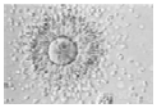
Maternal Diabesity, Oocyte Quality and Reproductive Outcomes



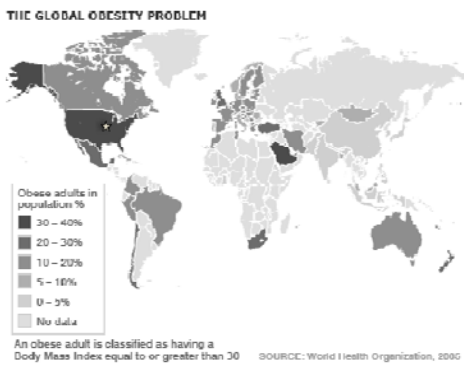
Kelle H. Moley, MD
Professor and Vice Chair
Obstetrics and Gynecology
Washington University in St Louis

Washington University in St.Louis

Financial Disclosure

◊ Member, Scientific Advisory Board of Ovascience

Global obesity trends among adults



Obesity and Women

- Over 35% of all reproductive age women in the US are obese and about 1/3 of them have some degree of glucose intolerance=Diabesity
- Diabesity poses specific health related consequences
  - Diabetes, CVD, Cancer
  - Ovulatory disorder
  - Longer times to conception even if young and ovulatory
  - Adverse reproductive outcomes

Flegal KM, JAMA 2010
Rohler, JCEM 2009

Obesity and Reproductive Outcomes

TABLE 1
Epidemiologic studies of adverse reproductive outcomes in obese women

Outcome	Odds ratio (95% CI)	Reference	Type of study	Patients, n
Subfertility	2.2 (1.8–2.6)	Nohr et al[12]	Prospective cohort	4901
Miscarriage	1.67 (1.25–2.25)	Metwally et al[5]	Metaanalysis	2257
Various fetal anomalies	1.2 (1.03–1.4, cleft lip and palate); 2.24 (1.86–2.69, spina bifida)	Stothard et al[6]	Metaanalysis	868, 1188
Large for gestational age	2.3 (1.9–2.7)	Nohr et al[12]	Prospective cohort	4901
Preeclampsia	1.6 (1.1–2.25, obese vs nonobese); 3.3 (2.4–4.5, morbidly obese vs nonobese)	Weiss et al[68]	Prospective cohort	15,225, 14,829
Obesity in the offspring at age 1 y (BMI ≥95th percentile)	1.9 (1.3–2.6)	Nohr et al[12]	Prospective cohort	4901

BMI, body mass index; CI, confidence interval.
Jungheim. Obesity and reproduction. Am J Obstet Gynecol 2010.

Obesity and Reproductive Outcomes

Obesity and congenital anomalies

	Overweight		Obesity	
Congenital anomaly	**Odds ratio (95% CI)**	***P* value**	**Odds ratio (95% CI)**	***P* value**
Neural tube defects	1.2 (1.04–1.38)	.01	1.87 (1.62–2.15)	< .001
Cardiovascular anomalies	1.17 (1.03–1.34)	.02	1.3 (1.12–1.51)	.03
Cleft lip and palate	1.0 (0.87–1.15)	> .99	1.2 (1.03–1.4)	.02
Anorectal atresia	1.19 (0.91–1.54)	.2	1.48 (1.12–1.97)	.006
Craniosynostosis	1.24 (0.98–1.58)	.07	1.18 (0.89–1.56)	.25
Diaphragmatic hernia	0.95 (0.72–1.26)	.72	1.28 (0.95–1.71)	.1
Gastroschisis	0.83 (0.39–1.77)	.63	0.17 (0.1–0.3)	< .001
Hydrocephaly	1.28 (0.93–1.75)	.13	1.68 (1.19–2.36)	.003

Adapted from Stothard KJ et al.[6]
Gunatilake. Obesity and pregnancy: Am J Obstet Gynecol 2011.

