Timing of Ovulation Induction Matters in Women with Low Functional Ovarian Reserve

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Conflict Statement

Dr. Gleicher is listed as co-inventor on a number of pending patent applications claiming diagnostic and therapeutic benefits from determination of CGG repeat numbers and ovarian *FMR1* genotypes and sub-genotypes.

Dr. Gleicher is co-inventor of awarded U.S. patents, claiming therapeutic benefits for supplementation of DHEA in women with diminished ovarian reserve, a topic discussed in this talk. Other patent applications in regards to DHEA and other fertility-related claims, with no relationship to this talk, are pending. Dr. Gleicher receives royalties from, and owns shares in Fertility Neutraceuticals, LLC, a distributor of a DHEA product.

Dr. Gleicher is co-inventor of three pending patent applications claiming potential therapeutic benefit for anti-Müllerian hormone (AMH) in infertile women. Dr. Gleicher owns shares in OvaNova Laboratories, LLC.

Outline

- Historical perspectives
- Motivation for study
- Investigation in oldest women (>43 years)
- Investigation in women with POA
- Investigation of best lead-follicle sizes for hCG trigger
- Conclusion: Individualization of care

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Lead Follicle Sizes

- > 18 mm with gonadotropins
- 20-22 mm with Clomid
- European colleagues somewhat earlier than U.S.
- Sense that older women should be retrieved somewhat earlier

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Our center's 2010-2012 age-specific clinical IVF outcome data by "intent to treat"*/** for women 40 years and older

Age (years)	Live birth rate (%)	Clinical pregnancy rate (%) if different
40	15.4	
41	42.9	
42	6.3	18.8
43	0.0	16.7
44	1.4	5.4
45	2.7	5.4
46-53	0.0	

* "Intent to treat" reflects denominator of per cycle start for each age group. A total of 233 IVF cycles are reported. Because of an ~20% cycle cancellation rate before embryo transfer, reports based on patients reaching embryo transfer of at least 1 embryo would demonstrate ~ 20% higher clinical pregnancy and live birth rates.

** Miscarriages reflect only established clinical pregnancies, confirmed by ultrasound by presence of at least one intrauterine gestational sac. Chemical and ectopic pregnancies are not considered in here presented data.

Gleicher et al; in press

We, therefore, wondered whether we could find out by molecular means what differentiates younger from older follicles

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	Group 1 Donors N=31	Group 2 Intermediate age infertility patients N=64	Group 3 Older infertility patients N=41
Average age (years)	24.4±3.0^{ab}	34.1±3.0^{ac}	44.3±1.5^{bc}
FSH (mIU/mL)	6.3±0.9^{a}	7.6±1.8^{b}	10.3±1.5^{ab}
AMH (ng/mL)	3.1±0.6^{a}	2.8±0.5^{b}	0.28±0.1^{ab}
Number of follicles/cycle	22.5±8.3^{abc}	10.5±7.1^{ad}	6.8±5.1^{bcd}
Number of oocytes retrieved/cycle	15.5±7.0^{abc}	2.1±6.6^{ad}	3.6±3.2^{bcd}
Number of artretic oocytes retrieved/cycle	1.3±1.2^{a}	0.9±1.3	0.6±0.9^{a}
Number of embryo ≥ 4 cells	15.5±6.5^{abc}	7.1±6.5^{ad}	3.6±3.1^{bcd}
Pregnant rate/cycle	16 (51.6%)	22 (34.4%)	3 (7.3%)
Progesterone/estradiol ratio	0.26±0.08^{a}	0.5±0.15^{b}	1.96±0.47^{ab}

Values with a-d in their superscripts in same row differ significantly (P<0.05).

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- The P4/E2 ratio was significantly higher in the oldest group
- A first hint at “premature luteinization”

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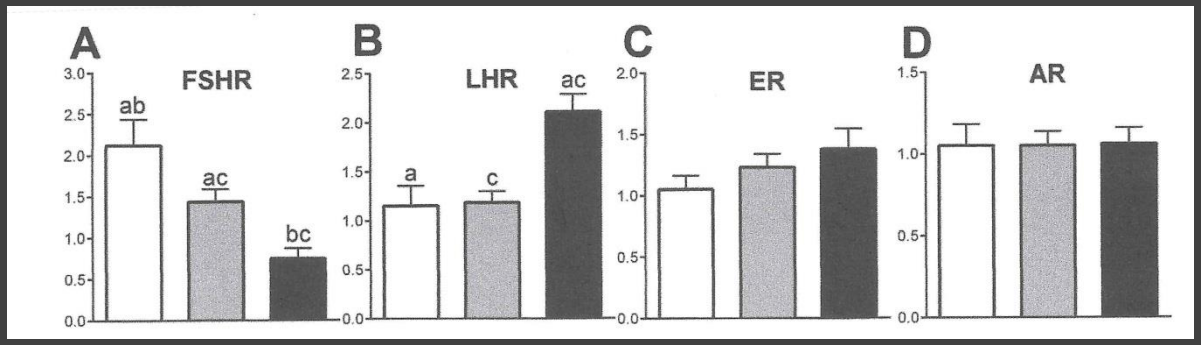
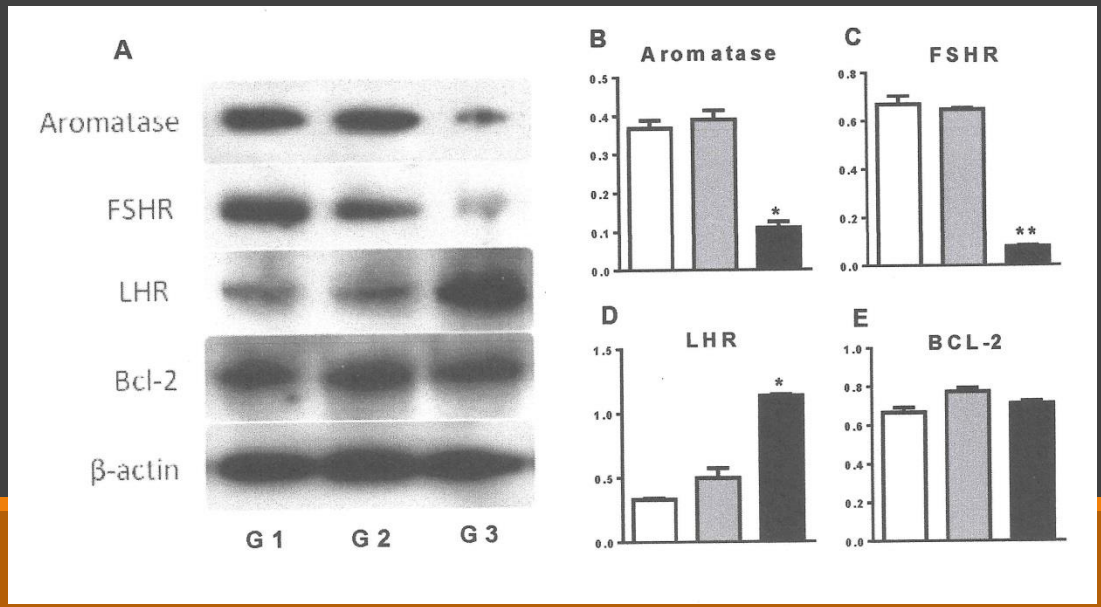
Impact of Maternal Aging on Gene Expression in GCs



