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TV5 & TV6

The Canadian Fertility and Andrology Society

Preimplantation Genetic Testing for Aneuploidy in Patients with Low Embryo Numbers: Benefit or Harm?

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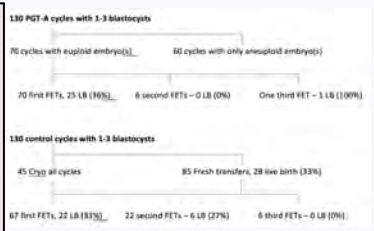
Objective: We sought to explore the utility of preimplantation genetic testing for aneuploidy (PGT-A) in a poor prognosis group of women with few embryos available for transfer.

Introduction: The primary assumption behind PGT-A is that many embryo transfers fail or lead to miscarriage because of aneuploid embryos, so eliminating aneuploid embryos will improve IVF outcomes. Theoretically, the benefits of PGT-A and euploid embryo transfer are to increase the live birth rate per embryo transfer, reduce the risk of miscarriage and shorten the time taken to conceive. In practice, PGT-A cycles eliminate many potential embryo transfers because of an aneuploid, diagnosis. A higher pregnancy rate per embryo transfer may be observed with PGT-A tested embryos but if there are any false positive diagnoses, the cumulative pregnancy rate per cycle could be lower than the alternative of not testing. In a large randomized controlled trial, PGT-A with transfer of euploid embryos was shown to be associated with a higher ongoing pregnancy rate (OPR) per embryo transfer in older women, though this benefit was not seen when OPR was analyzed per oocyte retrieval (Munne 2019). In one positive study, cumulative live birth rates were no different between PGT-A and controls although first embryo transfer live birth rates were higher in the PGT-A group (Rubio 2017). In another study analyzing over 1200 women with 3 or more blastocysts, the cumulative live birth rate was significantly lower in PGT-A compared with controls (Yan 2021). The question arises as to which patients stand to benefit most from PGT-A and which patients may not benefit, or worse, may be harmed by unnecessarily discarding embryos that could become healthy pregnancies. Of particular interest is the subpopulation of patients with a small number of embryos, where a false positive PGT-A result would yield a disproportionate negative impact on live birth outcome.

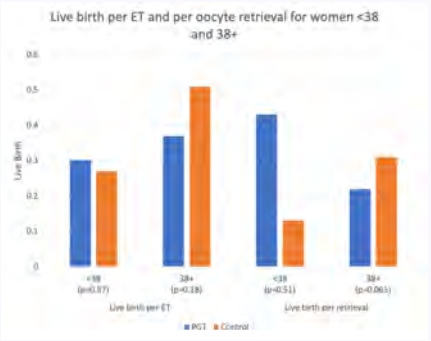
Design: This was a retrospective matched cohort study examining records for first or second-cycle IVF patients with 1 to 3 blastocysts in IVF cycles conducted in 2019 and 2020 and had embryo transfers up until April 2021. The study group comprised 130 patients who underwent PGT-A on all embryos. The control group included 130 patients matched by age, BMI, and blastocyst number and quality who did not undergo PGT-A during the same time-period. Donor oocyte recipients were excluded, as were patients with endometriosis, 2 or more miscarriages, or mosaic embryos.

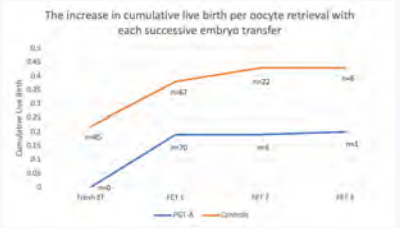


Results: There were no statistically significant differences between the groups in age, BMI, oocyte number, MII oocytes, normally fertilized oocytes or blastocysts. Further, the total number of embryos in each group was identical.

In the PGT-A group, 60 of the 130 cycles (46%) yielded no euploid embryos available for transfer. In the remaining 70 cycles, 90 embryos were euploid and available for transfer. Since no PGT-A testing was done in the control group, there were many more embryos available for transfer. At the time of the data analysis, 180 embryo transfers were performed in the control group (68% of total embryos) vs 77 in the PGT-A group (86% of total embryos).

The clinical pregnancy rate and live birth rate per embryo transfer were no different in the PGT-A and control groups. The miscarriage rate at 23% was the same in both groups. However, with many more transfers in the control group, including 85 fresh transfers, significantly higher cumulative live birth rates per oocyte retrieval were seen in the control group (0.43 vs 0.20).





Conclusion: Our data suggest that PGT-A is not beneficial in women with 3 or fewer embryos. In younger women, pregnancy outcomes per embryo transfer were comparable in PGT-A vs controls but 25% of PGT-A cycles had no euploid embryos and did not proceed to embryo transfer. In older women (38+), the PGT-A group trended to a higher per embryo transfer live birth rate for the few transfers that occurred, but a lower cumulative live birth rate per oocyte retrieval. In these older women, the impact of the large number of cycles with no embryos for transfer (69%) has a disproportionate adverse effect. Finally, our data showed that one of the largest contributions to cumulative live birth rate came from fresh embryo transfers which are not possible in the PGT-A group. The time to pregnancy, rather than being prolonged, may actually be shortened in the control group due to the potential for fresh transfer, and the lack of cancelled cycles due to no euploid embryos. Our data suggests PGT-A has limited utility in the subpopulation of women with few embryos and may cause harm.

GATA binding to the P450 aromatase (*Cyp19a1*) promoter is required for *Cyp19a1* expression and fertility in female mice

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Abstract

GATA factors are a family of transcriptional regulators that control gene expression by binding to consensus nucleotide sequences (A/TGATAA/G, called GATA motifs) that are present throughout the genome. Although originally identified and studied for their roles in the development of the heart and blood and immune cells, GATA factors are now known to be essential in a wide variety of tissues where they act as critical regulators of developmental and tissue- and/or cell-specific gene expression. Dysregulated GATA function has also been linked to several human diseases, including major pathologies affecting women's health such as polycystic ovary syndrome, endometriosis, and breast cancer. In the testis and ovary of most vertebrate species, GATA factors (primarily GATA1/4/6) are exclusive to somatic cells (granulosa, theca, Sertoli, Leydig). Consistent with their strong presence in the gonads, loss-of-function studies in mice have shown GATA factors to be critical for urogenital ridge development, sex determination, steroidogenesis, and fertility in both males and females. While we know that GATA factors are important for reproductive function, we have yet to fully understand their mechanism of action. Knockout studies have generated a list of genes that are likely targeted by GATA factors. The promoter sequences of some of these target genes are also activated by GATA factors in cell line-based assays. Their validation as genuine direct targets of GATA factors has unfortunately been a slow and difficult process. However, the recent availability of CRISPR-based genome editing technologies has made the task of selectively targeting promoter regulatory elements technically more feasible. In the present study, we have used a similar genome editing strategy to inactivate the GATA motifs suspected to control expression of the P450 aromatase (*Cyp19a1*) gene which codes for an enzyme required for estrogen production. *Cyp19a1* GATA mutant male mice did not exhibit any observable phenotypes. In contrast, all adult mutant females were infertile. Ovaries from *Cyp19a1* GATA mutants were filled with hemorrhagic cysts and were devoid of corpora lutea, indicating an absence of ovulation. The uterine horns of the same mice were severely undeveloped, suggesting a lack of circulating estrogens. Indeed, we observed a dramatic decrease in *Cyp19a1* gene expression in mutant ovaries and a corresponding increase in plasma levels of both testosterone and luteinizing hormone. These observations are highly reminiscent of the phenotypes described for *Cyp19a1* knockout mice. Collectively, the data indicate that GATA factors bind to the *Cyp19a1* promoter to drive gene expression, a prerequisite for estrogen biosynthesis and fertility in females. Supported by CIHR project grant PJT-166131.

Context

- GATA factors are a family of transcriptional regulators that control gene expression by binding to consensus nucleotide sequences (A/TGATAA/G, called GATA motifs) that are present throughout the genome.
- In the testis and ovary of most vertebrate species, GATA factors (primarily GATA1/4/6) are exclusive to somatic cells (granulosa, theca, Sertoli, Leydig).
- Consistent with their abundance in the gonads, loss-of-function studies in mice have shown GATA factors to be critical for urogenital ridge development, sex determination, steroidogenesis, and fertility in both males and females.
- Dysregulated GATA function has also been linked to several human diseases, including major pathologies affecting women's health such as polycystic ovary syndrome, endometriosis, and breast cancer.
- Knockout studies have generated a list of genes that are likely targeted by GATA factors. The promoter sequences of some of these target genes are also activated by GATA factors in cell line-based assays. Their validation as genuine direct targets of GATA factors has so far been limited.
- In this study, we have used CRISPR-based genome editing to selectively delete two GATA binding motifs in the *Cyp19a1* PII promoter.
- The *Cyp19a1* gene codes for the aromatase enzyme which catalyses the transformation of androgens to estrogens.
- Female mice lacking *Cyp19a1* are infertile and present an underdeveloped uterus. Their ovaries are abnormal; they show hemorrhagic cysts and lack the presence of corpora lutea (no ovulation).
- CYP19A1 activity is also essential in humans for the function of many organs (brain, bones, heart, etc.) and homeostasis (immunity, metabolism).

Hypothesis

- The *Cyp19a1* PII promoter contains two binding sites for the GATA transcription factors. We hypothesize that endogenous *Cyp19a1* gene expression is regulated by direct binding of GATA factors, especially GATA4 and GATA6.