Obesity and Offspring Outcomes

TABLE 1
Epidemiologic studies of adverse reproductive outcomes in obese women

Outcome	Odds ratio (95% CI)	Reference	Type of study	Patients, n
Subfertility	2.2 (1.8–2.6)	Nohr et al[17]	Prospective cohort	4901
Miscarriage	1.67 (1.25–2.25)	Metwally et al[6]	Meta-analysis	2257
Various fetal anomalies	1.2 (1.03–1.4, cleft lip and palate); 2.24 (1.86–2.69, spina bifida)	Stothard et al[5]	Meta-analysis	863; 1188
Large for gestational age	2.3 (1.9–2.7)	Nohr et al[17]	Prospective cohort	4901
Preeclampsia	1.6 (1.1–2.25, obese vs nonobese); 3.3 (2.4–4.5, morbidly obese vs nonobese)	Weiss et al[60]	Prospective cohort	15,225; 14,629
Obesity in the offspring at age 1 y (BMI >95th percentile)	1.9 (1.3–2.6)	Nohr et al[17]	Prospective cohort	4901

BMI, body mass index; CI, confidence interval.
Jungheim, Obesity and reproduction. Am J Obstet Gynecol 2010.

Obesity and Adverse Pregnancy Outcomes: Proposed Mechanisms

TABLE 2
Proposed mechanisms by which obesity may affect early stages of reproductive development

Stage of development	Proposed mechanism	Possible effects on reproduction	Evidence	References
Oocyte	Abnormal hypothalamic GnRH pulsatility; abnormal follicular environment	Poor oocyte quality; impaired ovulation	Human serum samples; human IVF specimens; animal specimens	Jain et al[61]; Robker[62]; Robker et al[47]; Jungheim et al[41]
Preimplantation embryo	Poor oocyte quality; impaired embryonic metabolism and quality; epigenetic modification	Impaired implantation; miscarriage; fetal anomalies and growth abnormalities	Animal specimens; human IVF specimens	Jungheim et al[41]; Carroll et al[63]; Metwally et al[64]; Eng et al[65]
Implantation	Abnormal endometrium	Impaired implantation; miscarriage; fetal growth abnormalities	Human endometrial biopsies	Mozzanega et al[42]

GnRH, gonadotropin-releasing hormone; IVF, in vitro fertilization.
Jungheim, Obesity and reproduction. Am J Obstet Gynecol 2010.

Clinical evidence supporting oocyte as target:

- Failure to achieve a live birth increases with higher BMI, significantly with the use of autologous oocytes ($P < 0.0001$), and to a greater extent among women < 35 years of age ($P < 0.0001$).
- Higher BMI is associated with an increased failure to achieve a clinical intrauterine gestation; this risk was overcome with the use of **DONOR OOCYTES**.

Luke et al., Hum Reprod 2011

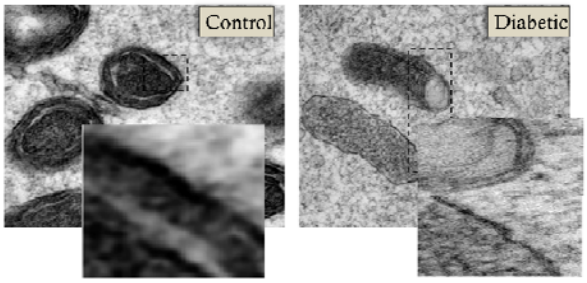
Maternal metabolism and the oocyte: Animal Model Data

Abnormal maternal physiology and the oocyte: Type 1 diabetes

- Animal models of Type 1 diabetes
  - Smaller oocytes, impaired maturation, increased granulosa cell apoptosis
  - Poor reproductive outcomes: growth restriction and congenital anomalies
  - Abnormal mitochondria morphology, mtDNA copy number, spindle defects/chromosome misalignment