FSHR ↓

LHR ↑

Confirmed by western blot



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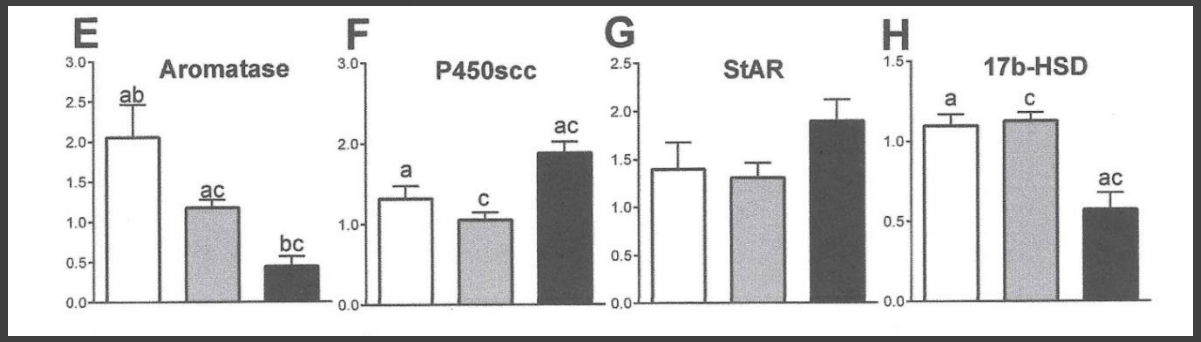
Impact of Maternal Aging on Steroidogenic Activity in GC