Results

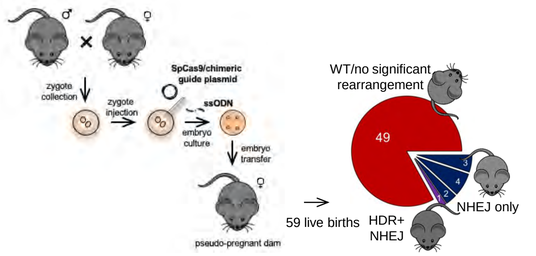

Figure 1. Generation of genetically modified mice using CRISPR/Cas9-based genome editing. A SpCas9/chimeric guide plasmid was used to target and induce a double strand break near the GATA binding elements in the mouse *Cyp19a1* PII promoter; a single-stranded oligodeoxynucleotide (ssODN) was used as a donor for homology-directed repair (HDR) of the DNA, introducing the mutation of the GATA element (TGATAG → TCCTAG). A total of 59 mice were born from genetically modified embryos: 49 were wild type, 9 showed a 36bp deletion of the GATA elements on one or both alleles resulting from non-homologous end joining (NHEJ) and one bore the desired deletion along with a 37bp deletion.

Figure 2. Schematic representation of the proximal region of the mouse *Cyp19a1* PII promoter. Potential transcriptional factors binding sites are indicated on the wild type sequence (WT). The 36bp deletion resulting from NHEJ present in the mutated sequence is schematized by the red triangle. The mutation includes a double GATA motif and one binding site for NR5A family (SF-1/LRH-1) members.

MUT Females Show Abnormal Ovarian Histology

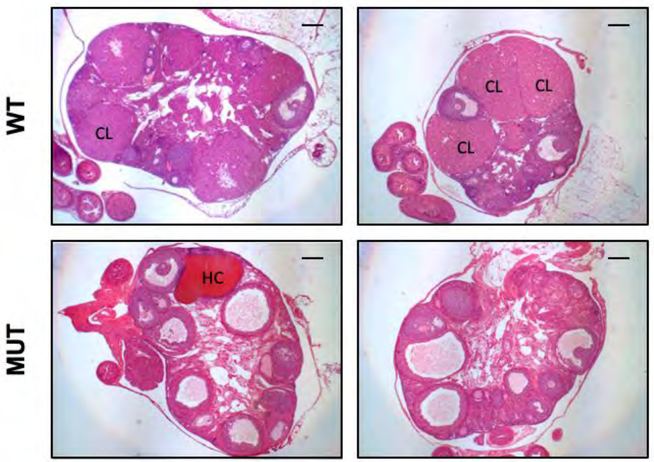

Figure 3. Histological comparison between WT and p*Cyp19a1*^{del36} mice ovaries. Photos of p90 adult mice ovaries magnified 50X and colored with hematoxylin and eosin. We can see that the wild type (WT) mice show multiple corpora lutea (CL) while the p*Cyp19a1*^{del36} (MUT) mice show none. The p*Cyp19a1*^{del36} mice show hemorrhagic cysts (HC) while the WT mice show none. Scale bar = 200 µM.

Estrogen (E2) Treatment Rescues Female Reproductive Tract Histology

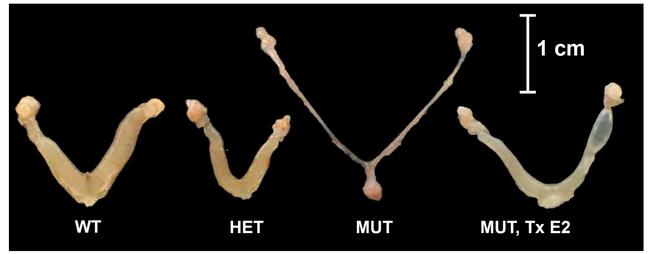

Figure 6. Gross dissection of the reproductive tracts of wild type (WT), heterozygous (HET), p*Cyp19a1*^{del36} (MUT), and p*Cyp19a1*^{del36} mice treated with estradiol (MUT, Tx E2). Female mice were sacrificed at 90 days of age and their reproductive tracts were retrieved. The tracts from the WT and HET mice show a normal phenotype while the tract form the MUT mouse appears underdeveloped and juvenile in appearance. Treatment with E2 restores reproductive tract histology in the MUT animal. In contrast to females, male p*Cyp19a1*^{del36} (MUT) mice are fertile and do not show any phenotype at a similar age.

Cyp19a1 Gene Expression is Decreased in MUT Ovaries

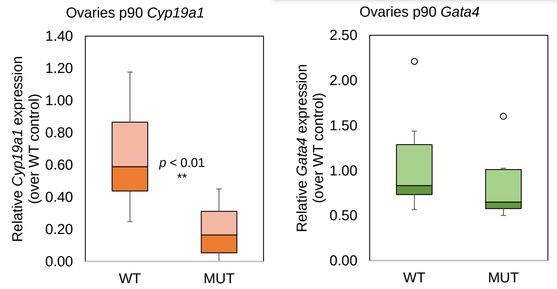

Figure 4. Quantification of *Cyp19a1* and *Gata4* mRNA transcripts in p90 ovaries from WT and mutant mice. *Cyp19a1* transcripts are 73% less abundant in the p*Cyp19a1*^{del36} (MUT) mice than in the wild type (WT) mice. The abundance of *Gata4* transcripts is not significantly different between the WT and MUT mice. Quantitative data were normalized using reference genes *Polr2a*, *Ppia* and *Rplp0* and expressed as fold change over WT ± S.E.M. The primers used for Asterisks indicate a significant difference between the WT and MUT mice.

Hormone Levels are Abnormal in MUT Females

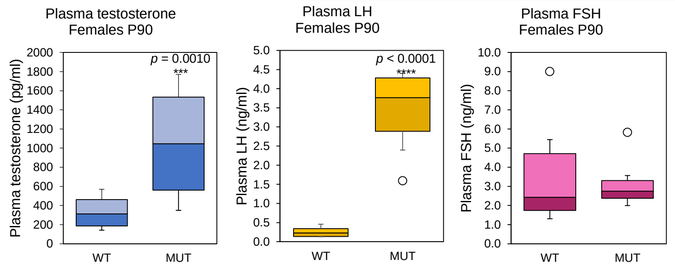

Figure 5. Serum testosterone and LH levels are about 3 times higher in adult p*Cyp19a1*^{del36} mice than in WT mice. ELISA assays were used to quantify serum testosterone (T), luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels in adult wild type (WT) and homozygous p*Cyp19a1*^{del36} (MUT). Serum T and LH levels are about 3 times higher in MUT mice than in WT mice while the serum FSH levels are not significantly different. Asterisks indicate a significant difference between WT and MUT female mice.

Summary and Conclusion

- Using a CRISPR-Cas9 genome-editing techniques, we successfully created transgenic mice with a 36bp deletion removing the double GATA motif and one SF-1 binding site in the proximal promoter of the *Cyp19a1* gene (p*Cyp19a1*^{del36}).
- All female p*Cyp19a1*^{del36} mice examined were infertile. They showed an underdeveloped reproductive tract. Their ovaries are abnormal; they show hemorrhagic cysts and lack the presence of corpora lutea (no ovulation).
- We also observed a dramatic decrease in *Cyp19a1* gene expression in mutant ovaries and a corresponding increase in plasma levels of both testosterone and luteinizing hormone.
- These observations are highly reminiscent of the phenotypes described for *Cyp19a1* knockout mice.
- Collectively, the data indicate that GATA and NR5A actors bind to the *Cyp19a1* promoter to drive gene expression, a prerequisite for estrogen biosynthesis and fertility in females.

Acknowledgments

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- JP is supported by studentships from FRQS, Fondation du CHU de Québec and CRDSI.

Does gonadotropin composition affect outcomes in women with Polycystic Ovarian Syndrome (PCOS)?

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Introduction:

Pathophysiologically, PCOS is characterized by increased LH pulsatility (higher frequency in all forms and higher pulse amplitudes in non-obese individuals) lending to higher serum LH levels. In turn, the increased LH promotes higher androgen levels potentially negatively affecting follicle/oocyte quality. Theoretically then, FSH-only preparations for IVF stimulation in PCOS may have better outcomes. Our study was intended to evaluate IVF outcomes in PCOS patients receiving IVF stimulation with FSH+/-LH.

Methods:

- Single university centre retrospective. Cohort study conducted between January 2013 and December 2019
- Comparing outcomes of PCOS patients receiving freeze-all GnRH-antagonist IVF cycles with GnRH-agonist trigger with either r-FSH alone or r- FSH and hMG stimulation
- 2003 Rotterdam diagnostic criteria used to identify study participants
- data was analyzed using T-test based on Levene's test for equality of variances or chi-squared tests. The two groups were matched for baseline demographics preventing the need for logistic regression analysis

Results:

Overall, 44 women received r-FSH and LH(from hMG) while 38 received r-FSH alone. There were no significant differences (p>0.05) in baseline characteristics other than serum TSH (1.79±1.03 vs. 3.63±8.26 mIU/L, p=0.05), which was not clinically significant (Table 1).

Patient Baseline Characteristics in the Treatment Groups

Variable	FSH and LH (n=44)	FSH only (n=38)	P Value
Gravida	0.64±1.123	0.55±0.795	0.129
Parity	0.16+0.428	0.26±0.601	0.063
Age (Years)	30.75±2.821	30.21±3.550	0.345
Male Age	33.88±5.003	35.71±6.834	0.247
Duration of infertility	3.029±2.256	3.308±2.549	0.607
FSH	6.063±1.725	5.4528±1.524	0.980
LH	9.440±6.681	7.794±5.242	0.454
Estradiol	247.457±349.9224	216.411±146.610	0.560
Prolactin	10.439±5.424	10.849±4.314	0.639
TSH	1.793±1.034	3.633±8.262	0.050
Total Testosterone (nmol/L)	1.863±1.158	1.441±0.687	0.098
Free Testosterone (nmol/L)	0.804±0.577	0.642±0.417	0.212
AFC	45.86±21.266	44.55±17.299	0.856
Sperm Volume	2.878±1.336	2.576±1.411	0.671
Sperm Concentration	44.785±39.153	35.724±32.329	0.265
Sperm Motility	44.342±23.730	38.171±30.212	0.038

When comparing the r-FSH alone group to the r-FSH and LH activity group, there were no differences between peak serum estradiol, maximum endometrial thickness, total oocytes collected, number of MII oocytes obtained, 2PNs and blastocysts frozen. There was a difference in stimulation length (8.3±1.6 vs. 7.3±3 days/p=0.004), total dose of FSH (1271.45±376.79 vs. 1407.72±645.34 IU/L/p=0.02), and follicles stimulated (37±16 vs. 36±10/p=0.008). (Table 2).