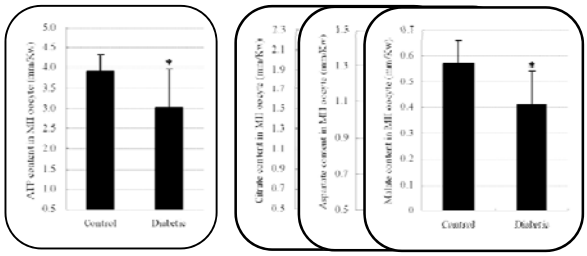
Wang Q et al, Mol Endo 2009
Ratchford AM et al., A J Phys Endo Metab; 2007
Chang AS, et al, Endocrinology; 2005
Moley KH et al, J Reprod Fertil; 1991
Wyman A, et al, Endocrinology; 2008
Wang Q et al, PLoS ONE, 2010

Mitochondrial Ultrastructure of Diabetic Oocyte

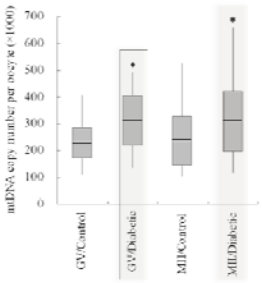


Wang et al., 2009, Mol Endo, 23:1603-12

Metabolic Dysfunction in Diabetic Oocyte

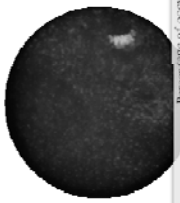


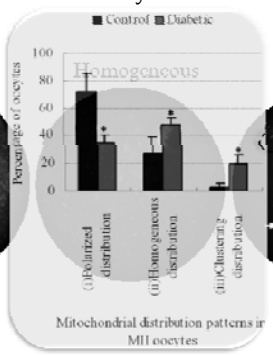
Increased mtDNA Content in Diabetic Oocyte



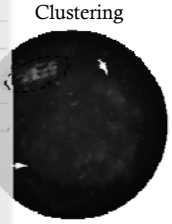
Disrupted Mitochondrial Distribution in Diabetic Oocyte

Polarization



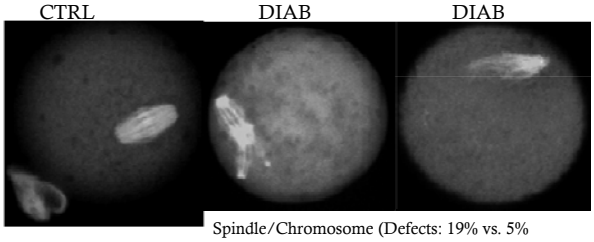


Clustering



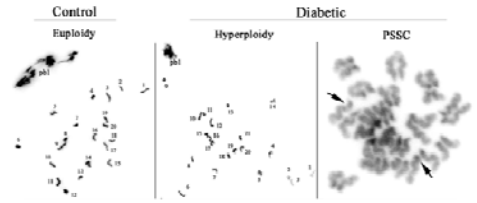
Meiotic Defects in Diabetic Oocyte

CTRL DIAB DIAB



Spindle/Chromosome (Defects: 19% vs. 5% Ctrl)

Aneuploidy in diabetic oocytes



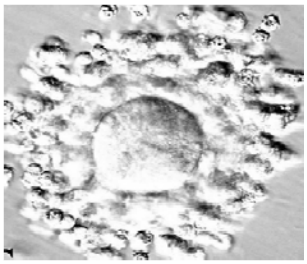
Group	Number of Mice	Number of Cells	Euploidy	Aneuploidy	PSSC
Control	10	45	43 (95.6%)	2 (4.4%)	0 (0.0%)
Diabetic	12	37	30 (81.1%)*	7(18.9%)*	4(10.8%)*

*Note:PSSC, prematurely seperated sister chromatid; *, p<0.05 versus controls.

Summary: Maternal diabetes causes

- ✧ Structural, spatial and metabolic dysfunction of mitochondria in oocytes;
- ✧ Spindle defects and chromosome misalignment result in aneuploid embryos;
- ✧ These defects in oocytes probably contribute to the reproductive problems experienced by type I diabetic women.

