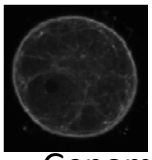
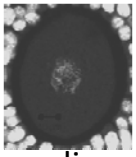
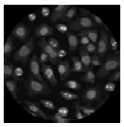
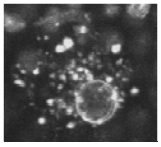




Genome Integrity in Mammalian Oocytes

ESHRE
Workshop on mammalian folliculogenesis and oogenesis
April 19-21
Stresa Italy



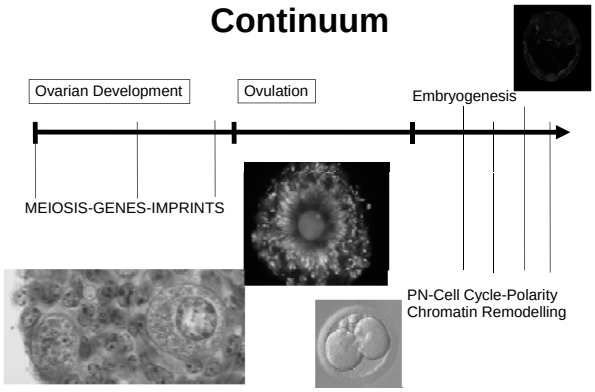


2003 Workshop Lisbon

Genome Integrity

- Structure-is chromatin in open or closed state and organized around the NE and nucleolus
- Function- can it remodel during growth, meiosis, reprogram after fertilization
- Metabolic-can it meet the demands of DNA damage repair in terms of energy, expression of repair factors, and translocation in response to damage stimulus

Oocyte Qualiy Is A Developmental Continuum



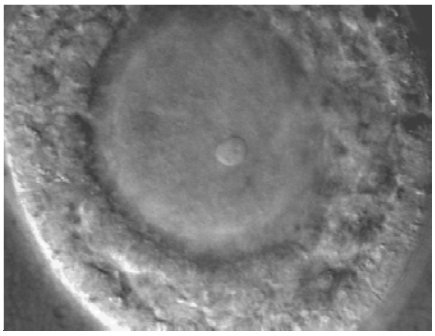
Outline

- What does “Genome Integrity” mean for oocytes?
- What is the DNA Damage Response (DDR)?
- Is DDR active during oogenesis and folliculogenesis?
- What is the impact of ovarian irradiation on genome integrity?

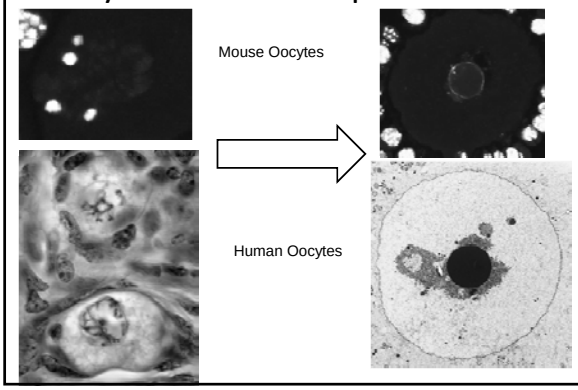
A Brief History

- Chromatin remodels during growth and maturation-unique cell cycle and question of checkpoints
- Age affects OQ and aneuploidy is a problem to humans
- Lied 3 environment exposes us to DDR

Oocyte Nucleus is Dynamic!



Oocyte Chromatin:Open or Closed?



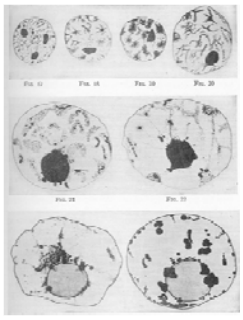
The SN/NSN picture is incomplete

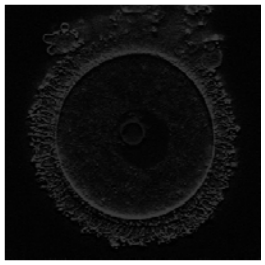
- When it comes to NSN or SN
- Chromatin is not static
- Is influenced by somatic cell contact
- Changes in response to hormonal stimulation
- Changes in response to induced DNA damage
- Open and closed states of chromatin are much more than a reflection of transcriptional status