For primary outcomes, There were no significant differences in: pregnancy rates after first transfer, clinical pregnancy rates after first transfer, live birth rates after first transfer and cumulative clinical pregnancy rates.
However, cumulative live birth rates per cycle favoured no LH activity (77% vs. 56%/ p=0.044).

Table 2: Cycle Characteristics and IVF Stimulation Outcomes

Variable	FSH and LH (n=44)	FSH only (n=38)	P Value
Cycle Length (Days of FSH)	7.27±3.371	8.34±1.632	0.004
Total dose of FSH (IU)	1407.72±645.399	1271.45±376.788	0.02
Peak E2 (pg/mL)	13231.318±6858.882	11716.158±5085.505	0.220
Endometrial thickness (mm)	10.174±1.768	10.421+±2.390	0.257
Total Follicles	36.91±15.88	35.95±9.71	0.008
Oocytes Collected	26.95±9.36	28.42±9.731	0.837
Number of MII oocytes	20.02±7.94	21.55±8.153	0.861
Number of 2PN	14.70±7.675	16.71±7.067	0.986
Number of embryos frozen?	7.95±4.851	7.71±5.03	0.571

Table 3: Pregnancy Outcomes

	FSH and LH (n=44)	FSH only (n=38)	P value
Cumulative clinical pregnancy	16	17	0.441
Cumulative Clinical Pregnancy Rate	36.3%	44.7%	0.441
Cumulative Live Birth			
Cumulative Live Birth Rate	56%	77%	0.044
	FSH and LH (n=33)	FSH only (n=29)	P Value
Number of pregnancies following first transfer	20	16	
	60.6%	55.2%	0.665
Number of clinical pregnancies following first transfer	14	11	
	42.4%	37.9%	0.719
Live births following transfer	11	7	
Live birth rate following transfer	33.3%	24.1%	0.426

Conclusion:

On review of the current literature, the present study is one of few examining IVF and pregnancy outcomes in PCOS patients receiving GnRH antagonist cycles with recombinant FSH alone or combined with LH activity. Although the study population is small in number, a significant difference in cumulative live birth rate was observed favouring recombinant FSH alone versus combination. This appears to be biologically plausible with the the theoretical negative effect of excess LH on oocyte or embryo quality potential and further emphasizes the need for larger and better powered studies to examine IVF stimulation outcomes in PCOS patients with FHS+/-LH activity.

A comparison of the developmental competence of in vitro-produced mouse embryos under different O_2 concentrations with in vivo-developed blastocysts

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Introduction

- In clinical IVF programs, embryos undergo extended culture until the blastocyst stage (~6 days) under 5% or 2% O_2 concentrations to facilitate success of single embryo transfer.
- Modest success rates of these culture systems warrant further research to refine culture conditions.
- Implementation of 2% O_2 systems is an emerging field that must be critically evaluated for stress responses of embryos to excessive hypoxia.
- O_2 availability and ROS production may influence transcriptional and epigenetic regulation during preimplantation embryo development.

Objective and Hypothesis

- **Objective:** To characterize stress-related cellular responses and developmental competence of mouse blastocysts developed *in vitro* by culturing embryos under 5% and 2% O_2 in comparison to *in vivo*-derived blastocysts using a CD1 mouse model.
- **Hypothesis:** 2% O_2 culture compromises developmental competence through activation of embryonic stress response and apoptosis genes relative to 5% O_2 and *in vivo*-derived blastocysts.

Experimental Methodology

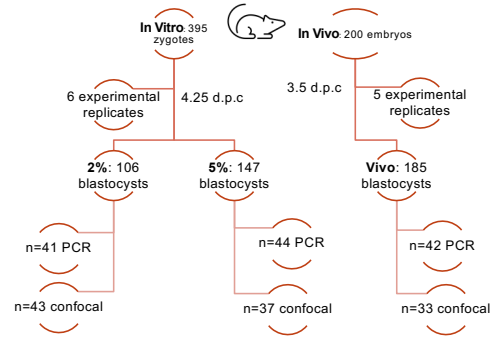

Figure 1. Experimental design

Figure 2. Experimental setup for pre-implantation embryo culture: Sealable glass jars connected to premixed gas cylinders containing either 5% O_2 /5% CO_2/90% N_2 or 2% O_2/5% CO_2/93% N_2 and incubated within a tissue culture incubator set to 37˚C with high humidity.

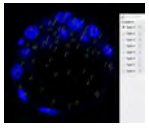

Figure 3. Example of total cell count workflow on FIJI using the cell counter tool on a mouse blastocyst. Each cell within each z-stack was counted and counter marks remained between stacks to ensure accurate counting.

- CD1 mouse zygotes were cultured 4.25 d.p.c under either 5 or 2% O_2.
- For the *in vivo* cohort, CD1 females with plugs were maintained for 3.5 d.p.c and blastocysts were harvested.
- Blastocysts were either transferred to 4% PFA for fixation or to Extraction Buffer for RNA extraction and subsequent cDNA synthesis for RT-qPCR.
- Caspase-3 immunofluorescence and DAPI staining was used to assess apoptosis and cell counts via spectral confocal microscopy.
- Blastocyst development rates were compared using a Brown-Forsythe and Welch One-Way ANOVA with Dunnett's T3 multiple comparisons test.
- Gene expression results, total cell count, ratio blastocoel area, ratio mean diameter, mean gray intensity, and mean integrated density were analyzed using a One-Way ANOVA with Tukey's post-hoc test.

Results

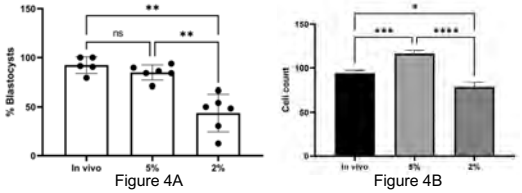

Figure 4A Figure 4B

- Figure 4: A) The mean % of blastocyst development for the *in vivo* group and zygotes cultured under 5% O_2 were higher than culture under 2% O_2; and B) embryos cultured under 5% O_2 had more cells within each blastocyst relative to those cultured under 2% O_2 and *in vivo*-derived blastocysts.

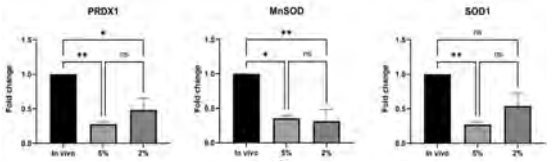

Figure 5A Figure 5B Figure 5C

- Figure 5: Expression levels of A) PRDX1, B) MnSOD, and C) SOD1 mRNAs were higher in blastocysts from the in vivo group relative to cultured embryos.

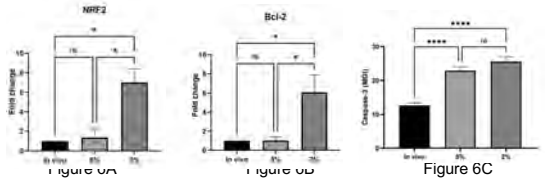

Figure 6C

- Figure 6: Expression levels of A) NRF2 and B) Bcl-2 mRNAs were significantly higher in embryos cultured under 2% O_2; and C) *In vivo*-derived blastocysts had lower Caspase-3 expression relative to embryos cultured under 5 or 2% O_2.

Figures 4-6: *P < 0.05; ** P < 0.01; *** P < 0.001; **** p < 0.0001

Conclusions

- In vitro culture of mouse embryos under 2% O_2 compromised developmental competence of resulting blastocysts relative to culture under 5% O_2 or in vivo-derived blastocysts through altered expression of stress-response genes; embryos may respond to oxidative stress via an NRF-2 mediated pathway.
- In vitro embryo culture under 5% or 2% O_2 attenuates antioxidant gene expression and results in greater apoptotic response relative to *in vivo*-derived blastocysts.
- 5% O_2 systems may be optimal for culture of mouse embryos, warranting critical re-evaluation of culturing human embryos under continuous 2% O_2 concentrations during clinical IVF programs.