How does maternal diabetes exert its effects on the oocyte?



Maternal diabetes adversely impacts cumulus cells contributing to poor oocyte quality

Our evidence

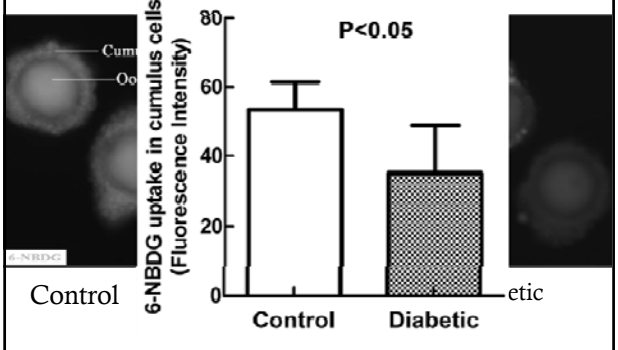
- Glucose transporter expression and transport-**DECREASED**
- Mitochondria status in cumulus cells-**ABNORMAL**
- Gap junction communication-**IMPAIRED**

Wang et al, PLoS, ONE, 2010

New data using fluorescent tagged glucose analog NDBG

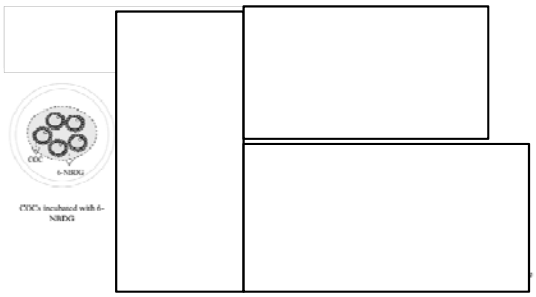
Wang et al, Endocrinology 2012

Decreased live 6-NBDG uptake in diabetics



Control etic

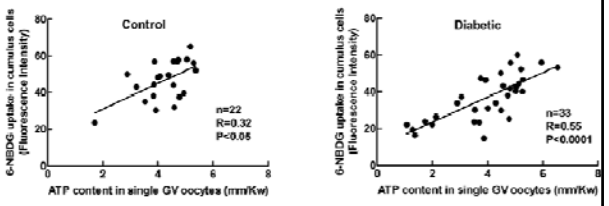
Wang et al, Endocrinology 2012



Live imaging to determine the link between glucose uptake, ATP and spindle formation

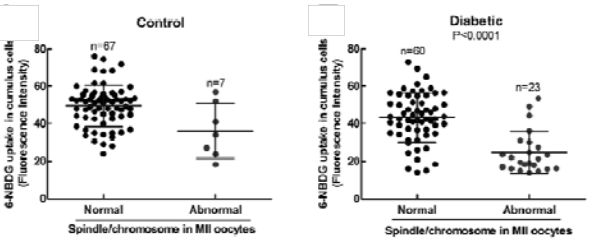
Wang et al, Endocrinology 2012

6-NBDG uptake was significantly lower and correlated with decreased ATP in same the same oocyte

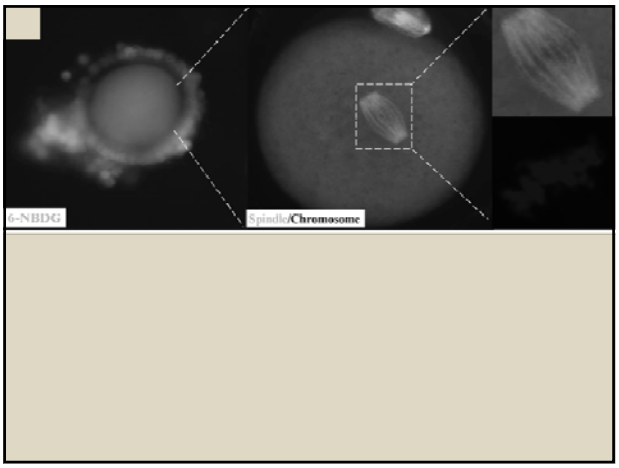


Wang et al, Endocrinology 2012

Strong correlation between decreased glucose uptake and abnormal spindles in diabetics only