Abbreviations are short cuts to the truth

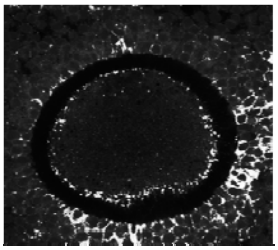
- NSN-non surrounded nucleus
- NSN-not silly nomenclature
- NSS-not stressful Stresa
- NSC-non stim cycles
- NSI-non strategic initiatives
- NSI-negligible social impact
- NSI-negligible scientific impact

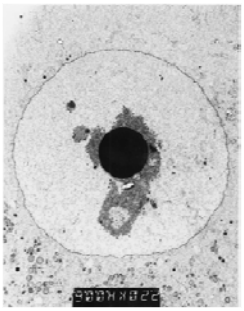
Pincus 1939 More than NSN SN





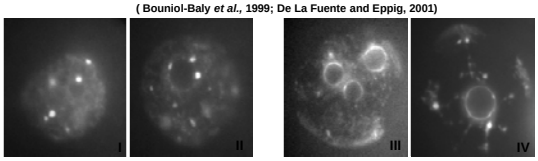
Why chromatin is elusive!





Germinal Vesicle Chromatin Patterns

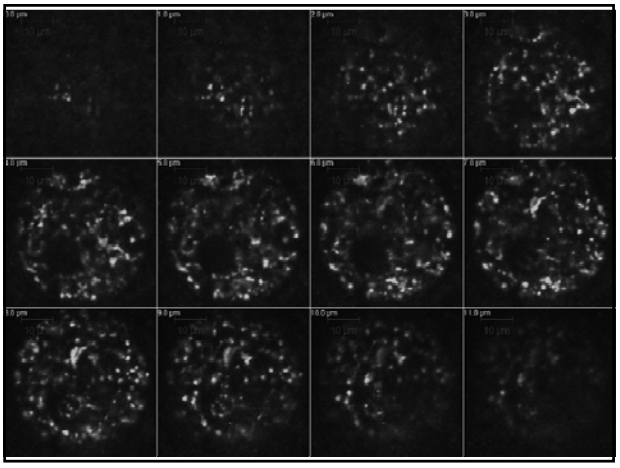
(Bouniol-Baly *et al.*, 1999; De La Fuente and Eppig, 2001)



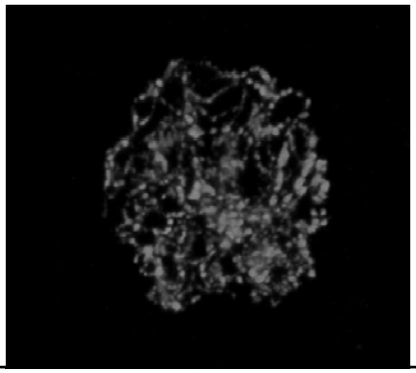
Non-Surrounded Nucleolus (NSN) **Surrounded Nucleolus (SN)**

	C^+		$C^{+/-}$		C^-		All Types		
	NSN	SN	NSN	SN	NSN	SN	NSN	SN	N
Unprimed	28.3 (8.8)	71.7	44.6 (2.2)	55.4	42.1 (4.5)	57.9	38.3	61.7	876
Primed	6.7 (3.5)	93.3*	22.9 (2.1)	77.1*	28.5 (3.1)	71.5*	19.4	80.6*	838

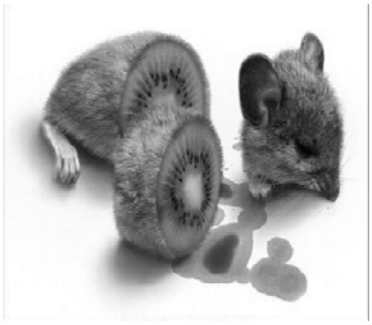
Mean Percent of Oocytes (± SEM) for 12 experiments; two-tailed Z-test, p<0.0005