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Characterization of a novel mouse Cre line exclusive to Leydig cells

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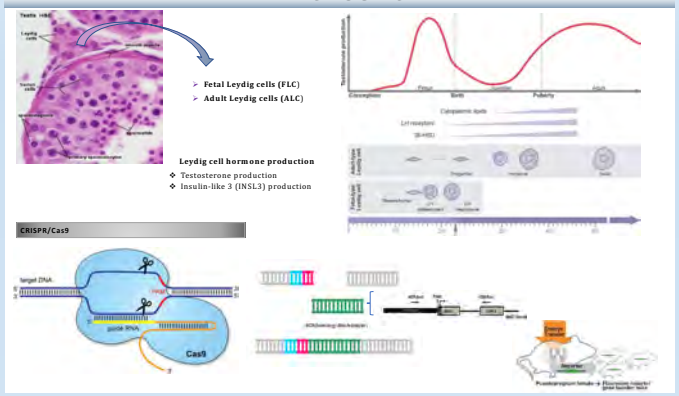
ABSTRACT

In the testis, Leydig cells regulate male sex differentiation and fertility through the production of the Insulin-like 3 (INSL3) and testosterone hormones. INSL3 regulates testis descent during fetal life and is a highly specific marker of Leydig cells. Testosterone controls virilization of the male fetus and the initiation and maintenance of spermatogenesis. At least two distinct populations of Leydig cells are responsible for the production of these two hormones during fetal and postnatal life: fetal Leydig cells (FLCs) and adult Leydig cells (ALCs). FLCs atrophy shortly after birth. ALCs derive from undifferentiated precursors that are believed to be present during fetal life but start to differentiate prior to puberty. Whether the different Leydig cell populations share a common origin and whether they contribute to the development of each other, however, remain unknown. These important questions, along with the study of Leydig cell gene function, have long been hampered by the lack of suitable mouse models that can exclusively target Leydig cells. Since INSL3 is unique to Leydig cells of both fetal and adult populations, we have used CRISPR/Cas9 gene editing to knock-in an improved Cre recombinase (iCre) into the *Insl3* locus. Constitutive iCre mice were crossed with mice containing a lox-stop-lox-TdTomato reporter transgene. TdTomato is visualized as a red fluorescent protein when the intervening stop codon is removed by the Cre recombinase. Testes were harvested at different developmental timepoints and then frozen and cut into 6 µm sections. Red fluorescent cells were detected in both FLCs and ALCs (confirmed by CYP17A1 immunofluorescence) and at all the corresponding fetal and postnatal ages where they are normally active. No fluorescence was observed in any other fetal or adult tissue examined (hypothalamus, adrenal, pituitary, heart, skeletal muscle, liver, lung, brain, stomach, gut, skin). The Leydig cell-exclusive iCre driver will be a useful tool for generating fluorescent Leydig cells that can then be isolated from mice at different times during development by simple FACS sorting. The iCre line will also be invaluable for performing targeted loss-of-function studies (gene knockouts) in Leydig cells. This new Cre line represents a significant contribution to the andrology field that will allow us to finally address fundamental questions about the biology and gene function of Leydig cells that have remained unanswered for decades (supported by CIHR MOP-81387).

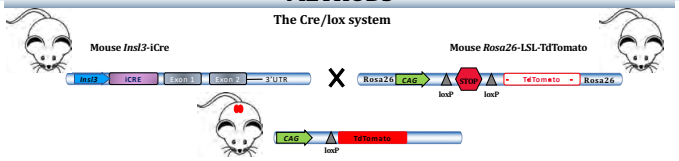
INTRODUCTION



OBJECTIVE

The objective of this study is to perform a detailed characterization of the developmental and post-natal expression/activity profile of iCre recombinase in a novel Leydig exclusive transgenic mouse line.

METHODS



RESULTS

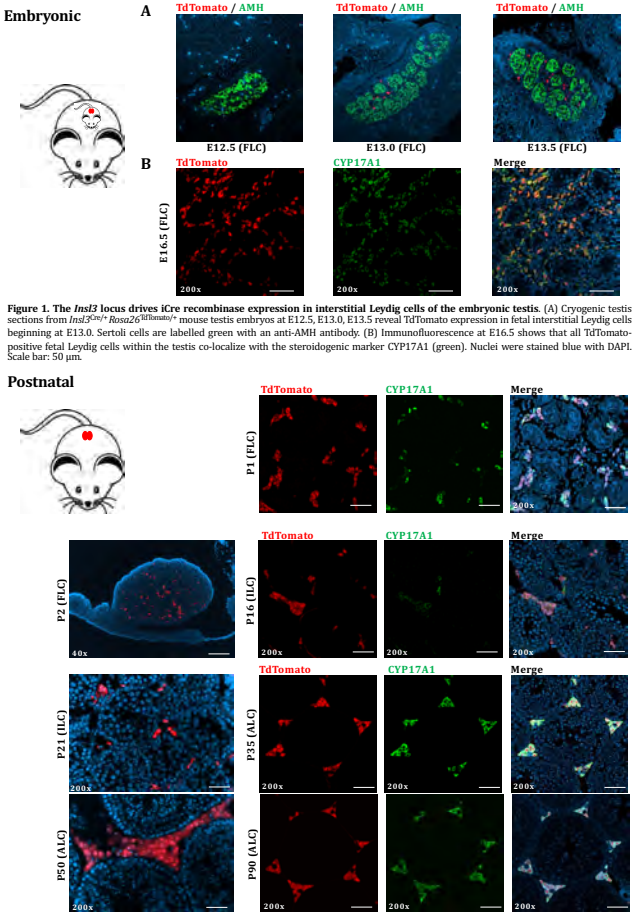


Figure 1. The *Insl3* locus drives iCre recombinase expression in interstitial Leydig cells of the embryonic testis. (A) Cryogenic testis sections from *Insl3*$^{iCre/+}$*Rosa26*$^{TdTomato/+}$ mouse testis embryos at E12.5, E13.0, E13.5 reveal TdTomato expression in fetal interstitial Leydig cells beginning at E13.0. Sertoli cells are labelled green with an anti-AMH antibody. (B) Immunofluorescence at E16.5 shows that all TdTomato-positive fetal Leydig cells within the testis co-localize with the steroidogenic marker CYP17A1 (green). Nuclei were stained blue with DAPI. Scale bar: 50 µm.

Figure 2. The TdTomato fluorescent protein remains exclusive to Leydig cells in the mouse postnatal testis. Cryogenic sections from *Insl3*^{iCre};*Rosa26*-TdTomato$^{flox/+}$ mouse testis from different postnatal ages were analysed for the presence of TdTomato (red). Immunofluorescence for CYP17A1 (green) was assessed at differents ages. Nuclei were stained blue with DAPI. Scale bar: 50 µm or 200 µm.

No ectopic TdTomato expression in other tissues

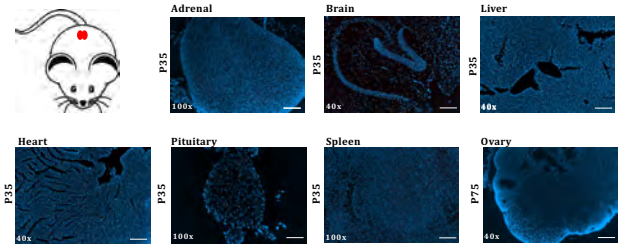


Figure 3. Cre-mediated recombination is undetectable in extratesticular tissues from *Insl3*^{iCre};*Rosa26*-TdTomato$^{flox/+}$ transgenic mice. Representative cryosections show that TdTomato is absent several peripheral tissues tested from *Insl3*^{iCre};*Rosa26*-TdTomato$^{flox/+}$. Nuclei were stained blue with DAPI. Scale bar, 50 µm or 100 µm.

Without iCre, TdTomato is not expressed

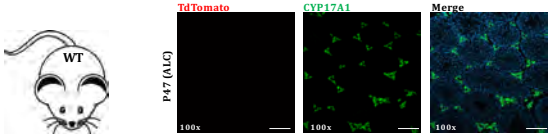


Figure 4. Absence of TdTomato fluorescence in Leydig cells from *Insl3*$^{+/+}$;*Rosa26*-TdTomato$^{flox/+}$ adult transgenic mice. Immunofluorescence was performed on cryogenic testis sections for CYP17A1 (green). No expression of TdTomato (red) is found in Leydig cells in the absence of iCre expression. Nuclei were stained blue with DAPI. Scale bar: 100 µm.

SUMMARY & CONCLUSIONS

The presence of TdTomato only in Leydig cells indicates that the iCre recombinase is expressed and active exclusively in these cells at all ages during fetal and postnatal life and therefore in both populations of Leydig cells. Moreover, we show that iCre expression appears as early as E13.0 in the embryonic testis demonstrating the extraordinary potential of this transgenic mouse to knockout genes in fetal Leydig cells soon after their commitment, which occurs at E12.5. This is the first mouse model expressing an iCre recombinase exclusively in Leydig cells. This new mouse model will be invaluable to perform experiments that were impossible until now, including the isolation of Leydig cells by FACS (both FLC and ALC) and the inactivation of genes of interest exclusively in Leydig cells. These mice represent a significant contribution to the field and will help answer fundamental questions about the biology and function of Leydig cells.

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ACKNOWLEDGEMENTS

We would like to thank the members of our lab for their critical inputs and funding agencies for their support.

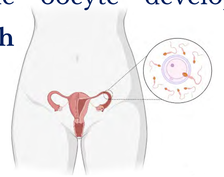
The Impact Of Maternal Metabolic Stress On Embryo Transcriptome: Implication Of Epigenetics

Julie-Pier Robichaud, Marc-André Sirard

Centre de Recherche en Reproduction, Développement et Santé Intergénérationelle (CRDSI), Département des Sciences Animales, Université Laval, Quebec

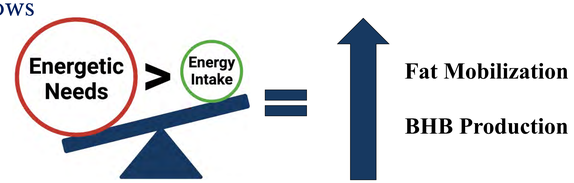
Environment

The **environment** in which the oocyte develops influences **fertility and oocyte health**



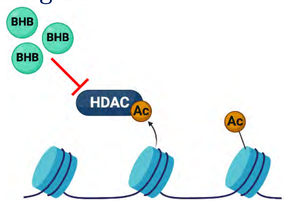
Metabolism

Negative Energy Balance leads to an increase in **BHB** due to **higher mobilization of fat** during conditions of clinical ketosis in dairy cows



Metaboloepigenetics

Metabolites such as **BHB** can regulate genome expression by modulating epigenetic regulators :