Wang et al, Endocrinology 2012

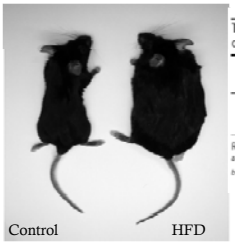


Conclusion of diabetes work

- Maternal diabetes affects oocyte and cumulus cell via metabolic changes in part due to communication difficulties
- COCs are adversely affected by the diabetic environment which directly affects oocyte ATP and spindle formation
- This may be the cause of poor pregnancy outcome in these patients as well as others with high rates of poor outcomes

This may include oocyte from obese, PCOS and/or aged women

Obesity and Reproductive Outcomes: A High Fat Diet (HFD) Model of Obesity



Control HFD

TABLE 1. Characteristics of female mice on a regular diet *vs.* a high-fat diet after 16 wk of feeding

	Regular diet (n = 10)	High-fat diet (n = 10)
Body weight (g)	27.04 ± 0.8	46.92 ± 1.8[a]
Glucose	97 ± 8.9	145.7 ± 3.6[a]
FFA	0.43 ± 0.06	0.90 ± 0.17[b]

Results are expressed as means ± SEM.
[a] $P < 0.001$ *vs.* control.
[b] $P < 0.03$ *vs.* control.

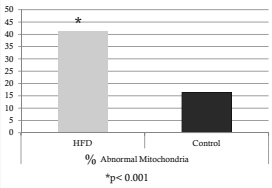
Jungheim, Endo 2010

Oocyte Size and Maturation

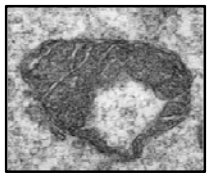
- Increased apoptosis: TUNEL nuclei---17.6 ± 1.2% Control vs 59.64 ± 2.2% Obese
- Smaller size: Significantly smaller diameter in GV and MII oocytes from Obese vs Control
- Delay to maturation: Significantly lower % of oocytes reaching GVBD in Obese vs Control

Jungheim; Endo, 2010

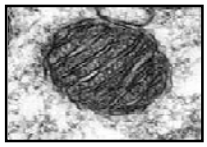
Obesity: altered oocyte mitochondria structure



*p< 0.001



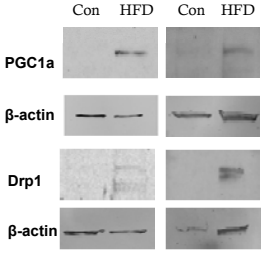
HFD

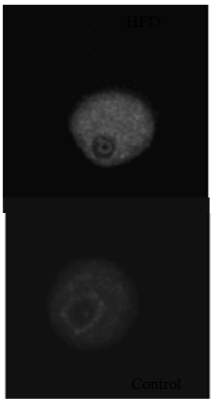


Control

Luzzo et al, unpublished

Abnormal mitochondrial protein expression related to biogenesis and fission:

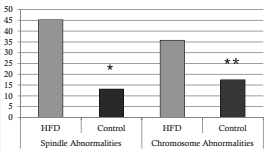




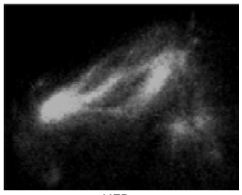
Altered Drp1 localization

Luzzo et al, unpublished

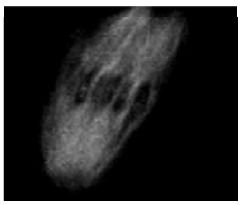
Meiotic Spindle and Chromosomal Abnormalities