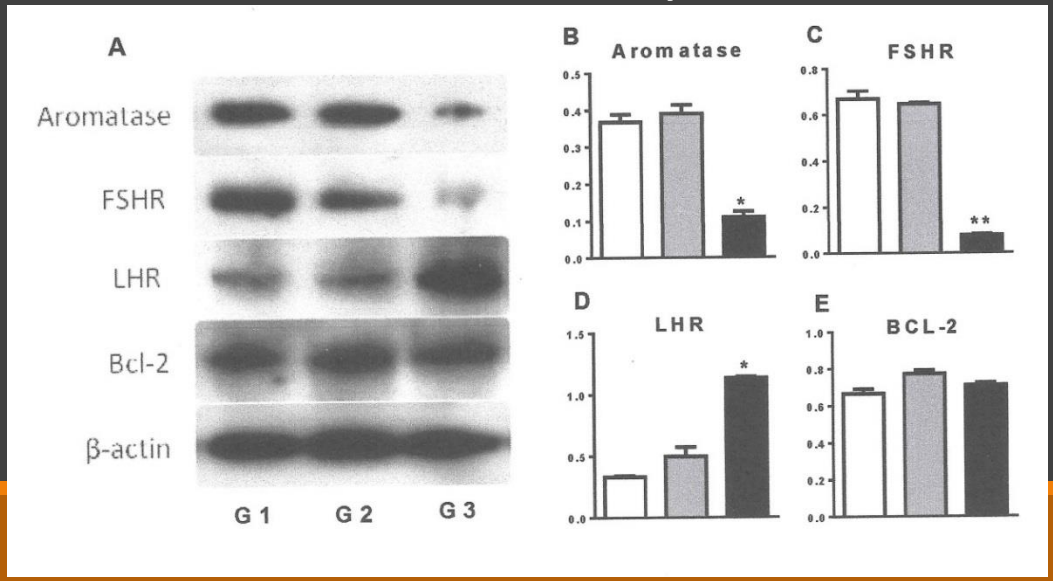


Aromatase ↓

17b/HSD ↓

P450scc ↑

Confirmed by western blot



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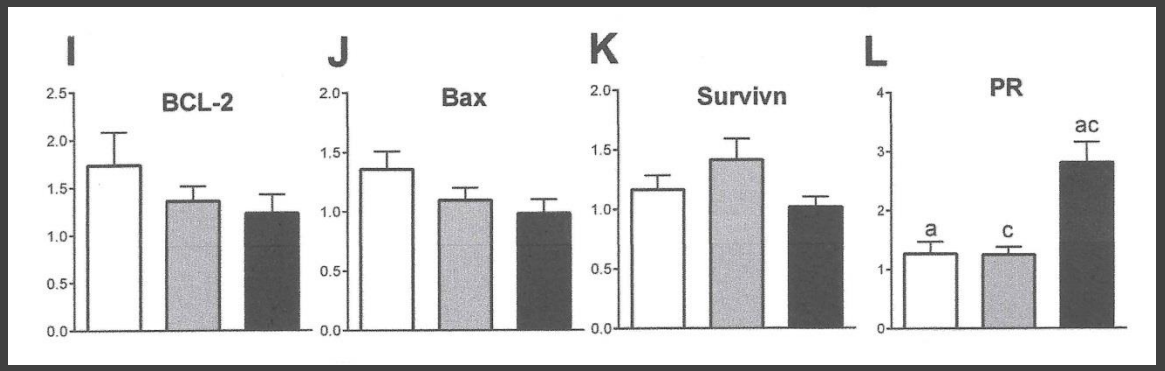
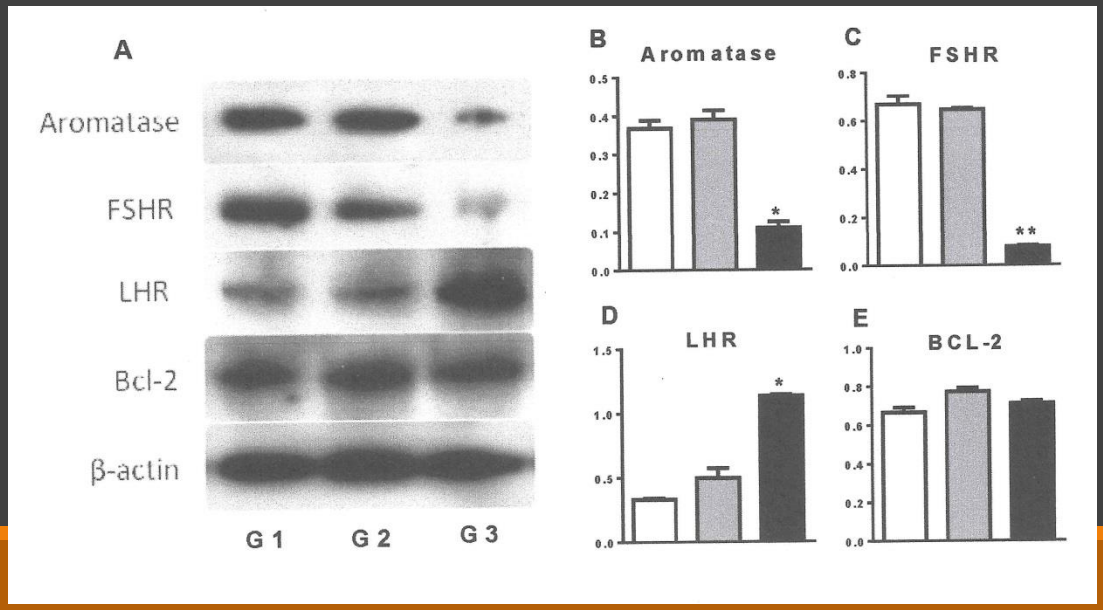
Impact of Maternal Aging on Apoptosis



No differences were found. Confirmed by western blot



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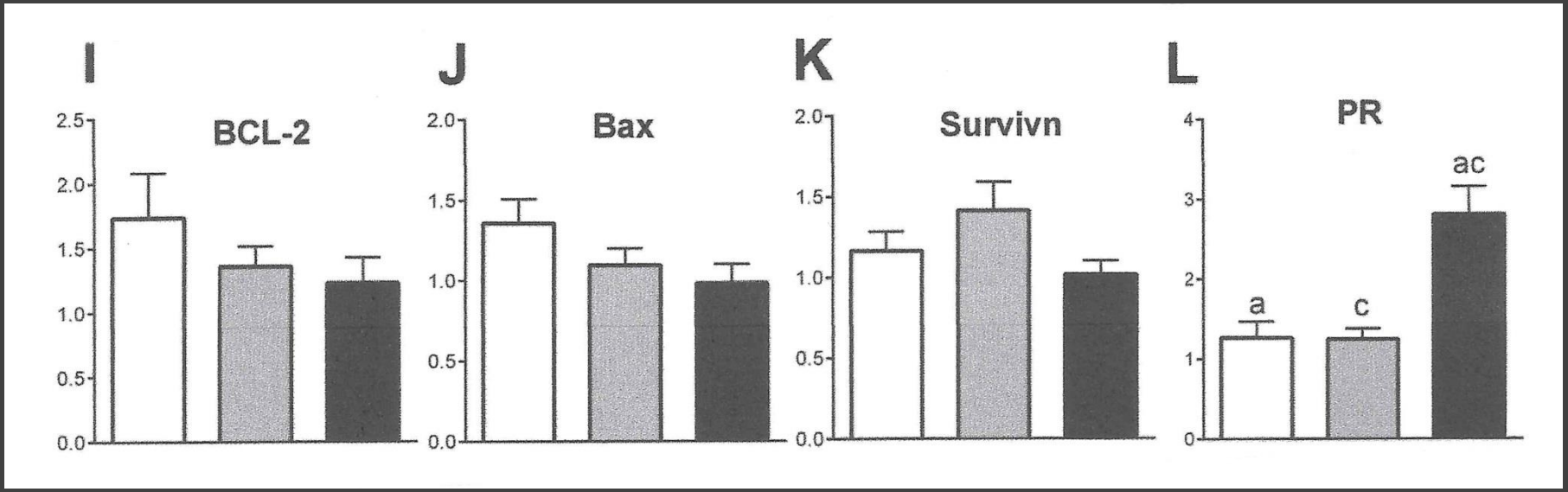
Conclusion

- **Increased LHR expression**
- **Reduced FSHR expression**
- **Reduced Aromatase expression**

with advancing age are supportive of **premature luteinization of GCs in older women**

Progesterone receptor (PR)

This was further confirmed by older women who also demonstrated higher PR expression