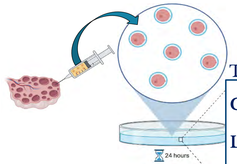
By inhibiting deacetylation, **BHB** can **activate genome expression**

Goal

Investigate the potential implication of epigenetics in the transmission of maternal metabolic information to the embryo in vitro

Methodology

1- Ovary punction & Maturation



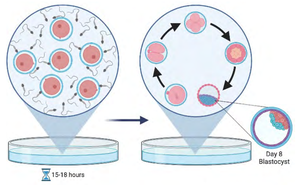
Treatments

CTL : 5.5mM glucose

LG : 2.75mM glucose

LG + BHB : 2.75mM glucose + 1.8mM BHB

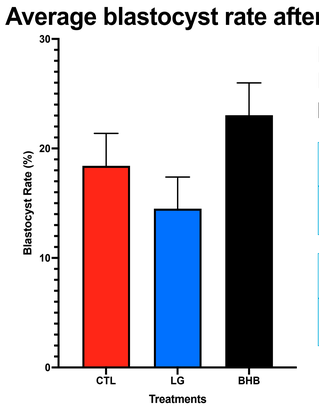
2- IVF & embryo development



3- Genomic extraction & Sequencing

Results

1- BHB exposure may have compensatory effect on blastocyst rates



One-Way ANOVA	P-Value	Significance
All Treatments	**0,0873**	**ns**

T-Test	P-Value	Significance
LG vs. BHB	**0,318**	*****

n=9

Fig. 1 : Blastocyst rate of 265 oocytes (from 78 bovine ovaries) treated with 5.5mM glucose (CTL), 2,75mM glucose (LG) or 2,75mM glucose with 1.8mM BHB (LG + BHB). *Data is presented as average value ± standard deviation*

Results

2- Glucose availability influences transcriptome of blastocysts

Contrasts Studied : CTL vs. LG; CTL vs. BHB; LG vs. BHB

Number of molecules (p-value < 0,05)	CTL VS. LG	CTL VS. BHB	LG VS. BHB
Upregulated	49	28	16
Downregulated	17	22	27
Total	66	50	43

Upregulated in BHB – **Downregulated** in LG

Cellular Function			
Cell Adhesion	CYTIP, KIRREL2	**Lactation**	OAS2
Metabolic Processes	GRB14, HOGA1, LANCL3, SARDH	**Sperm motility**	CFAP251, IQUB
Differenciation & Growth	NPAS2, INHA	**Gamete Formation**	IHO1
Tyrosine phosphatase signaling	PTPRR	**Ubiquitination**	ASB2
G protein-coupled signaling	ADGRL4	**RNA Binding**	PABPC5

Conclusion

- **BHB** seems to influence **blastocyst rates**
- **Glucose availability** has a more important **impact on transcriptome;** BHB exposure seems to minimize the impact on the developmental rate

This project allows us to further understand how metabolic conditions such as obesity could impact the health of the embryo during IVF

Acknowledgements

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Isabelle Laflamme
Hélène Martin
Meihong Shi

CRSNG NSERC

Pregnancy complications and placental histology in in-vitro fertilization pregnancies with initial low serum β-HCG levels

Hadas Ganer Herman[1,3], Alexander Volodarsky-Perel[1,3], Tuyet Nhung Ton Nu[2], Alexandre Machado-Gedeon[1], Yiming Cui[1], Jonathan Shaul[1], Michael H. Dahan

[1]Department of Obstetrics and Gynecology and [2]Department of Pathology, McGill University, Montreal, Quebec, Canada.

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Introduction:

The correlation between initial low b-HCG and pregnancy viability is much established, yet less so regarding outcomes in viable pregnancies.

We aimed to assess obstetric outcomes and placental findings following in vitro fertilization (IVF) with an initial low serum β-human chorionic gonadotropin (β-HCG).

Materials and Methods:

- Retrospective cohort of live singleton deliveries after IVF between 2009 and 2017 at a single university medical center.
- Inclusion - pregnancies following IVF, exclusion - oocyte recipients, preimplantation genetic testing and pregnancies with two initial gestational sacs.
- Obstetric outcomes and placental findings were compared between:
  - pregnancies for which day 16 embryo age β-HCG following transfer was low, defined as the lower 10th percentile for the cohort (low β-HCG group)
  - patients with an initial β-HCG above the lower 10th percentile (control group).
- Placental examination was performed for all deliveries during the study period, irrelevant of complication status, and findings categorized according to the Amsterdam Placental Workshop Group Consensus.
- Continuous and categorial variables were compared as appropriate and presented as mean ± SD or n (%) as appropriate. Multivariate regression analysis employed to control for confounders.

Results :

- The lower 10th percentile of β-HCG results was 149 mUI/mL. There were 103 cases in the low β-HCG group, and 928 in the control group.

Table 1 – patient demographics and cycle characteristics

	Lower β-HCG group n=103	Controls n-928	P
Age, (years), mean ± SD	35.8 ± 4.2	35.5 ± 4.2	0.43
BMI (kg/m2), mean ± SD	24.9 ± 2.3	25.0 ± 2.2	0.73
Gravidity, median (range)	2 (1-9)	2 (1-16)	0.43
Parity, median (range)	0 (0-3)	0 (0-4)	0.36
Paternal age, mean ± SD	38.0 ± 5.3	39.3 ± 6.0	0.04
Fresh embryo transfer, n (%)	66 (64.0%)	583 (62.8%)	0.80
Intracytoplasmic sperm injection, n (%)	63 (66.3%)	612 (74.0%)	0.04
Blastocyte transferred, n (%)	68 (66.0%)	703 (75.7%)	0.03

- Maternal demographics were non-significant between the groups, yet paternal age was higher and ICSI and blastocyte transfer were more common in the control group.

Table 2 – obstetric outcomes and placental findings

	Lower β-HCG group n=103	Controls n-928	P
Preterm birth, n (%)	16 (15.5%)	76 (8.1%)	0.01
Birth weight (grams), mean ± SD	3183 ± 589	3302 ± 606	0.06
Low birth weight, n (%)	12 (12.0%)	64 (7.1%)	0.07
Single umbilical artery, n(%)	4 (3.8%)	6 (0.6%)	0.01
Velamentous cord insertion , n (%)	20 (19.4%)	72 (7.7%)	<0.001
Maternal vasculopathy, n (%)	11 (10.6%)	45 (4.8%)	0.001

- An increased rate of preterm births deliveries was found in the low β-HCG group, 15.5% vs. 8.1%, p=0.01, which maintained significance after adjustment for confounders.
- Placentas in the low β-HCG group were characterized by a higher rate of velamentous cord insertion, 19.4% vs. 7.7%, p<0.001, and single umbilical artery 3.8% vs. 0.6%, p=0.01

Table 3 – Adjusted odds ratio of lower β-HCG for preterm birth, single umbilical artery, velamentous cord insertion and maternal vasculopathy

	OR (95%CI)
Model I - Preterm birth	2.08 (1.12-3.861-
Model II – Single umbilical artery	9.04 (2.14-38.17)
Model III - Velamentous cord insertion	3.11 (1.74-5.56)
Model IV - Maternal vasculopathy	2.80 (1.36-5.75)

OR: odds ratio, C.I: confidence interval
Adjusted for paternal age, intracytoplasmic sperm injection and blastocyte transfer

Conclusions:

- **IVF pregnancies with an initial low β-HCG were associated with a two-fold increase in the risk of preterm birth and placental gross and histological changes.**
- **It may thus be considered to follow such cases by high-risk pregnancy specialists.**

Association of Female Age and Semen Parameters with Euploid Blastocyst Rate

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INTRODUCTION

Elective single euploid embryo transfer has become the method of choice to reduce the risk of multiple births and miscarriages. Recent report from the Society for Assisted Reproductive Technology (SART) has shown an increasing number of patients across all age groups continue to choose elective single embryo transfer (eSET), which critically decreases the risk of multiple births, with its use increasing to 78.4% of transfer cycles in 2020, up from 75% in 2019. Maternal age, ovarian stimulation, in vitro gamete manipulation, gamete aging, high oxygen tension during culture, oocyte immaturity, Severe male factor infertility and poor sperm parameters are correlated with embryo aneuploidy rate.

OBJECTIVE

The objective of this study was to determine the associations between maternal age, sperm concentration and sperm motility in couples undergoing ICSI and PGT-A cycles at a single tertiary care facility.

MATERIALS AND METHODS

The data from 89 couples (89 cycles) from Jan 2021 to March 2022 were analyzed. A total of 345 blastocysts (157 euploid and 188 aneuploid) were available. Female age ranged from 25 to 45 years. The couples were divided into 8 groups: A (cycles with female age < 35 years and sperm concentration < 5 M/mL), B (Female age < 35 years and sperm concentration ≥ 5 M/mL), C (Female age ≥ 35 years and sperm concentration < 5M/mL), D (Female age ≥ 35 years and sperm concentration ≥ 5M/mL), E (Female age < 35 years and sperm motility < 25%), F (Female age < 35 years and motility ≥ 25%), G (Female age ≥ 35 years and sperm motility < 25%), and H (Female age ≥ 35 years and sperm motility ≥ 25%). For each group total number of blastocysts, and percent euploid blastocysts were calculated. Comparisons between groups were made using t-test.

RESULTS

Female age is the single most important factor affecting the blastocyst aneuploidy rates in vitro. The differences between groups based on sperm count and motility were not statistically significant. However, significantly more euploid blastocysts were available for women younger than 35 years of age as compared to those couples where female partners were ≥ 35 years of age ($p < 0.05$).