*p<0.0001
** p< 0.05



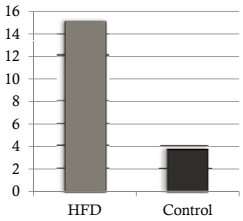
HFD

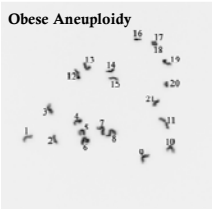


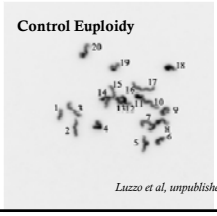
Control

Luzzo et al, unpublished

Increased aneuploidy in oocytes from obese mice:

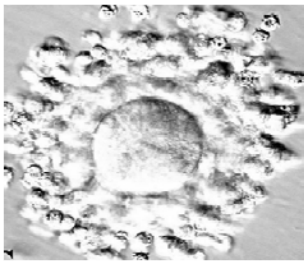




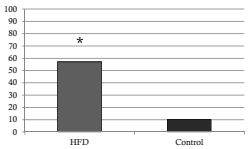


Luzzo et al, unpublished

How does obesity impact cumulus oocyte complex?

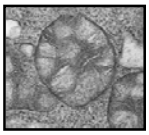


Obesity: altered cumulus cell mitochondria structure

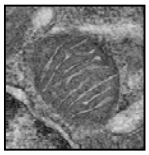


% abnormal mitochondria

*p < 0.001



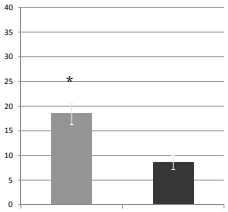
HFD



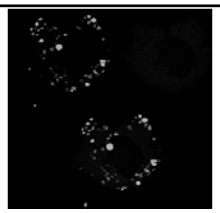
Control

Luzzo et al, unpublished

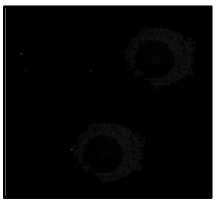
Increased apoptosis in cumulus cells of Obese Mice



% TUNEL positive nuclei



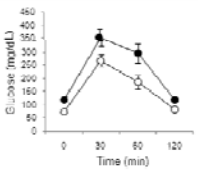
HFD



Control

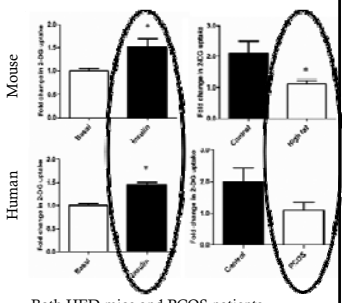
Luzzo et al, unpublished

Obesity leads to insulin-resistance in cumulus cells



New HFD model in ICR mice
fed for 1 month

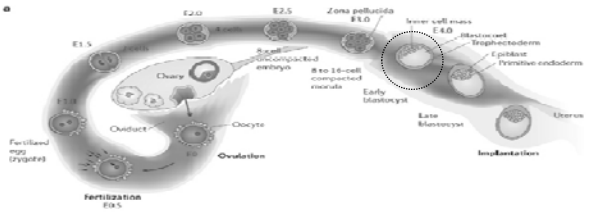
Human and mouse cumulus cells demonstrate insulin sensitivity



Both HFD mice and PCOS patients demonstrate insulin-resistant cumulus cells

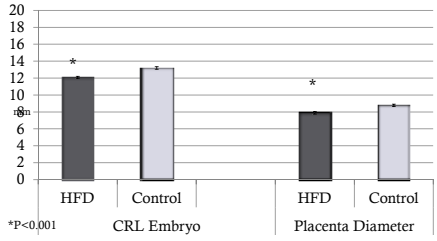
Purcell et al, Endocrinology 2012

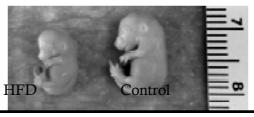
Embryo transfer studies



- Blastocysts have decreased IGF1R expression
- Transferred blastocyst stage embryo from obese to non-obese