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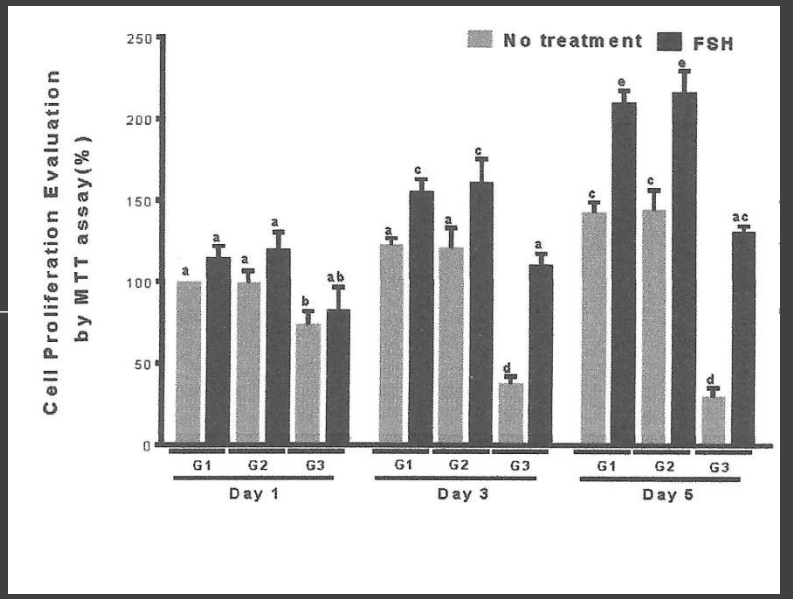
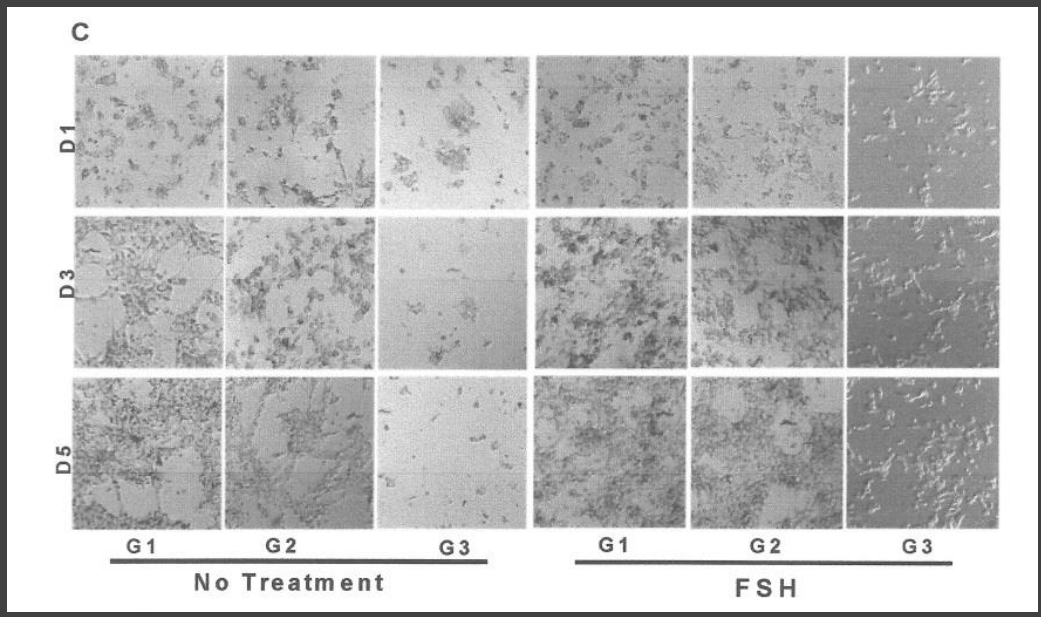
For further confirmation, we now went into a GC culture system where cells were evaluated on days 1, 3, and 5 in presence/absence of FSH

Impact of Maternal Aging on GCs in Culture

In absence of FSH, cell proliferation declines rapidly



Growing patterns are distinctively different.



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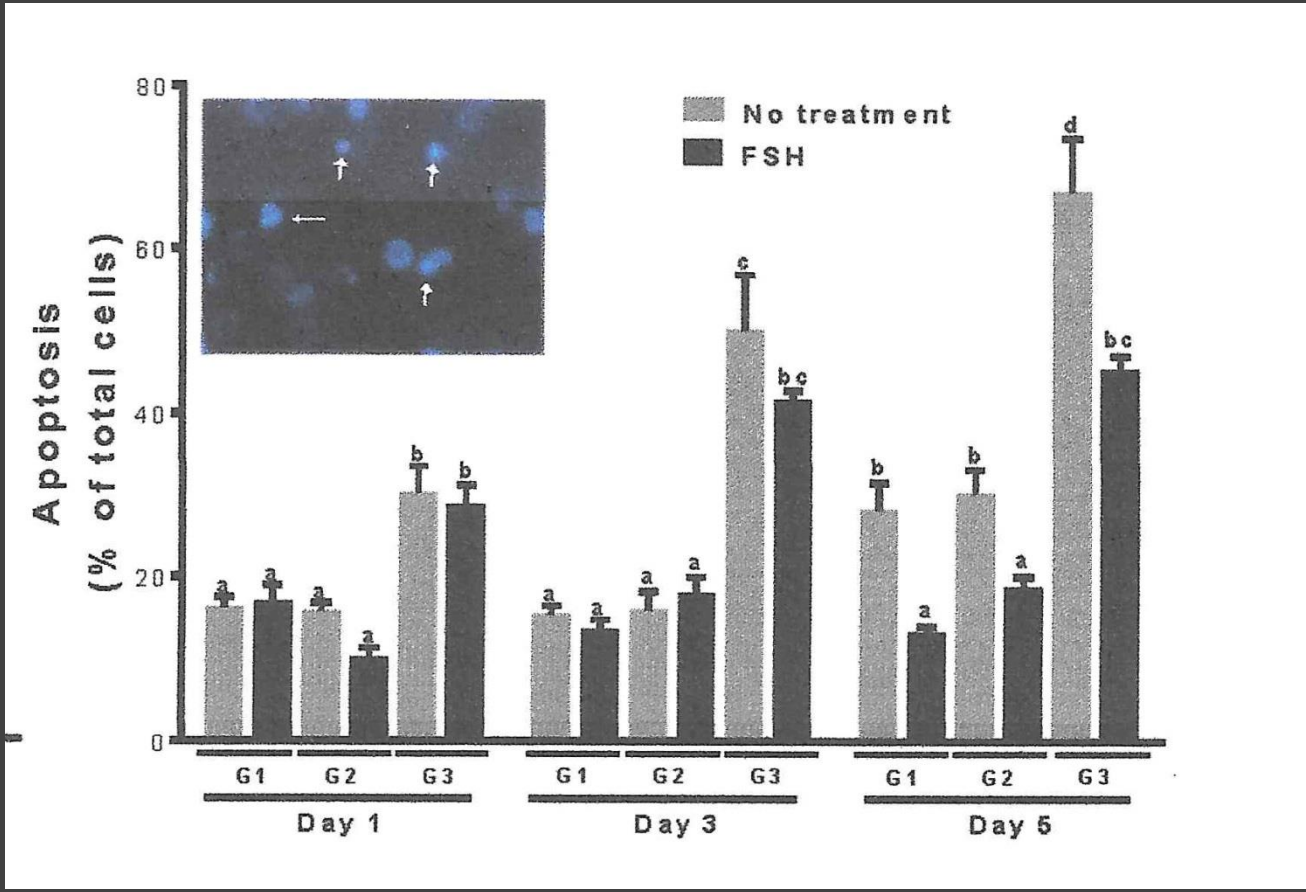
Impact of Maternal Aging on Apoptosis During Culture