Table 1: Comparison of Euploid Blasts in different groups

F. Age (Yrs)	Group	Sperm parameter	Patients (N=89)	Total Blasts	No. Euploid	% Euploid	Mean ± SD Euploid
<35	A	Conc <5 M/mL	16	104	50	48.1	3.1 ± 2.8
	B	Conc ≥5 M/mL	22	126	58	46.0	2.6 ± 2.4
≥35	C	Conc <5 M/mL	24	78	25	32.0	1 .0 ± 1.1
	D	Conc ≥5 M/mL	27	97	24	24.7	1.1 ± 1.2
<35	E	Motility <25 %	15	85	56	65.9	3.7 ± 3.0
	F	Motility ≥25 %	18	122	47	38.5	2.6 ± 2.1
≥35	G	Motility <25 %	27	111	30	27	1.1 ± 1.2
	H	Motility ≥25 %	29	87	24	27.6	0.8 ± 1.1

CONCLUSION

The results of this study indicate that females ≥ 35 years of age are likely to have significantly lower number of euploid blastocysts. While poor sperm concentration and motility were not significantly associated with aneuploidy rates, they might have synergistic effect on embryo aneuploidy rates.

Thinner endometriums in IVF cycles, how do they affect third trimester pregnancies and placentas?

Hadas Ganer Herman[1,3], Alexander Volodarsky-Perel[1,3], Tuyet Nhung Ton Nu[2], Alexandre Machado-Gedeon[1], Yiming Cui[1], Jonathan Shaul[1], Michael H. Dahan[1]

[1]Department of Obstetrics and Gynecology and [2]Department of Pathology, McGill University, Montreal, Quebec, Canada.
[3]The Sackler faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

Introduction:

We aimed to assess obstetric outcomes and placental findings following in vitro fertilization (IVF) with a thinner endometrial lining.

Materials and methods:

- This was a retrospective cohort of live singleton deliveries after IVF between 2009 and 2017, at a single university medical center.
- We included only pregnancies following autologous IVF cycles.
- Obstetric outcomes and placental findings were compared between IVF pregnancies with a maximum endometrium thickness <9 mm (thinner endometrium group), and cases with an endometrium ≥9 mm (control group).
- Placental examination was performed for all deliveries during the study period, irrelevant of complication status. Placental pathologic findings were categorized according to the Amsterdam Placental Workshop Group Consensus. Outcomes were placental findings, including anatomic, inflammatory, vascular malperfusion and villous maturation lesions and obstetric and obstetric outcomes.
- Multivariate regression analysis was employed to control for confounders.

Results:

- Included were 292 cases in the thinner endometrium group, and 765 in the control group.

Table 1 – patient demographics and cycle characteristics

	Thinner endometrium n= 292	Controls n-765	P
Age, (years), mean ± SD	35.9 ± 4.2	35.4 ± 4.2	0.06
Gravidity, mean ± SD	2.3 ± 1.7	2.1 ± 1.3	0.04
Parity, mean ± SD	0.40 ± 0.60	0.38 ± 0.63	0.67
Uterine fibroids, n (%)	26 (8.9%)	61 (7.9%)	0.62
Diminished ovarian reserve, n (%)	52 (17.8%)	72 (9.4%)	<0.001
Male factor, n (%)	79 (27.0%)	273 (35.6%)	0.007
Fresh embryo transfer, n (%)	138 (47.2%)	522 (68.2%)	<0.001
Single embryo transfer, n (%)	224 (76.7%)	547 (71.5%)	0.08
Blastocyte transferred, n (%)	235 (80.4%)	554 (72.4%)	0.007

- Maternal demographics were similar between the groups, except for a diagnosis of diminished ovarian reserve which was more common in the thinner endometrium, p<0.001, and male factor, which was more common in the control group, p=0.009. Live births following fresh transfer were more common in the control group, p<0.001, while a higher rate of blastocyte transfers occurred in the thinner endometrium group, p=0.007.

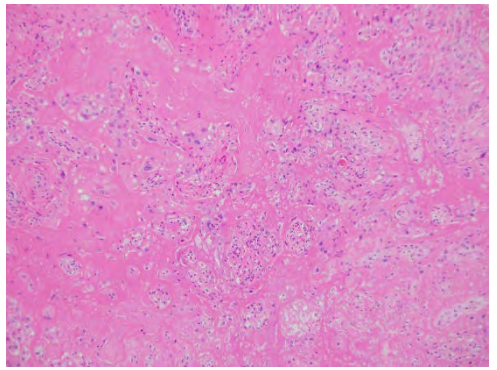
Table 2 – obstetric outcomes and placental findings

	Thinner endometrium n= 292	Controls n-765	aOR
Placental complications, n (%)	67 (22.9%)	117 (15.2%)	aOR 1.65 (1.08-2.52)
Birth weight (grams), mean ± SD	3243 ± 625	3310 ± 591	β -100.76 (-184.4- -17.0)
Placental thickness (cms), mean ± SD	1.82 ± 0.53	1.99 ± 0.64	β -0.14 (-0.25— 0.02)
Bilobated placenta, n (%)	11 (3.7%)	6 (0.7%)	aOR 3.05 (1.00-9.32)
Maternal vascular malperfusion , n (%)	133 (45.5%)	289 (37.7%)	aOR 1.44 (1.03-2.03)

Placental complications - preterm delivery, preeclampsia, low birth weight or placental abruption. aOR – adjusted odds ratio - for categorial variables, β – for continuous variables. Adjusted for maternal age, gravidity, diminished ovarian reserve, male factor fresh transfer and blastocyte transfer.

- An increased rate of placenta accreta was found in the thinner endometrium group, 3.7% vs. 1.1%, p=0.005, and overall rate of obstetric complications – 22.9% vs. 15.2%, p=0.003. In a linear regression analysis adjusting for confounders, thinner endometrium was found significantly associated with lower birthweight β -101.3 grams, 95% CI (-185.0 to -17.6) grams, p=0.01. Placentas in the thinner endometrium group were notable for reduced thickness, a higher rate of bilobated placentas and a higher rate of maternal and fetal malperfusion lesions.

Perivillous fibrin deposition (maternal malperfusion lesion) as demonstrated by Hematoxylin and Eosin staining



Wider implications of the findings:

Live births following IVF with a thinner endometrium are associated with a higher rate of placental mediated obstetric complications and lower birthweights. Placentas after transfer to thinner endometriums were characterized by gross anatomical and histological malperfusion lesions. Excess obstetric risks should be considered while planning an embryo transfer with thinner endometrium. Additional studies are needed to determine the effect of cycle cancellation and of preventive treatment such as Micropirin.

Advanced paternal age together with a low sperm motility is associated with a higher aneuploidy rate in preimplantation embryos

Obstetrics & Gynaecology
UNIVERSITY OF TORONTO

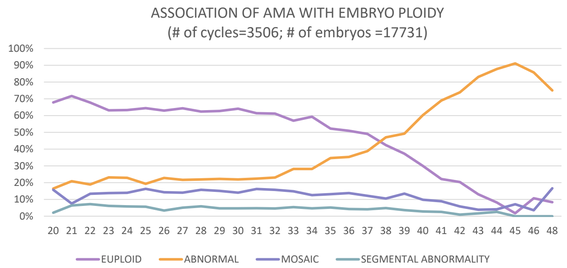
Rina Abramov[1], Ran Antes[1], Mitko Madjunkov[1,2], Svetlana Madjunkova[1,4], Clifford Librach[1,2,3,5]

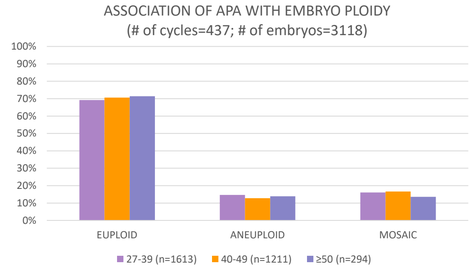
[1]CReATe Fertility Centre, Toronto, Canada; [2]Department of Obstetrics and Gynecology; [4]Department of Laboratory Medicine and Pathobiology; [3]Institute of Medical Sciences; [5]Department of Physiology, University of Toronto, Toronto, Canada

CReATe Fertility Centre
GENETICS

INTRODUCTION

The influence of paternal reproductive age (APA) and sperm characteristics on embryo ploidy is less studied than maternal reproductive age (AMA). It is well known that embryo aneuploidy is one of the most important factors for implantation failure. The evaluation of sperm parameters in relation to paternal age and embryo ploidy has not been reported systematically in a large cohort size.





OBJECTIVE

Objective of our study was to evaluate the correlation between APA, sperm count, motility and chromosomal aberrations using a large sample size analyzed by high resolution PGT-A.

MATERIALS AND METHODS

This is a retrospective cohort study with IRB approval, performed at the CReATe Reproductive Genetics Laboratory, Toronto, Canada, between January 2019 and December 2021. A total of 5890 blastocysts from 1436 cycles (965 patients) analyzed by high resolution NGS PGT-A (10Mb resolution; 30%-70% reported as mosaicism) were included in the study. Clinical, laboratory and demographic data were obtained for statistical analysis. Embryos were allocated into four groups by paternal age: Group A 20-29 (n=150); Group B 30-39 (n=3073); Group C 40-49 (n=2244); Group D≥50 (n=423). Multiple logistic/ordinal regressions were conducted to evaluate euploidy, mosaicism and aneuploidy rates between age groups in relation to sperm counts (low <5mil/ml; medium 5-15mil/ml; high >15mil/ml) and motility (<40%; >40%).

CONCLUSION

In summary, we found an association between older paternal age having low motility with significantly increased aneuploidy; but no association between older paternal age having a low sperm count with aneuploidy. The results of this study provide important information to assist fertility practitioners when providing pre-conception counseling to patients, particularly for couples in which the male partner belongs to an older age group.

ACKNOWLEDGEMENTS

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The authors thank ALL CReATe Fertility Centre Staff and patients.
All authors confirm that there are no conflicts of interest.