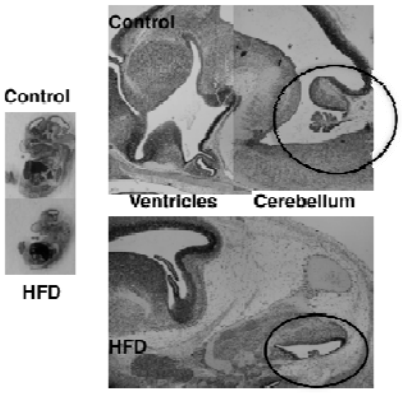
Blastocyst transfer studies from HFD to control





Luzzo et al, unpublished

Abnormal ventricle and choroid plexus development

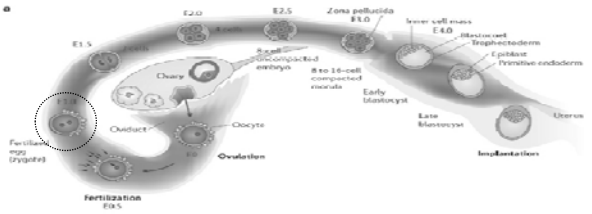


10/10 dams
87 pups

4/10 mice
27 pups

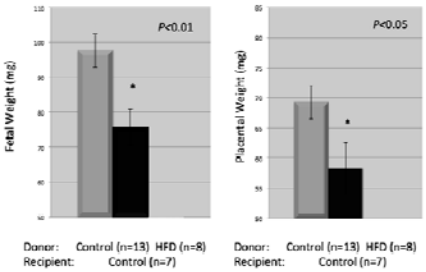
Luzzo et al, unpublished

Embryo transfer studies



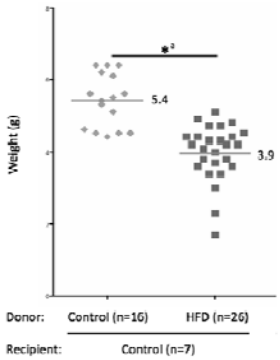
- One-cell zygote transfer from obese to non-obese mouse

One cell zygote transfer—fetal growth lag

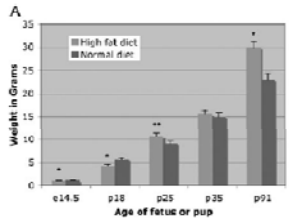


Collaboration with Sasson and Simmons from UPenn

Smaller pups at birth and 3 weeks of age



Obesity and Offspring Growth



- At 13 weeks pups from obese mice: glucose intolerance, increased cholesterol, and higher body fat %.
- Suggests early development of metabolic syndrome

Jungheim et al , Endo 2010

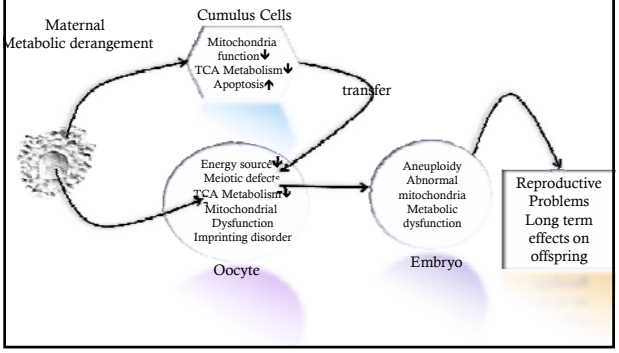
Conclusions

- ✧ Maternal diabetes has adverse effects on pregnancy as early as the oocyte maturation step
- ✧ Energy metabolism of the oocyte is compromised, possibly due to metabolic perturbation in the cumulus cells

Conclusions

- ✧ Energy depletion results in abnormal spindle formation and chromosome misalignment which may manifest as miscarriages in diabetic women
- ✧ Mitochondrial dysfunction may carry over to the next generation resulting in malformations, growth retardation and metabolic syndrome in the offspring

