Younger women with diminished oocyte reserve are not more prone to meiosis errors leading to spontaneous abortion than their age peers with normal oocyte reserve

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Summary

Purpose: To determine the relative effect of age vs ovarian oocyte reserve on miscarriage rates. *Materials and Methods:* Miscarriage rates (loss of pregnancy established by ultrasound) from pregnancies achieved through in vitro fertilization-embryo transfer (IVF-ET) were evaluated according to four age groups (≤ 35, 36-39, 40-42, and 43-44 years) and four day 3 serum follicle-stimulating hormone (FSH) groups (≤ 11, 12-14, 15-17, and > 17 mIU/ml). All cycles were supplemented with progesterone in the luteal phase. *Results:* With each advancing age group irrespective of the FSH levels, there was a progressive increase in miscarriage rates. In contrast, no difference in miscarriage rates were seen as FSH levels increased (and thus ovarian reserve decreased) in women up to 42 years of age. Only in the group aged 43-44 years was there an association with diminished oocyte reserve and miscarriage. *Conclusions:* Since low FSH stimulation protocols were purposely used for the group with diminished oocyte reserve, the data are consistent that the conclusion regarding poor oocyte quality in women with diminished oocyte reserve was mostly iatrogenic and related to the use of high-dosage FSH stimulation trying to create more oocytes for retrieval.

Key words: Oocyte reserve; Day 3 serum FSH; Miscarriage rates; Advancing age; Ovarian hyperstimulation.

Introduction

From the early days of in vitro fertilization-embryo transfer (IVF-ET), most IVF centers reported very poor pregnancy results following the transfer of embryos from women with diminished oocyte reserve even if they were younger [1-6]. Based on the demonstration that chromosomal analysis of 59 aborted fetuses, 19 of 33 (57%) with abnormal karyotypes had elevated day 3 serum follicle stimulating hormone (FSH) levels or elevated serum estradiol levels which were significantly higher than the 7 of 26 (27%) who had normal karyotypes. One study concluded that younger women with diminished oocyte reserve are more prone to meiosis errors leading to the oocyte quality of a woman of advanced reproductive age rather than her age peers [7]. Although in the modern IVF era, some of the top IVF-ET centers still report extremely low pregnancy rates despite marked improvement in IVF technology; many reach the conclusion that younger women with diminished oocyte reserve have not just poor quantity of oocytes but poor quality akin to women of advanced reproductive age who are known to be prone to meiosis errors. Thus they are likely to produce embryos with aneuploidy that either do not implant or spontaneously abort [8, 9].

However, other studies do not agree with these studies finding very low live delivery rates following IVF-ET in women with diminished oocyte reserve [10-12]. These IVF centers finding normal pregnancy rates in women with diminished oocyte reserve suggest that the reason for poor pregnancy results found by the other aforementioned studies was the inappropriate use of high FSH stimulation protocols [13].

The most likely cause of any given spontaneous abortion would be related to chromosome abnormalities in all women irrespective of oocyte reserve, especially in women supplemented with progesterone from early luteal phase throughout the first trimester as in women undergoing IVF-ET. The present study evaluated rates of spontaneous abortion according to age and according to degree of diminished oocyte reserve in women undergoing IVF-ET in which women with day 3 serum FSH > 11 mIU/ml were stimulated with mild FSH protocols.

Both authors reasoned that if no increase in spontaneous abortion rate was found according to those with or without diminished oocyte reserve, this finding would abrogate the widely held concept that oocytes from women with diminished oocyte reserve associated with high day 3 serum FSH levels are intrinsically more prone to meiosis errors and thus are highly unlikely to produce live pregnancies [9].