Older women demonstrated much more rapid increase in apoptosis



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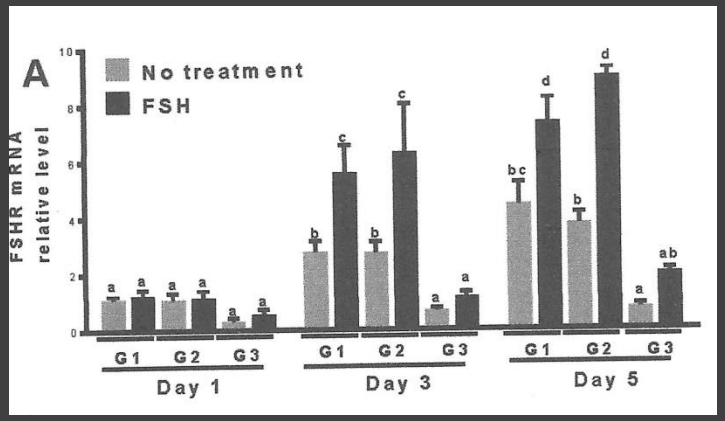
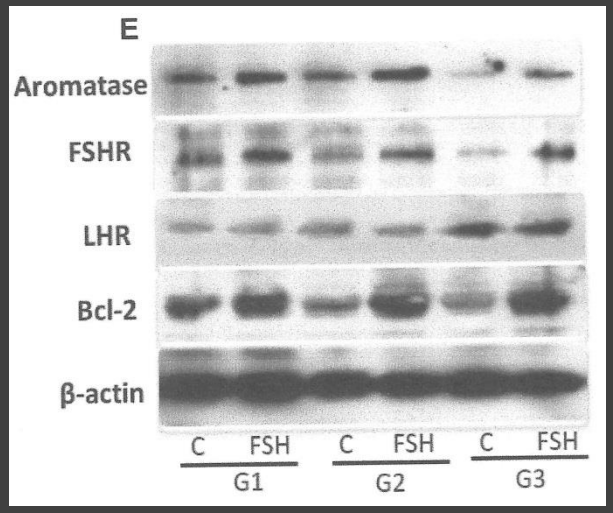
Conclusion

- FSH, thus at all ages demonstrates positive effects on GC proliferation and apoptosis of cultured GCs.
- This effect is, however, weaker in older women, going along with previously noted lower FSHR expression.

Impact of Maternal Aging on Gene Expression During Culture

- On Day-1 FSHR mRNA expression was low at all ages. It subsequently increased in younger but not in older women





- FSH in culture enhanced the response but less so in older women.

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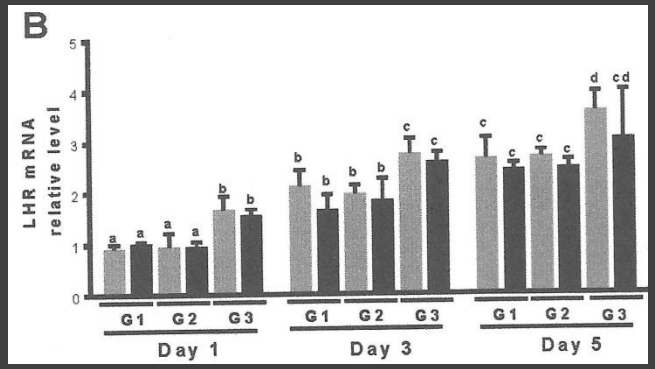
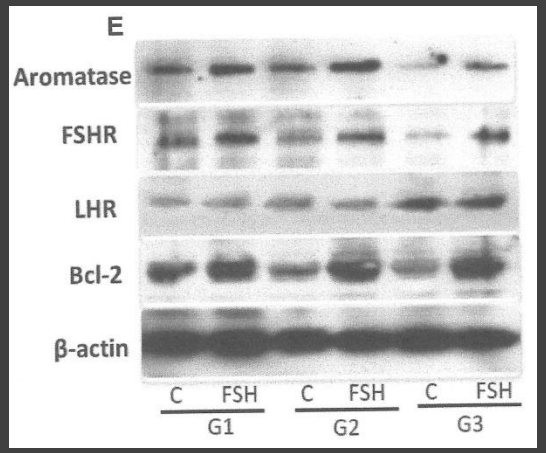
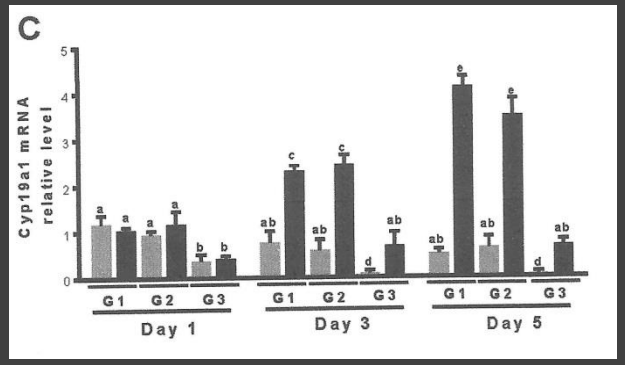
Impact of Maternal Aging on Gene Expression During Culture (cont.)

