et al.*, *Science*, 2007.

- **In female germ line – methylation occurs postnatally, following pachytene stage**

Moreno S *et al*, Biol. Reprod.,2001. – on rat; Coffigny *et al*, Cytogenetics; 1999. – on mouse

5-mC antibody



16 dpc **20 dpc**

- The results are consistent with the expression of DNMT enzymes involved in *de novo* methylation (La Salle S *et al*; Dev Biol; 2006.)

5-azacytidine

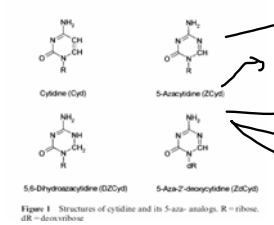
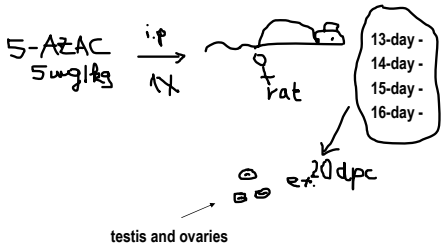


Figure 1 Structures of cytidine and its 5-aza- analogs. R = ribose. dR = deoxyribose

- Cyd base analogue
- Incorporates into the DNA instead of Cyd
- Irreversible inactivation of DNMT
- DNA demethylating agent
- 5-azacytidine - *Vidaza* - used in clinical practice for treating malignancies.

My experiment: *in utero* exposure of Fisher rat embryos to 5-azaC working hypothesis

Demethylating agent 5-azacytidine administrated during pregnancy in rat will affect FETAL TESTIS (or OVARY) development!



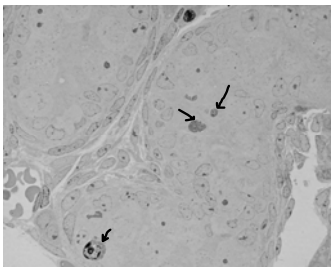
testis and ovaries

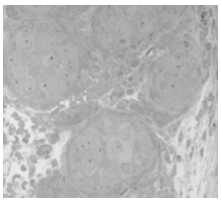
Methods

- Morphological changes – LM
- Ultrastructural changes in certain cells – TEM
- Immunohistochemistry – on Tokuyasu sections

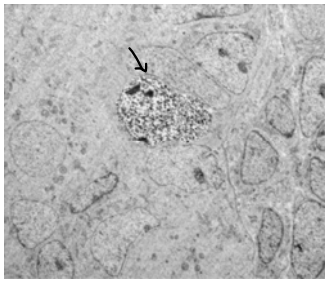
Results...presented by experimental days of treatment …