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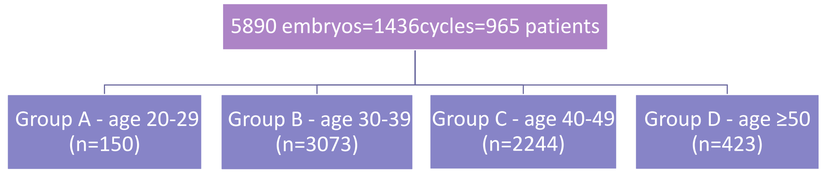
RESULTS



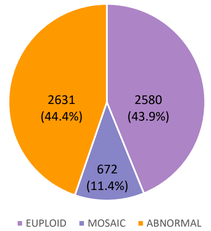


Fig 1: High resolution NGS PGT-A analysis of 5890 embryos showed 43.8% were euploid, 44.7% were aneuploid and 11.5% mosaic

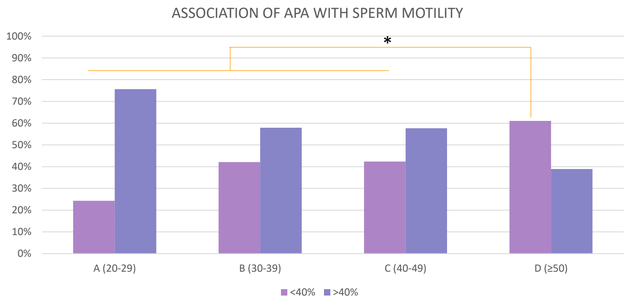


Fig 2: Increasing paternal age Group D was associated with a higher proportion of lower sperm motility (<40%) compared to Groups A, B and C (p<0.001).

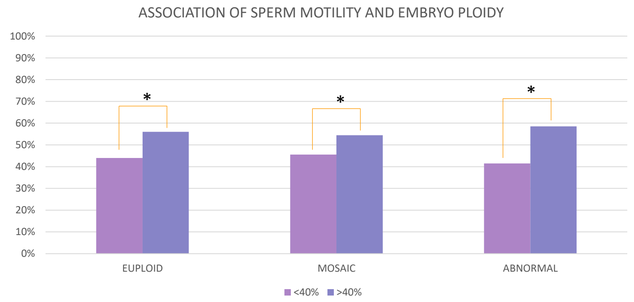


Fig 3: Increasing euploidy and mosaic rates and decreasing aneuploidy rates were observed between embryos injected with sperm having more than 40% motility (p<0.00001, p<0.017618 and p<0.00001, respectively).

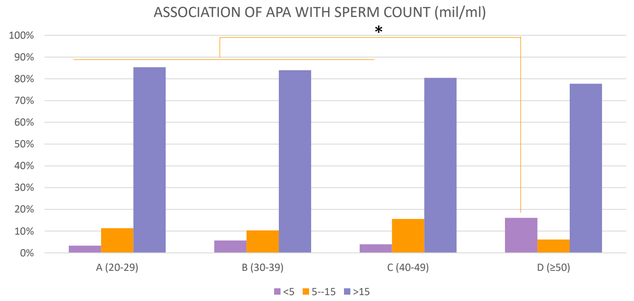


Fig 4: Advanced paternal age Group D had a higher proportion of low sperm count (<5mil/ml) compared to Groups A, B and C (p<0.00001).

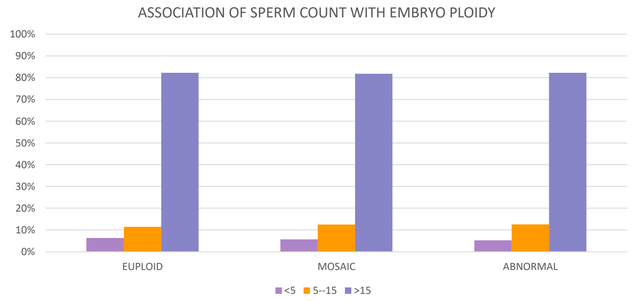


Fig 5: No association between embryo ploidy and sperm count was observed.

The Ethical, Legal and Social Implications (ELSI) of the CReATe Biobank; Recommendations to Improve the Future of Infertility Studies, Treatment and Outcomes

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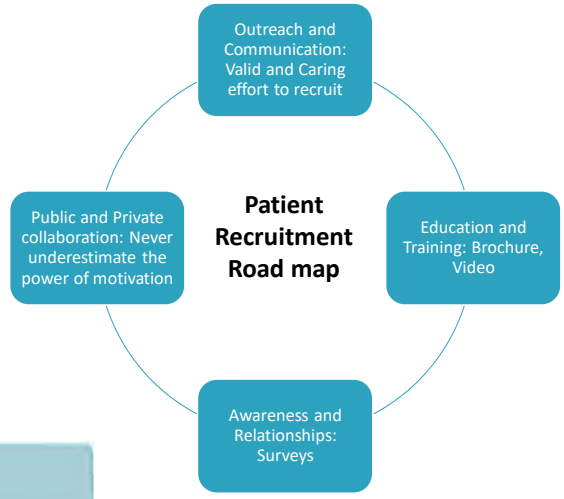
Objectives

The CReATe Biobank, established in 2015 as an adjunct to the CReATe Fertility Centre in Toronto, is among a handful of world-wide certified biobanks dedicated to fertility treatment-related specimens. It is however, Canada's first biobank to focus entirely on human reproductive biology-related samples. Its comprehensive and unique consent process for the collection of data, including preimplantation genetic screening results and pregnancy outcomes,

is what makes the CReATe Biobank stand out among others. Here, we outline the ELSI of the CReATe Biobank along with our recommendations to improve the future of infertility studies, treatment and outcomes.

Materials

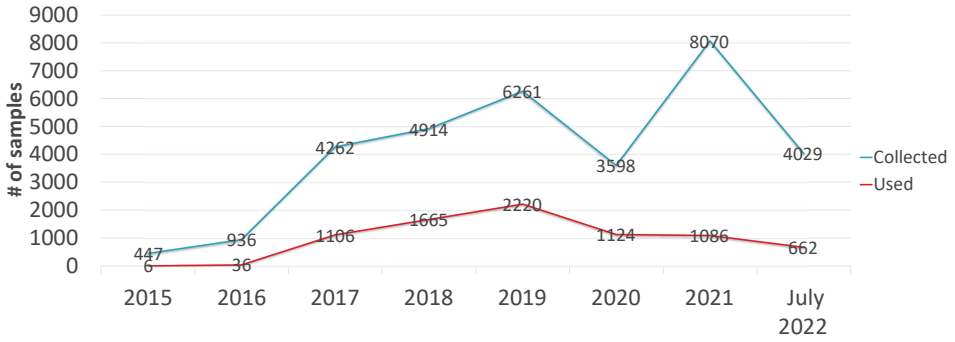
As a research entity, we have established a robust infrastructure to ensure compliance with the federal law of Canada (*The Assisted Human Reproduction Act of Canada* (*AHRA*) and Canadian ethical guidelines (*Tri-Council Policy Statement* (*TCPS2); Ethical Conduct for Research Involving Humans) and International Council for Harmonisation (ICH GCP*)). Our goal was to obtain a population as diverse and inclusive as possible. We re-wrote our one-size-fits-all Consent Form in early 2020 and developed six forms covering three diverse populations including intended parents, donors and surrogates. In the spring of 2022, we amended and streamlined the Consent forms again to address the ethical, legal and social implications of our biobanking processes.

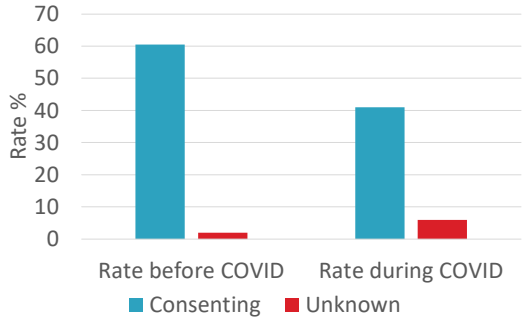


Results

The implementation of the new and very complex process coincided with the COVID Pandemic, during which all patient communication pivoted to a virtual platform. We reached out to 3,838 patients between August 2020 and April 2022 and observed a 53% drop in patient engagement during the pandemic. Our main challenge was unanswered phone calls and emails that had to be followed up in the clinic on the day of procedure. On the other hand, as per the fertility act, both partners' consents are required for embryo research. However, due to COVID related restrictions, follow ups were challenging and our TBD (to be determined) cases increased by 6-fold. The gap in provincial law and ethics board principles caused legal issues handling consents from individuals in polyamorous relationship where our forms only identified two persons as intended legal parents. As much as we want to build up an inclusive biobank, we also struggled in engaging non-Caucasian patients in the biobank. Finally, reaching out to anonymous sperm donors was a huge challenge and we never managed to recruit any patients in that category.

Biospecimen collection/ distribution rate over time





Patient Response

Conclusion

The ELSI of fertility biobanking are overwhelming for many reasons. There are copious moral and important questions asked whenever research is contemplated with gametes and embryos. In addition, government oversight and regulation have a tremendous impact on the ability we have to obtain legal consents. Continued discussion of fertility society with Health Canada can help finding less onerous means to use biological waste and non-waste products derived from non-invasive methods. Continued discussion with Members of Parliament and Senators to decriminalize portions of the *AHRA* will be welcomed as well so that those who fear the research related to the *AHRA*, will feel more comfortable. Engaging more stake-holders such as consulting agencies and fertility lawyers will also benefit our engagement percentages.