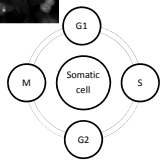
Bovine oocyte: Open Chromatin

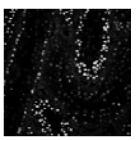


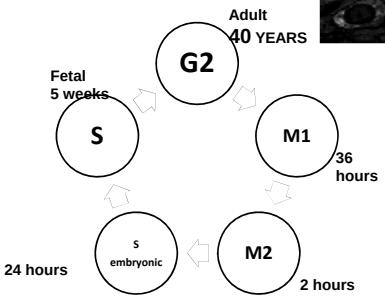
What is inside is what counts

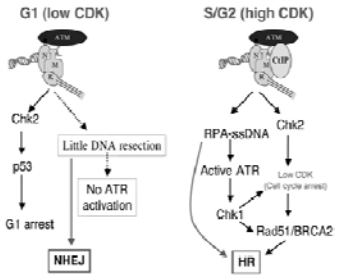


The oocyte has an atypical cell cycle







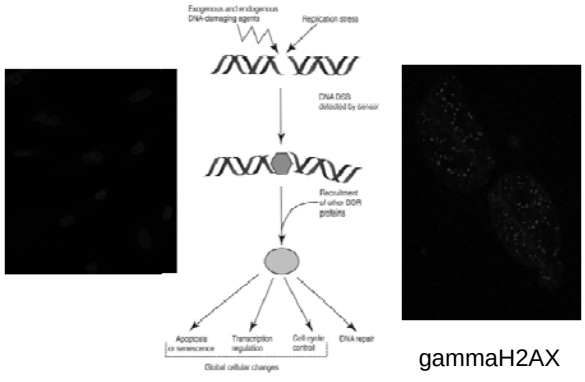


3 stories

- Why current views of oocyte chromatin remain in the dark ages

Why eggs are in a precarious situation-long storage, dynamic chromatin state, and require an intrinsic DDR response

- What happens when DNA damage is imposed on oocytes-just the beginning of a long story
- **DDR=DNA Damage Response**



gammaH2AX

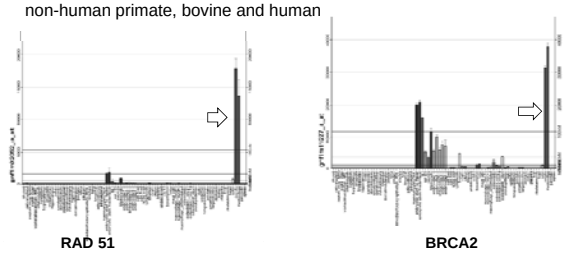
More than just a focus: The chromatin response to DNA damage and its role in genome integrity maintenance

Jiri Lukas, Claudia Lukas and Jiri Bartek

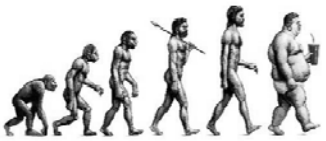
NATURE CELL BIOLOGY VOLUME 13 | NUMBER 10 | OCTOBER 2011

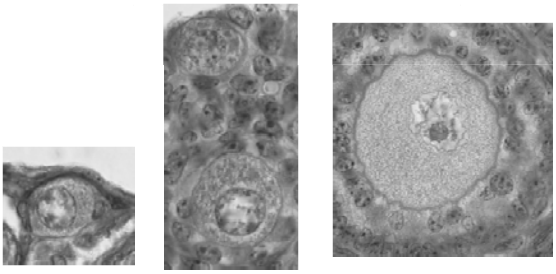
The players in somatic cell DDR are expressed in oocytes

- In Silico at mRNA level
- In both homologous recombination (HR, accurate) and non-homologous end joining (NHEJ, error-prone) pathways
- Post pachytene…Dictyate/diplotene…detected in mouse, rat, non-human primate, bovine and human



RAD 51 **BRCA2**





Some of the players we have studied

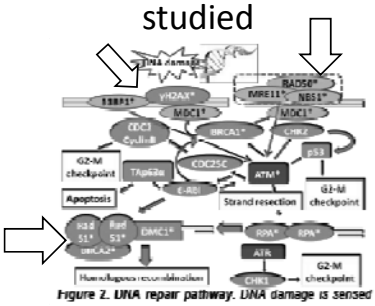


Figure 2. DNA repair pathway. DNA damage is sensed

At what stages of oogenesis does the DDR operate?