Materials and Methods

The first pregnancies achieved by women undergoing IVF-ET during a ten-year period at one university based IVF center were stratified into four groups based on the age of the female partner: ≤ 35, 36-39, 40-42, and 43-44 years. Each of the four

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Table 1. — *Pregnancy and spontaneous abortion rates by age and baseline FSH levels in women aged ≤ 39 years.*

Age at time of retrieval (years)	≤ 35				36-39			
Baseline FSH (mIU/ml) levels	≤ 11	12-14	15-17	> 17	≤ 11	12-14	15-17	> 17
No. transfers	2120	111	37	88	1313	120	47	93
No. pregnancies (beta-hCG > 100 mIU/ml)	805	41	17	44	434	50	20	46
% pregnancies/transfers	38.0	36.9	45.9	50.0	33.1	41.7	42.6	49.5
No. clinical pregnancies (ultrasound at six weeks)	717	36	15	39	369	44	14	35
% clinical pregnancy/transfer	33.8	32.4	40.5	44.3	28.1	36.7	29.8	37.6
No. chemical	73	4	2	4	58	6	5	9
No. ectopic	15	1	0	1	7	0	1	2
No. viable pregnancies	654	33	15	34	319	37	11	28
% viable pregnancy/transfer	30.8	29.7	40.5	38.6	24.3	30.8	23.4	30.1
No. miscarriages/clinical pregnancies	97	5	2	6	78	9	3	8
% miscarriage/clinical pregnancy	13.5	13.9	13.3	15.4	21.1	20.5	21.4	22.9
No. live deliveries	617	31	13	33	288	35	11	27
% delivered	29.1	27.9	35.1	37.5	21.9	29.2	23.4	29.0

Table 2. — *Pregnancy and spontaneous abortion rates by age and baseline serum FSH levels in women aged 40-44 years.*

Age at time of retrieval (years)	40-42				43-44			
Baseline FSH (mIU/ml) levels	≤ 11	12-14	15-17	>17	≤ 11	12-14	15-17	> 17
No. transfers	737	103	30	65	121	30	18	25
No. pregnancies (beta-hCG > 100 mIU/ml)	220	36	13	37	41	11	4	14
% pregnancies/transfer	29.9	35.0	43.3	56.9	33.9	36.7	22.2	56.0
No. clinical pregnancies (ultrasound at six weeks)	176	31	11	23	32	8	3	8
% clinical pregnancy/transfer	23.9	30.1	36.7	35.4	26.4	26.7	16.7	32.0
No. chemical	40	5	2	10	8	3	1	6
No. ectopic	4	0	0	4	1	0	0	0
No. viable pregnancies	136	19	9	15	26	3	0	2
% viable pregnancy/transfer	18.5	18.4	30.0	23.1	21.5	10.0	0.0	8.0
No. miscarriages/clinical pregnancies	56	14	4	9	13	6	3	7
% miscarriage/clinical pregnancy	31.8	45.2	36.4	39.1	40.6	75.0	100.0	87.5
No. live deliveries	115	17	7	14	19	2	0	1
% delivered	15.6	16.5	23.3	21.5	15.7	6.7	0.0	4.0

groups were further subdivided into four groups based on their day 3 serum FSH level (mIU/ml) during their screening cycle: ≤ 11, 12-14, 15-17, and > 17 mIU/ml.

The clinical pregnancy rates as determined by a gestational sac at six weeks were determined for each of these subgroups. The spontaneous abortion rate were equal to the percentage of women with a gestational sac who did not deliver at least one live baby.

All women with a day 3 serum FSH of ≥ 12 mIU/ml were treated with mild ovarian stimulation ranging from 150 units of FSH from days 3-5 or a completely natural cycle according to antral follicle count and level of day 3 serum FSH as previously described [12, 13]. Most cycles used gonadotropin releasing hormone (GnRH) antagonists.

The majority of IVF-ET cycles in women with normal day 3 serum FSH level used traditional controlled ovarian hyperstimulation commencing at 225 or 300 IU of FSH. They more often used GnRH antagonists, but a significant minority used the GnRH agonist leuprolide acetate from the mid-luteal phase. All women were treated with varying vaginal progesterone from the day after oocyte retrieval throughout the first trimester.

Only IVF cycles transferring on day 3 were included in the study to allow uniformity. Also, to prevent wrongly placing a woman with diminished oocyte reserve into the normal FSH category, women with elevated day 3 serum estradiol > 50 pg/ml but whose serum FSH was ≤ 11 mIU/ml, were not included in the study.

Statistical comparisons between groups were performed by either chi-square analysis or Fisher's exact test.