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EMPIRICAL USE OF GH IN IVF IS USELESS

THE LARGEST RANDOMISED CONTROLLED TRIAL

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The Canadian Fertility and Andrology Society's 68th Annual Meeting
September 22nd - 24th, 2022
in Halifax, Nova Scotia

Introduction

Based on the available evidence, there is an uncertainty regarding the benefice of use of GH therapy in IVF. The literature lacks a well-designed high quality RCT with an adequate sample size to answer the question in the general IVF population. The present study was undertaken to determine the efficacy and safety of adjuvant GH therapy in expected normal responders. It is, to our best knowledge, the largest RCT to address this topic of interest.

Objective

To determine the efficacy and safety of adjuvant growth hormone (GH) therapy in GnRH antagonist cycles on reproductive outcomes in the general In-Vitro fertilization (IVF) population.

Materials and Methods

This is a phase III open label randomised controlled trial involving a total of 288 patients who underwent an antagonist IVF cycle at OVO fertility center in Montreal, Canada, between June 2014 and January 2020. The study protocol was registered with Health Canada and approved by VERITAS IRB. The intervention group consisted of patients who received daily 2.5 mg subcutaneous injections of GH starting day 1 of ovarian stimulation until the day of oocyte retrieval, while the control group received standard ovarian stimulation without any adjuvant therapy. All embryo transfers (ET), fresh and/or frozen, resulting from this single IVF cycle were included in an intention-to-treat (ITT) and per-protocol (PP) analyses. The primary outcome was clinical pregnancy rate, while the number of retrieved oocytes and good quality embryos, maturation, fertilization, implantation and miscarriage rates and safety endpoints were recorded as secondary outcomes.

	GH group (n=144)	Control group (n=144)	Overall (n=288)
Age (years)	38.2 (2.6)	37.8 (2.6)	38.0 (2.6)
BMI (Kg/m2)	24.67 (3.91)	25.56 (4.09)	25.11 (4.02)
AMH (ng/ml)	2.48 (2.65)	2.53 (2.54)	2.51 (2.59)
FSH (IU/L)	6.58 (2.43)	6.68 (2.22)	6.63 (2.33)
AFC	15.36 (10.69)	15.03 (9.69)	15.19 (10.18)
Type of infertility, n (%)			
Primary infertility	73 (50.7%)	64 (44.4%)	137 (47.6%)
Secondary infertility	71 (49.3%)	80 (55.6%)	151 (52.4%)
Duration of infertility (years)	3.5 (2.8)	3.2 (2.1)	3.3 (2.4)
Cause of infertility, n (%)			
Female factor	60 (41.7%)	46 (31.9%)	106 (36.8%)
Male factor	39 (27.1%)	45 (31.3%)	84 (29.2%)
Mixed factor	14 (9.7%)	14 (9.7%)	28 (9.7%)
Unexplained infertility	22 (15.3%)	34 (23.6%)	56 (19.4%)
Single women/same-sex couple	9 (6.3%)	5 (3.5%)	14 (4.9%)
Prior IVF cycle, n (%)			
No	80 (55.6%)	71 (49.3%)	151 (52.4%)
Yes	64 (44.4%)	73 (50.7%)	137 (47.6%)
Number of prior IVF cycles	0.7 (1.1)	1.0 (1.6)	0.9 (1.4)
Number of prior embryos transferred	0.7 (1.2)	0.8 (1.4)	0.8 (1.3)

Table 1. Baseline characteristics of patients according to the intervention and control groups

Note: The group characteristics are expressed as mean (SD). BMI: Body Mass Index. AMH: Anti-Mullerian hormone. FSH: Follicular Stimulation Hormone. AFC: Antral Follicular Count

Inclusion Criteria

1. age between 30 and 42
2. GnRH antagonist protocol
3. Primary or secondary infertility
4. No previous IVF cycle using the same proposed protocol with Saizen
5. Anti-Mullerian Hormone (AMH) measured within the last 24 months prior to randomization

Exclusion Criteria

1. Contradiction to GH therapy
2. BMI ≥ 35 kg/m2
3. Simultaneous participation in another clinical trial
4. AMH < 0.5pg/ml
5. Known risk for gestational diabetes or diagnosed with diabetes

	GH group (n=144)	Control group (n=144)	P value
Total Dose of Gonadotropins (IU)	4602.9 (1504.9)	4657.1 (1357.3)	0.752
Number of Stimulation Days	11.4 (2.1)	11.7 (1.9)	0.118
Endometrial thickness (mm)	10.63 (2.82)	10.94 (2.85)	0.372
IGF-1 – Baseline (ng/nL)	139.6 (42.4)	132.0 (37.3)	0.110
IGF-1 - EoT (ng/nL)	229.6 (72.7)	125.1 (34.6)	<.0001
IGF-1 - Ratio (EoT/Baseline)	1.71 (0.55)	0.98 (0.24)	<.0001
(IGF-1 - EoT (ng/nL)) / (Utilizable Embryos)	122.5 (87.9)	61.7 (41.1)	<.0001
Cycle cancellation, n (%)	6 (4.2%)	3 (2.2%)	0.501
Insemination type, n (%)			
Standard IVF	19 (24.4%)	28 (31.1%)	0.718
ICSI	55 (70.5%)	59 (65.6%)	
Mixed	2 (2.6%)	1 (1.1%)	
PICSI	2 (2.6%)	2 (2.2%)	
Number of embryos transferred (fresh)	0.8 (0.8)	0.9 (0.7)	0.317
Number of embryos transferred (frozen)	1.7 (2.5)	1.7 (2.7)	1.000
Embryo age at transfer (fresh), n (%)			
Day 3 embryo	45 (57.7%)	51 (56.7%)	1.000
Day 5-6 embryo	33 (42.3%)	39 (43.3%)	
Embryo age at transfer (frozen), n (%)			
Day 3 embryo	21 (36.8%)	11 (25.6%)	0.282
Day 5-6 embryo	36 (63.2%)	32 (74.4%)	

Table 2. IVF procedural outcomes by ITT analysis.

Note: The group characteristics are expressed as mean (SD). E2: serum estradiol level at the day of trigger. Progesterone: serum progesterone level at the day of trigger. IGF-1-EoT: Insulin-like growth factor 1 serum level at the end of treatment. ICSI: intra-cytoplasmic sperm injection. PICSI: physiological intra-cytoplasmic sperm injection

Results

A total of 288 patients were recruited and randomly assigned at a 1:1 ratio to the GH or the control group. After removing the cycle cancellations and patients who did not undergo an ET, 105 patients remained in each group. The demographic characteristics in both groups were similar. The overall mean age was 38.0±2.6 years, the mean body mass index was 25.11±4.02 kg/m2 and the mean AMH was 2.51±2.59 ng/ml. The cycle characteristics were also similar between both groups. No differences were noted in terms of total dose of gonadotropin (4602.9 vs 4657.1 IU for the GH and control groups respectively, p=0.750), days of stimulation (11.4 vs 11.7 days, p=0.117) and endometrial thickness (10.63 vs 10.94 mm, p=0.372). The ITT and PP analyses detected similar results in terms of both IVF stimulation outcomes and reproductive outcomes. In the ITT analysis, no difference was noted in the number of follicles ≥15 mm (7.8 vs 7.1, p=0.212), oocytes retrieved (11.7 vs 11.2, p=0.613), mature oocytes (8.5 vs 8.6, p=0.851), maturation rate (73.8 vs 78.4%, p=0.06), fertilization rate (64.3 vs 67.2%, p=0.388), good quality embryos (2.5 vs 2.6, p=0.767), implantation rate (42.7 vs 50.8%, p=0.234), miscarriage rate (26.9 vs 29.5%, p=0.761) and clinical pregnancy rate (48.6 vs 58.1%, p=0.167). The number of embryos needed to achieve a clinical pregnancy was 2.9 vs 2.5 in the GH and control groups respectively, with no significant difference (p=0.322). Finally, no or only mild side effects related to GH injection were noted.

ITT analysis			
	GH group (n=144)	Control group (n=144)	P value
E2 (pmol/L)	7692.0 (5571.6)	8193.5 (5815.5)	0.472
Progesterone (nmol/L)	2.66 (1.37)	2.77 (1.30)	0.466
Number of Follicles ≥15 mm	7.8 (5.2)	7.1 (4.2)	0.212
Number of Follicles <15 mm	7.3 (6.4)	7.8 (6.7)	0.556
Number of Oocytes Retrieved	11.7 (8.5)	11.2 (7.9)	0.613
Number of Mature Oocytes	8.5 (6.2)	8.6 (6.3)	0.851
Maturation Rate, %	73.8% (21.6)	78.4% (17.6)	0.060
Fertilization Rate - All Types Included, %	64.3% (29.1)	67.2% (25.7)	0.388
Number of Embryos Available for Transfer	2.5 (2.4)	2.6 (2.6)	0.767

Table 3. Ovarian stimulation outcomes by ITT analysis

Note: The group characteristics are expressed as mean (SD)

ITT analysis			
Fresh and/or frozen embryos	GH group (n=144)	Control group (n=144)	P value
Implantation Rate, % (SD)	42.7% (48.8)	50.8% (49.5)	0.234
Clinical pregnancy Rate, n (%)	51 (48.6%)	61 (58.1%)	0.167
Miscarriage rate, n (%)	14 (26.9%)	18 (29.5%)	0.761
Overall Average Number of Embryos Transferred per First Clinical Pregnancy [Total Transferred/Total Clinical Pregnancies]	2.9 [166/57]	2.5 [162/64]	0.322

Table 4. Reproductive outcomes by ITT analysis in fresh and/or frozen embryo transfers

Conclusions

GH adjuvant therapy in GnRH antagonist cycles is a safe procedure; however, it does not improve the results of IVF stimulation, nor the reproductive outcomes, namely implantation, miscarriage, and clinical pregnancy rates.

Impact Statement

There is no benefit from adding GH therapy to ovarian stimulation in GnRH antagonist cycles for the general IVF population