- Meiotic Prophase
- In resting primordial follicles
- In growing follicles
- But not during meiotic maturation

BRCA2 deficiency in mice leads to meiotic impairment and infertili

Shyam K. Sharan[1,*], April Pyle[2], Vincenzo Coppola[1], Janice Babus[2], Srividya Swaminathan[1], Jamie Benedict[2], Deborah Bering[2], Betty K. Martin[2], Lino Tessarollo[1], Janice P. Evans[2], Jodi A. Flaws[2] and Mary Ann Handel[2]

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Accepted 24 September 2003

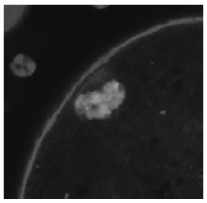
Cases of autonomous or induced DSBs

Autonomous

- Meiotic Prophase-leptotene,zygotene, pachytene(homologous recombination)
- Diplotene (acute after follicle formation; or later in adult life)
- Growth phase of oogenesis
- Other(nutrition, environmental exposure)

Induced

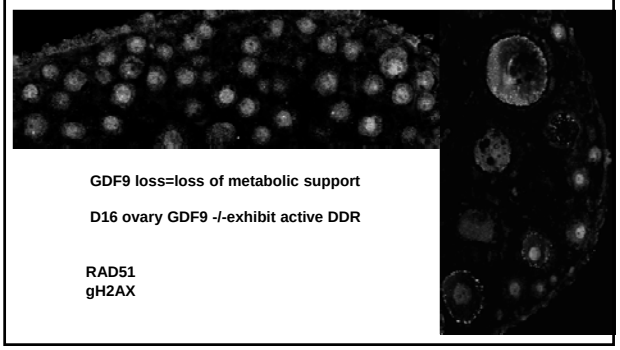
- Chemotherapy
- Cryopreservation
- Irradiation



Experimental models

- **Knock out mice (GDF9, FSHb)**
- Aging mice (E15-315 days)
- **Prepubertal Mice (15-21 days)-IR**
- **Rat Ovary-dioxin exposure**
- **Bovine Ovary-IR**
- **Bovine oocytes-IR**
- Cryopreserved Ovary (bovine, mouse)

Genetic models for follicle arrest

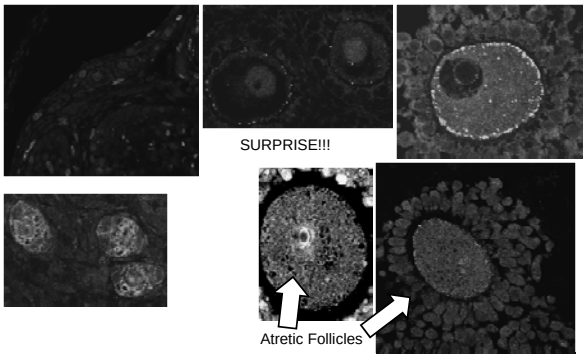


GDF9 loss=loss of metabolic support

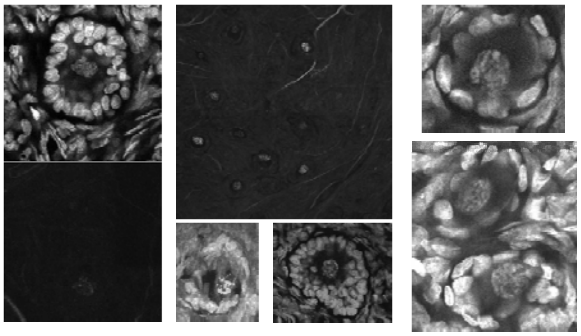
D16 ovary GDF9 -/-exhibit active DDR

RAD51
gH2AX

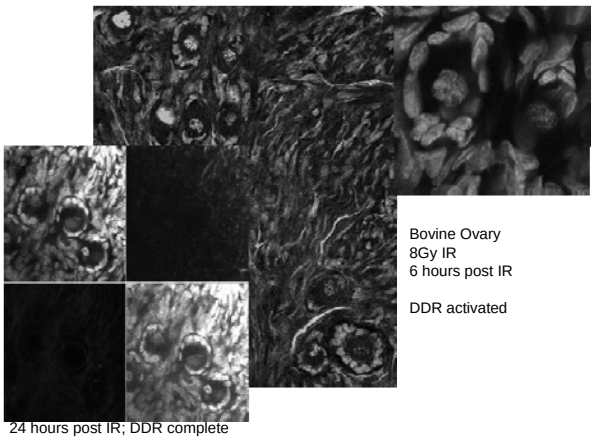
DDR in Oocytes: Induced or Constitutive ?