Results

The clinical (ultrasound evidence of pregnancy at six weeks), viable (live fetus at 12 weeks), live delivered pregnancy rates, and spontaneous abortion rates in women aged ≤ 39 are seen in Table 1. For women aged ≤ 35 or 36-39 years, there were no significant differences in spontaneous abortion rates between the four FSH groups or even a trend for higher spontaneous abortion rates with increasing day 3 serum FSH (p = NS, Fisher's exact test).

For women aged ≤ 35 years with normal day 3 serum FSH, the spontaneous abortion rate was 13.6% (97/717). For women aged 36-39 years with normal ovarian reserve, the spontaneous abortion rate was 21.1% (78/369). The spontaneous abortion rate was significantly higher in women aged 36-39 compared to those aged ≤ 35 years in women with normal oocyte reserve (p = 0.0017, chi-square analysis). Table 2 provides the same data for women aged 40-44 years. For women aged 40-42 years there was no significantly higher spontaneous

abortion rate in those women with serum FSH ≥ 12 mIU/ml (41.6%, 27/65) compared to women with normal FSH of ≤ 11 mIU/ml (31.8%, 56/176) (p = 0.0.21, chi-square analysis). There were no differences in spontaneous abortion rates in women aged 40-42 years with mildly diminished oocyte reserve (FSH 12-14 mIU/ml) vs severely depleted (FSH > 17) - 45.2% (14/31) vs 39.1% (9/23) (p = 0.78, Fisher's exact test).

For women aged 43-44 years, the spontaneous abortion rate was significantly higher for those women with increased day 3 serum FSH levels (84.2%, 16/19) vs those with normal serum FSH (40.6%, 13/32) with p = 0.006, chi-square analysis.

Considering women with normal FSH, there was a significantly higher spontaneous abortion rate comparing the four age groups, i.e., 13.5% (97/717) for women aged ≤ 35 vs 21.1% (78/369) for women aged 36-39 (p = 0.005, chi-square analysis) vs 31.8% (56/176) for women aged 40-42 (p = 0.009, chi-square analysis) vs 40.6% (13/32) for women aged 43-44 (p = 0.44, chi-square analysis).

Fetal karyotypes were available in 217 of the 320 products of conception. A female karyotype was seen in 124 of the 217 products of conception and a male karyotype in 93 abortuses. A chromosomal abnormality, i.e., trisomy, monosomy, triploidy, or tetraploidy was found in 71 of the 124 female fetuses (57.7%) vs 68 of 93 (73.1%) of male fetuses. The difference in male vs female could be related to possible maternal contamination. Looking just at male fetuses with abnormal karyotype, 40 of the 68 aborters (58.8%) were trisomies, 16 were monosomies (23.5%), ten were triploidies (14.7%), and two were tetraploidies (2.9%). Forty-one of the 61 (67.2%) women with normal oocyte reserve who had spontaneous abortions with male products of conception had chromosomal abnormalities vs 27 of 32 (81.7%) of those with diminished oocyte reserve (p = 0.13, chi-square analysis).

Discussion

Considering that chromosomal abnormalities are considered the most common cause of any given spontaneous abortion, it is a reasonable assumption that the cause of the majority of spontaneous abortions in these heavily progesterone supplemented women was fetal aneuploidy [14]. It is well-known that advanced maternal age is one of the most important associations with risk of aneuploidy. The present study certainly confirms this by showing a significantly higher spontaneous abortion rate with advancing age of the four age groups studied even in women with normal oocyte reserve.

However for the two younger groups (aged ≤ 39 years), there was no difference in spontaneous abortion rates within an age group according to the degree of oocyte reserve. There was not even a trend for higher spontaneous abortion rates in the group with the least ovarian reserve as manifested by a day 3 serum FSH of > 17 mIU/ml vs the normal reserve group with FSH ≤ 11 mIU/ml.

It was not until age 40-42 years that a significant increased rate of spontaneous abortion was found in the group with diminished vs normal ovarian reserve. However, even in this group, the differences were not great with a rate of 39.1% in the FSH group > 17 mIU/ml vs 31.6% for the FSH group ≤ 11 mIU/ml. The largest difference was found in the oldest group of 43-44-year-olds. Interestingly, the spontaneous abortion rate in the group of women aged 43-44 years with normal FSH was very similar to the group of 40-42-year-olds with diminished oocyte reserve.