- LHR mRNA expression increased much faster in older women.





- Though FSH in culture did not affect LHR expression, it did stimulate aromatase mRNA and protein expression.



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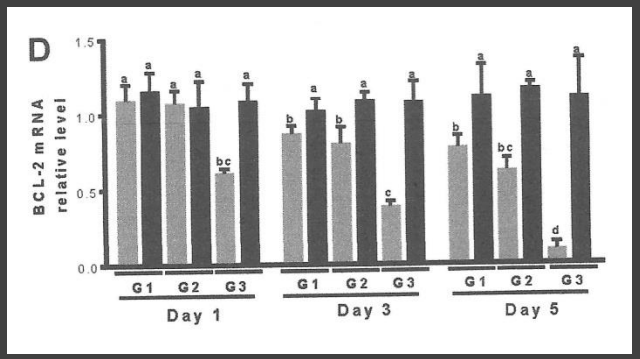
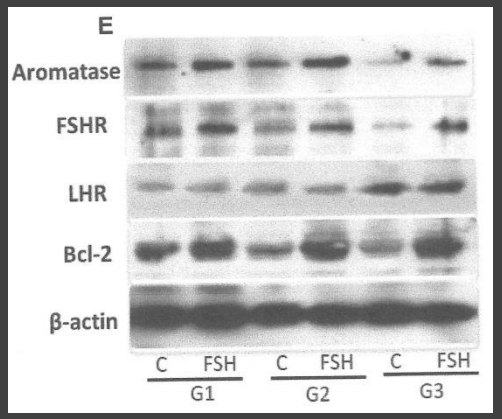
Impact of Maternal Aging on Gene Expression During Culture (cont.)

- Bcl-2 gene expression decreased at all ages, the fastest in oldest patients.





Concurring between protein expression by western blot and PCR results

- FSH, by inhibiting this decline, thus appears to inhibit apoptosis after all

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Conclusion

In vivo as well as *in vitro* results suggest that premature luteinization represents a central feature of the “old” follicle

This raised the question whether early oocyte retrieval would improve outcome by avoiding exposure of oocytes to premature luteinization?

Changes in IVF protocol in Women older than 43

- Retrieval at 14-16 mm follicle size (from 19-21 mm)
- 30 hours instead of 36 hours hCG to retrieval interval
- ET on day-2 (from day-3)

	Early retrieval group N=39	Normal retrieval group N=91	P value
Average age (years)	45±1.9	44.3±1.5	0.02
Number of follicles/cycle	7.2±4.7	7.3±5.6	0.947
Number of oocytes/cycle	6.3±5.1	5.9±4.9	0.703
Number of immature oocytes	2.4±2.5	1.1±1.6	0.006
Number of atretic oocytes retrieved/cycle	0.3±0.5	0.8±1.4	0.037
Number of good embryos/cycle	3.0±2.8	2.8±2.3	0.551
Percentage of cycles resulting pregnancies	12.8 (5/39)	7.7 (7/91)	N/A
Percentage of transferred cycles resulting in pregnancies	16.7 (5/30)	8.9 (7/28)	N/A
Implantation rate (%)	4.8	3.3	N/A