Summary



ACKNOWLEDGEMENTS


Emily Jungheim


Qiang Wang

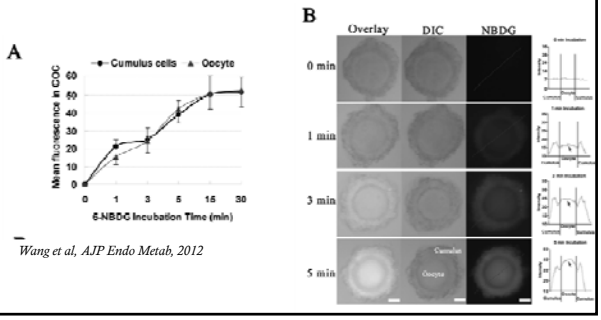

Maggie Chi


Kerri Marquard Luzzo


Scott Purcell

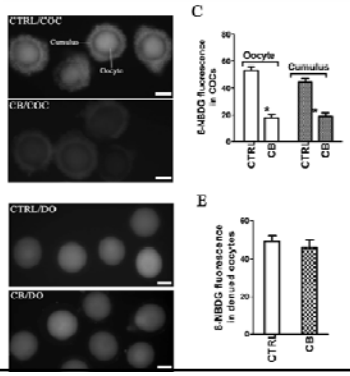
Funding from March of Dimes, NICHD, NIDDK and American Diabetes Association grants

An intracellular pathway for glucose transport into mouse oocytes



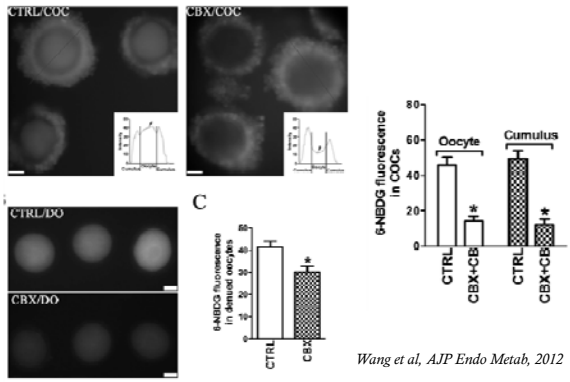
Wang et al, AJP Endo Metab, 2012

CB blocks glucose transport into CC, not oocytes



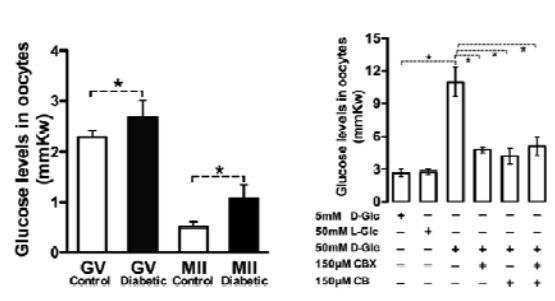
Wang et al, AJP Endo Metab, 2012

CBX blocks transport into oocyte, not CC



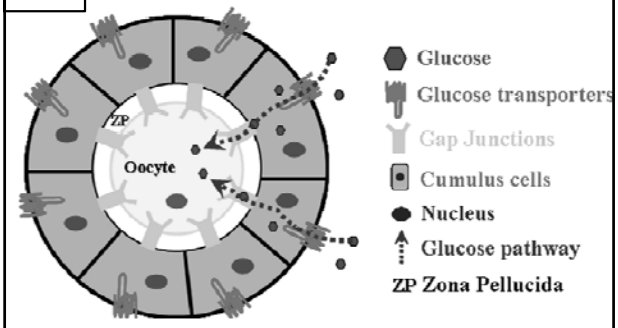
Wang et al, AJP Endo Metab, 2012

Oocyte glucose levels are increased in diabetic oocytes



Wang et al, AJP Endo Metab, 2012

Conclusions



Wang et al, AJP Endo Metab, 2012