These data are consistent with the concept that the oocytes with the least risk of meiosis errors are favored for selection of antral follicles from which the dominant follicle develops. These data are consistent with the concept that the etiology for younger women having diminished ovarian reserve is not from an acceleration of oocyte atresia in the natural order of the best ones first. Instead, these data are consistent with the concept that the main etiologic factor causing diminished oocytes reserve is a destructive process damaging significant portions of the ovaries; however what portion has been spared has the same proportion of oocytes with less risk of meiosis errors as their age peers, but only have a quantity remaining more akin to women of advanced reproductive age [15].

Tables 1 and 2 for women aged ≤ 42 years show no difference in live-delivered pregnancy rates in those with diminished vs normal oocyte reserve. These data are consistent with the concept that it is not the quality of the oocyte but the use of high FSH dosage of controlled ovarian hyperstimulation (COH) regimens that is responsible for the very low pregnancy rates reported by some of the most successful IVF centers for women with diminished oocyte reserve [12, 13]. One hypothesized mechanism of how high-dose stimulation protocols are responsible for very low pregnancy rates in women with diminished oocyte reserve is that the FSH receptors, which are very susceptible to down regulation, may be suppressed by a further rise in the serum FSH and some FSH dependent implantation factor is not produced leading to an embryo that looks normal but does not implant [12, 13, 15].

However, another possibility is somehow the high-dose FSH stimulation makes oocytes from women with diminished oocyte reserve to become more prone to meiosis errors, but in the presence of endogenous or mild exogenous FSH stimulation, these oocytes are not any more prone to meiosis errors than their age peers.

There is one inherent bias in these statistics which probably affects the pregnancy rate more than the spontaneous abortion rate. Our IVF center is known to take highly difficult cases with a poor prognosis. The majority of the women with elevated day 3 FSH have been refused IVF by other IVF centers because of their elevated day 3 FSH level. Thus frequently they are having their first IVF cycle with our group or possibly the second. In contrast, the normal FSH group has frequently failed after many cycles elsewhere or even in our own

practice either for unknown reasons or poor endometrial thickness, etc. Thus if this study was to determine the likelihood of live delivered pregnancies according to day 3 FSH levels, we would have only compared first IVF cycles. However for a miscarriage study we chose first pregnancies. The ideal study would use mild ovarian stimulation in all groups to exclude the confounding effect of hyperstimulation on pregnancy rates.

These data support the contention that of at least two possible mechanisms for diminished oocyte reserve, i.e., a destructive process damaging a large percentage of ovarian tissue, thus resulting in low number of remaining follicles, but the remainder of the ovarian tissue has the same percentage of normal oocytes as their age peers (just less of them). Alternatively another possibility is that there is a more rapid rate of atresia in the normal fashion, leaving not only less quantity of oocytes, but less quality ones more prone to meiosis errors. The former mechanism seems to be the more prevalent mechanism in women aged ≤ 39 years. When a woman reaches age 40-42 years based on these data, the percentage of women with a somewhat more rapid rate of atresia, as the mechanism of oocyte depletion, increases enough to cause a significant increase in spontaneous abortion rate. However, the spontaneous abortion rate is not that much higher thus suggesting that a good number of them have the destructive mechanism at their etiology. In contrast, in women aged 43-44 years, the majority with depleted oocyte reserve are just part of the bell-shaped curve and have defective oocytes. Nevertheless, live deliveries are still possible in a minority of the cases going through IVF.

There is still one more hypothesis that can explain these data. Aging of the mitochondria may explain the increased risk of aneuploidy with advancing age rather than advancing rise in day 3 FSH, or continued depletion of antral follicles, inhibin B, and the anti-Müllerian hormone. This theory contends that most oocytes are prone to meiosis errors but there may be one or at best a few with the best mitochondria that can allow proper chromosome segregation. However advancing age causes aging of mitochondria in all cells including the one or two oocytes in the cohort that have the "best" mitochondria, thus leading to errors of non-disjunction even in these "best" follicles. Indeed there are data suggesting that there are only an average of 1.8 normal embryos in a given group of embryos produced by an IVF-ET cycle, even in women with more embryos created by a high-dose FSH protocol vs women with less embryos produced by mild FSH stimulation [16].

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