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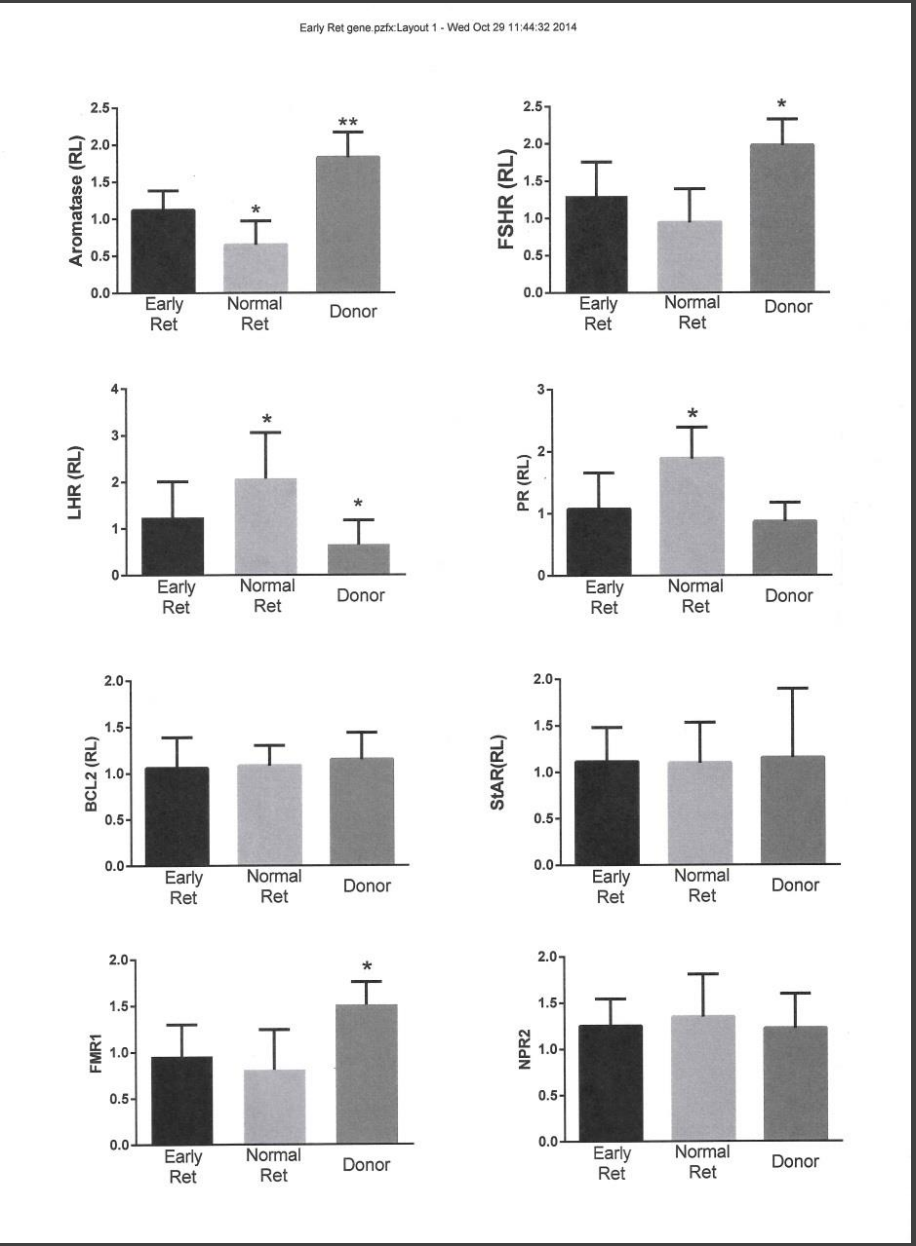
Molecular biology of follicles after early retrieval



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Aging-related premature luteinization of granulosa cells is avoided by early oocyte retrieval

Yan-Guang Wu[1], David H Barad[1,2,3], Vitaly A Kushnir[1,4], Emanuela Lazzaroni[1], Qi Wang[1], David F Albertini[1,5] and Norbert Gleicher[1,2,6]

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- Historical perspectives
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- Investigation in women with POA
- **Investigation of best lead-follicle sizes for hCG trigger**
- Conclusion: Individualization of care

Timing of retrieval determines IVF outcomes at all ages with low ovarian reserve because of premature luteinization of follicles

Yan-Guang Wu,[1,] David H. Barad,[1,2,3] Vitaly A. Kushnir,[1,4] Qi Wang,[1] Lin Zhang,[1] Sarah K. Darmon,[1] David F. Albertini[1,5] and Norbert Gleicher,[1,2,6,7*]

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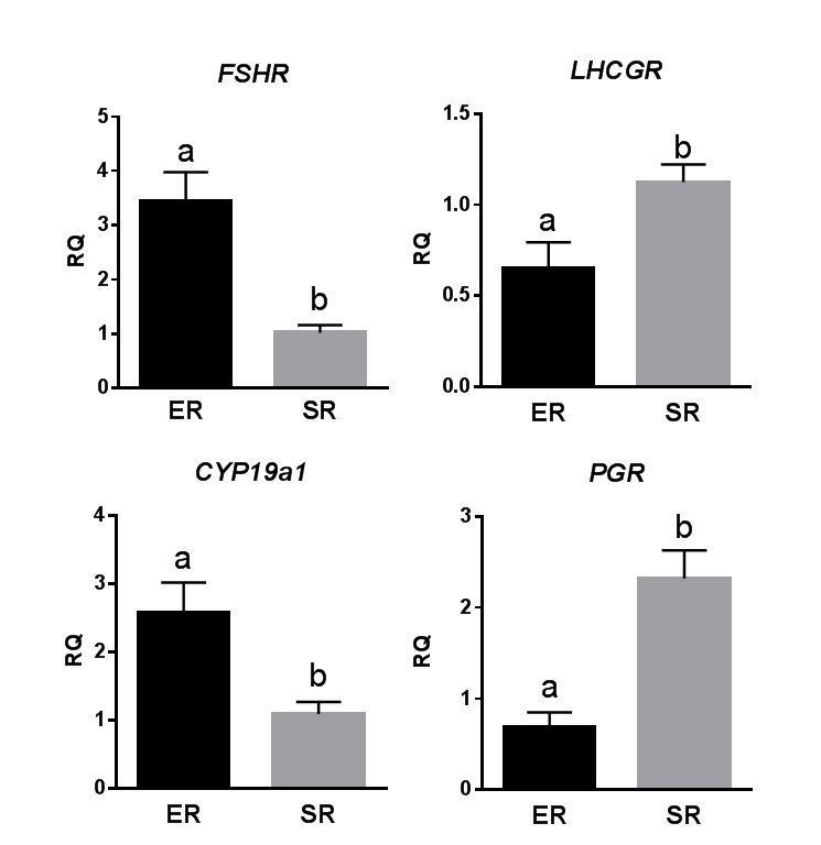
Patient population and IVF cycle characteristics of POA/oPOI patients

ER, early retrieval; SR, standard retrieval
Outcomes were similar, unless * reflects significant difference (P,0.05).