RESULTS: Day 13 pc – 5mg/kg 5-azaC

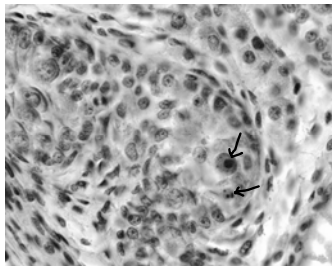


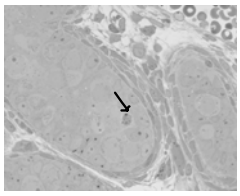


Day 13 pc – 5mg/kg 5-azaC, TEM

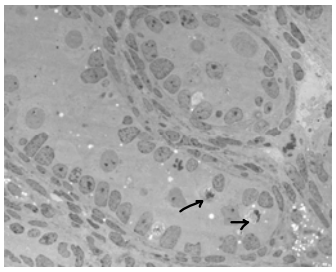


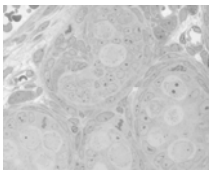
RESULTS: Day 14 pc – 5mg/kg 5-azaC



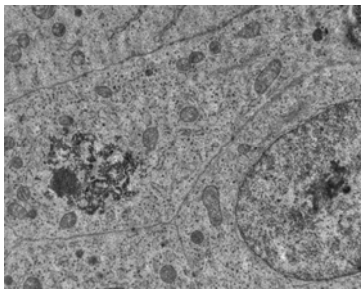


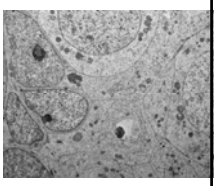
RESULTS: Day 16 pc – 5mg/kg 5-azaC



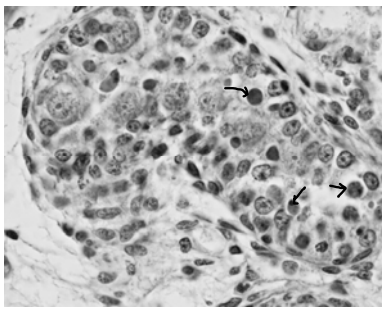


Day 16 pc – 5mg/kg 5-azaC, TEM

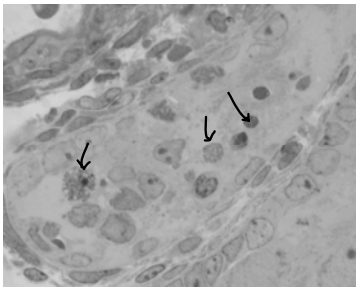


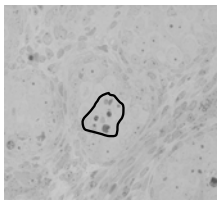


RESULTS: Day 15 pc – 5mg/kg 5-azaC

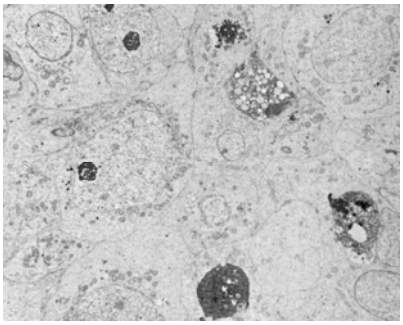


Day 15 pc – 5mg/kg 5-azaC



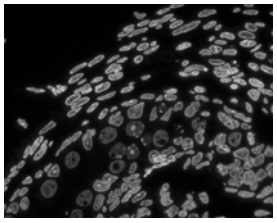


Day 15 pc – 5mg/kg 5-azaC, TEM

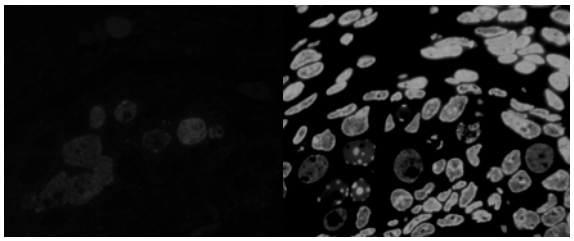


Different stages of apoptotic cells

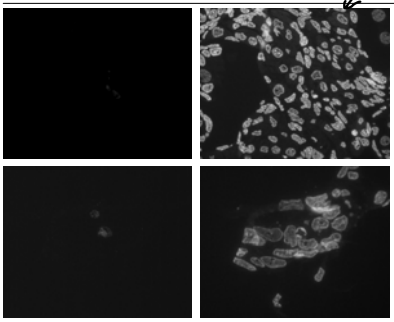
Cleaved caspase 3 immunoreactivity – day 15



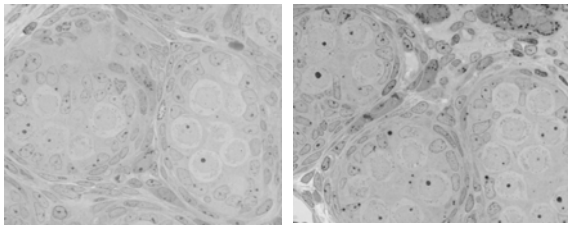
Cleaved caspase 3 immunoreactivity – day 15



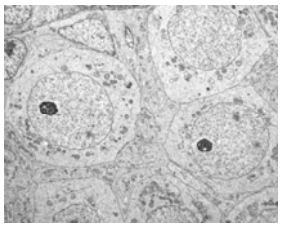
Cleaved caspase 3 immunoreactivity – day 13, 14

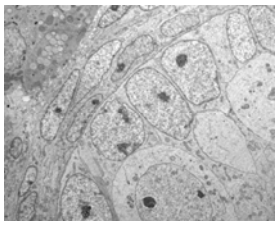


Control



Control, TEM





In control testis no cleaved caspase-3 labelling is present!

Conclusion

- **15th day of pregnancy as the most sensitive period to the action of demethylating agent on development of gonocytes in fetal rat testis**

- **Why? Role of methylation failure?**
- **Timing of apoptosis? In day 15 or other groups?**
- **Effect on later spermatogenesis?**

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THANK YOU!
QUESTIONS?