Bovine Ovary-post IR DDR



Control 5hr post IR 24hr post-IR

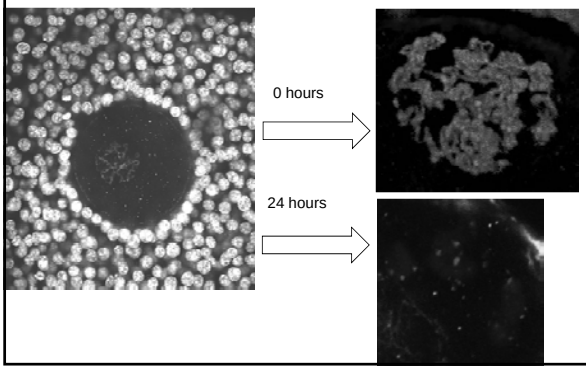


Bovine Ovary
8Gy IR
6 hours post IR

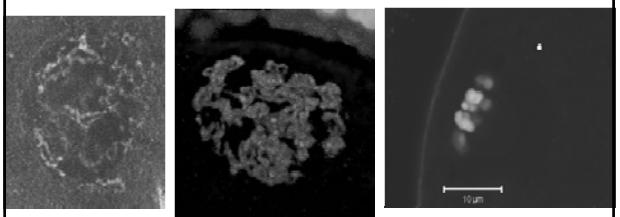
DDR activated

24 hours post IR; DDR complete

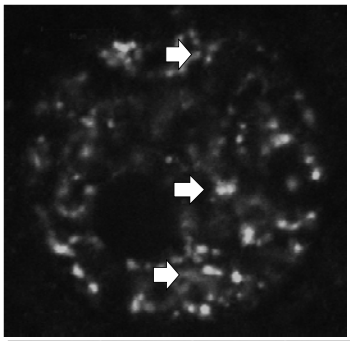
Bovin*e* oocytes-8Gy in situ

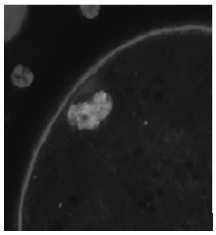


DNA lesion density decreases during meiotic progression

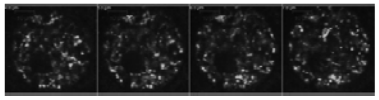


No IR T6 IR T30IR





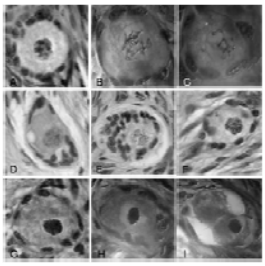
GV=33.7+/- 2.4
foci/nucleus
n=78
M2= 14.9=/- 5.1
n=93



Cell cycle progresses without resolution of DSBs

Future Directions

- Fertility Preservation
- OTC



- Protective strategies-adjuvant therapies targeting p63.

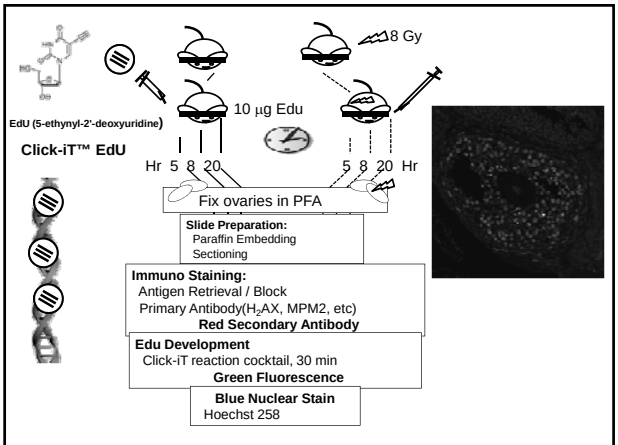
Take home message

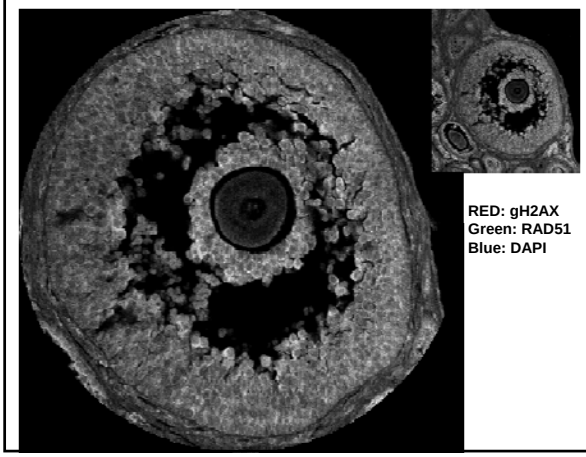
- The DNA Damage Response (DDR) is alive and well in mammalian oocytes
- It operates constitutively to maintain chromatin integrity during oocyte differentiation
- Ionizing radiation activates a robust response that leads to follicle loss and premature ovarian failure
- After induced DDR, or as a result of age-related accumulation of repair efforts, the genetic integrity of embryos and offspring may carry battle scars into the next generation.