IVF Parameters	ER	SR
Number of patients	24	13
Age (years)	39.4±0.6	38.5±0.6
Serum FSH (mIU/ml)	12.8±1.3	11.3±1.2
Serum AMH (mg/ml)	0.49±0.09	0.43±0.12
P4/E2 ratio on trigger day	2.49±0.37	3.16±0.68
Retrieved oocytes	3.4±0.6	5.7±1.3
Matured oocytes	2.7±0.44	3.4±0.9
% of mature oocytes	81.5±4.5	55.8±8.3*
Immature oocytes	0.45±0.1	0.7±0.2
% of immature oocytes	12.6±3.4	14.4±5.1
Atretic oocyte	0.32±0.2	1.3±0.3*
% of atretic oocyte	7.9±3.0	28.2±7.2*
Fertilized oocytes	2.2±0.4	3.1±0.7
% of fertilized oocytes	87.5±5.2	86.8±5.1
Total transferable embryos	1.6±0.2	2.5±0.3
High quality embryos	1.1±0.2	0.9±0.2
% high quality embryos	68.2±10.7	53.8±9.3
Clinical pregnancy rate	41.7% (10/24)	7.7% (1/13)*

Gene expression at mRNA level in GCs of POA/oPOI patients with ER and SR



FSHR, FSH receptor; *LHCGR*, LH receptor; *Cyp19a1*, P450 aromatrase; *PGR*, progesterone receptor; ER, early retrieval; SR, standard retrieval
a/b denote significant statistical difference (P,0.05).

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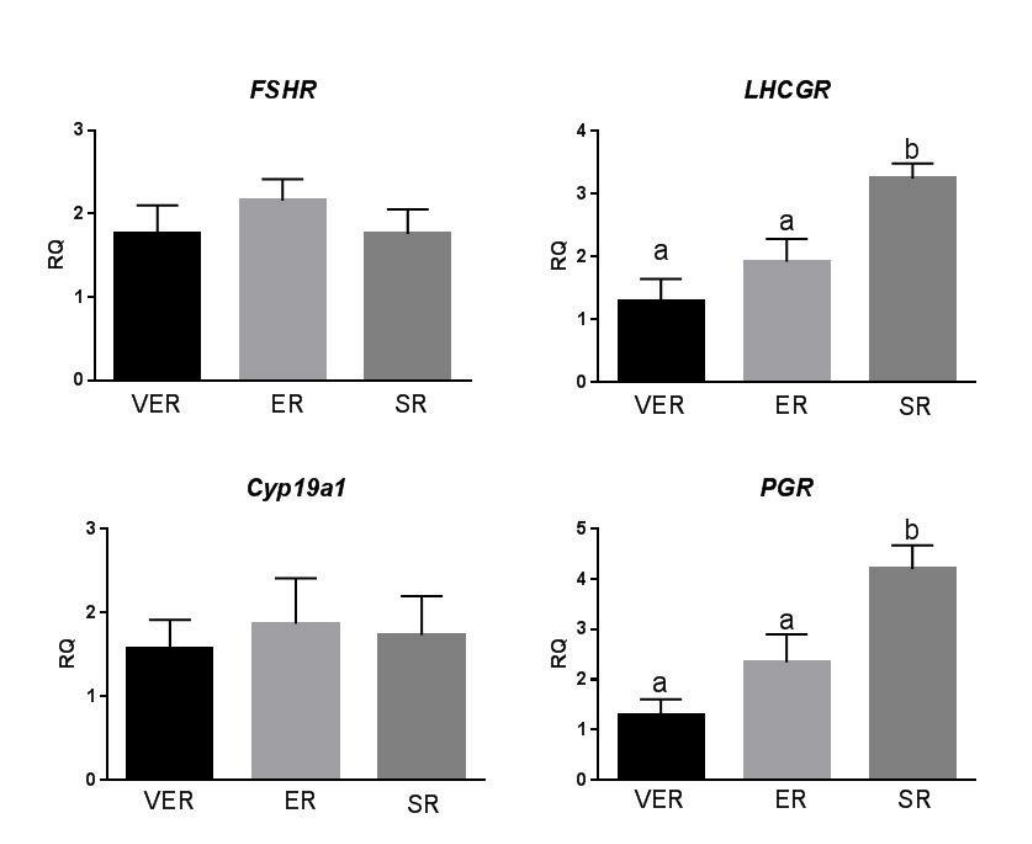
Patient and IVF cycle characteristics in women above age 43 with VER, ER and SR

VER, very early retrieval; ER, early retrieval; SR, standard retrieval

[a/a] denotes no significant difference
[a/b] denotes significant difference

	VER	ER	SR
Number of Patients	**17**	**24**	**15**
Average age (years)	44.9±0.3	44.6±0.3	45.0±0.6
FSH (mIU/ml)	12.6±3.3	9.3±1.0	12.8±5.9
AMH (ng/ml)	0.7±0.2	0.8±0.1	1.1±0.3
P4/E2 ratio on hCG trigger day	2.3±0.3	2.0±0.3	2.1±0.3
Retrieved oocytes	3.9±0.6	5.6±0.8	4.8±1.0
Mature oocytes	2.0±0.5	3.4±0.5	2.4±0.8
% of mature oocytes	53.5±8.7	66.0±5.5	51.1±10.5
Immature oocytes	1.7±0.4	1.4±0.3	2.0±0.8
% of immature oocytes	41.5±8.2	22.8±5.1	43.9±10.5
Atretic oocyte/patient	0.3±0.1	0.8±0.3	0.3±0.2
% of atretic oocyte	7.4±3.6	11.2±3.5	4.8±2.6
Fertilized oocytes	2.3±0.4	3.0±0.5	2.9±0.6
Total transferable embryos	2.0±0.3	2.7±0.4	2.3±0.6
High quality embryos	0.9±0.1[a]	2.3±0.4[b]	0.7±0.2[a]
% of high quality embryos	52.3±8.7[a]	83.8±5.8[b]	34.0±13.2[a]
Pregnant rate	5.9% (1/17)[a]	16.7% (4/24)[b]	6.7% (1/15)[a]

Gene expression at mRNA level in GCs with VER, ER and SR



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a/a denote no statistical difference.
a/b denotes significant statistical difference (P,0.05).

Conclusion

Individualization of care!

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