Friends, colleagues, and funding

- The lab..then and now

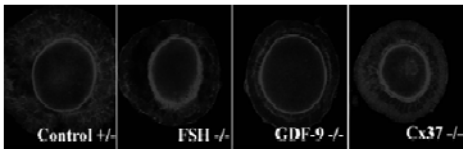
- Evelyn Flaherty Tilly-Telfer, Marie McLaughlin
- The Biogenesi Group-Cristina, Beatrice, Rubens, and of course Lucia and Gio
- NIH, March of Dimes, ESHE Fund, Hall Foundation





RED: gH2AX
Green: RAD51
Blue: DAPI

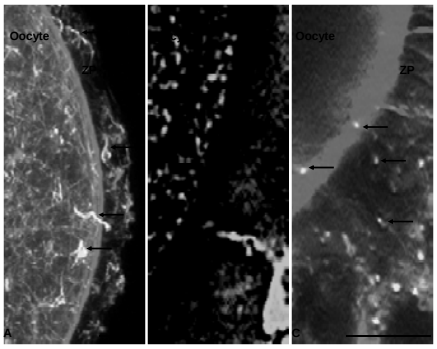
Lessons from KnockOuts



Follicle Arrest Arrest Arrest *Secondary*
Primary *Secondary*

Oocyte Partial Deficient Deficient
Competencies

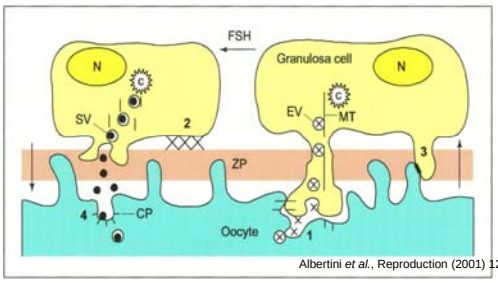
Properties of Microtubule TransZonal Projections (TZPs)



Microtubules
Endosomes

Mitochondria

Oocyte-Granulosa Cell Interface: Communication Modes

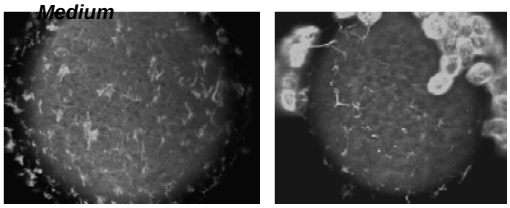


Albertini *et al.*, Reproduction (2001) 121

Hypothesis: **Granulosa cell-oocyte TZPs are modulated during follicle development by FSH.**

Optimization of TZP Detection

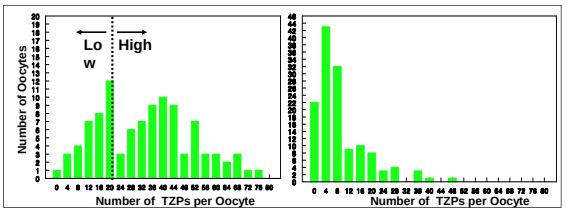
In Fix Medium ***In***



Mean TZP Density per Oocyte ± SEM:

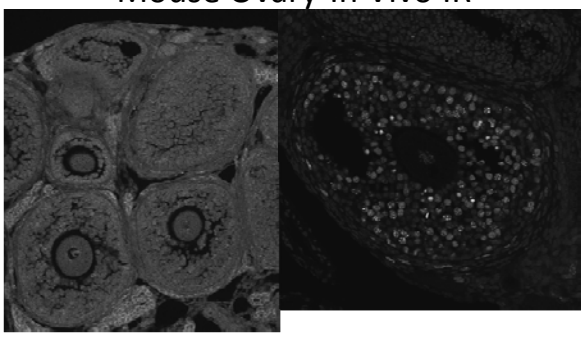
33.32 ± 1.38 (n= 146) **10.20 ± 0.77 (n=152)**

Density Distribution Plots of Oocytes Collected in F Medium



Collection in Fix (n=102) ***Collection in Medium (n=136)***

Mouse Ovary-In Vivo IR



Edu/gH2AX/DAPI 1.5hr post-IR 5hr post-IR