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ASSISTED REPRODUCTION

Elevated basal FSH and embryo quality: lessons from extended culture embryos

Raised FSH and blastocyst quality

MY Thum · E Kalu · H Abdalla

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Abstract
Background The relationship between elevated basal FSH and embryo quality remains a topic of heated discussion among practitioners of ART. Some authors suggest a negative effect of raised FSH on the quality of embryos and therefore on IVF treatment outcome. We postulate that women with elevated FSH who respond well to ovarian stimulation and have embryos to transfer, have the same chance of conceiving like women of a similar age with normal FSH. To test this hypothesis, we studied women with elevated basal FSH who made enough embryos to qualify for blastocyst culture and day 5 embryo transfer.
Methods Analysis of data collected prospectively, on women age 25–43 years, who underwent IVF between January 2005 and December 2006. The women were divided into: those with high FSH (≥10 IU/L) and women with normal FSH (<10 IU/L). We analysed data to show treatment outcome in the two groups, following embryo transfer on day3 and after transfer on day5. Outcome measures include number of oocytes retrieved, number of embryos available, implantation rate, pregnancy and live birth rate.
Results Among the 1,858 women who under-went a day3 transfer, 1,368 had basal FSH≤10 IU/L, and in 492 basal FSH was above 10 IU/L. The average number of oocytes retrieved was lower among women with elevated FSH (10.12±5.6 Vs 6.16±3.9). Women with a normal FSH, had a higher pregnant and live birth rate than those with elevated FSH (43.3% vs 27.9% p=0.021) and (30.8% vs 17.6% p=0.028) respectively. 398 women made enough embryos to qualify for extended embryo culture to blastocysts. Of these 366 had an FSH≤10 IU/L and 32 had FSH>10 IU/L. In this group, there was no significant difference in the pregnancy and live birth rates between women with elevated and those with normal FSH, (67.2% vs 65.6%) and (51.9% vs 43.8%) respectively. In this selected group of women where quantity is not an issue, the quality of embryos was same irrespective of whether the basal FSH was low or high.
Conclusion Women with elevated basal FSH who respond well to stimulation and generate a good number of oocytes / embryos have a chance of becoming pregnant and having a live birth similar to that of women of their age. Women should therefore not be denied the benefits of IVF based solely on the basal FSH level as a subset may respond well and therefore have a good chance of taking home a baby.

Keywords Basal FSH,IVF · Embryo quality · Blastocysts

M. Thum (✉) · H. Abdalla
Lister Fertility Clinic, Lister Hospital,
Chelsea Bridge Road,
London SW1W 8RH, UK
e-mail: mythum@doctors.net.uk

E. Kalu
Assisted Conception Unit, Kingston Hospital NHS Trust,
Galsworthy Road,
Kingston Upon Thames KT2 7 QB, UK
e-mail: ekalu@doctors.org.uk

Introduction

The inverse relationship between elevated early follicular phase follicle stimulating hormone (FSH) and diminished ovarian reserve is universally accepted. However the link between a raised FSH and quality of the resultant embryos remains a topic of heated discussion among practitioners of ART. Various authors have suggested a negative effect of elevated FSH on embryonic quality and outcome of IVF [1-3]. Others including papers from our unit, have argued that elevated basal FSH reflects a quantitative rather than qualitative declined of ovarian function [4]. In our previous

paper [4] we showed that the lower pregnancy and live birth rates among women with elevated basal FSH was due to the fewer number of oocytes produced rather than the quality of the oocytes. We postulated that if a patient with elevated FSH gets to the stage of egg collection and embryo transfer, their chance of becoming pregnant and having a live birth was similar to that of women of their own age, with a similar number of embryos generated. To test this hypothesis further, we studied women with elevated basal FSH, who under went extended embryo culture to blastocyst and day 5 embryo transfer. In our unit, only women who have 6 or more embryos on day 3, with at least 3 at 8-cell stage are offered extended embryo culture. Hence all women with elevated basal FSH who had blastocyst transfer had enough embryos on day 3 to have been eligible for extended culture. Based on our hypothesis, we expect that there will be no significant difference in the pregnancy and live birth rate following blastocyst transfer among women with elevated and those with normal basal FSH, if there is no significant difference in the number of available embryos in the two groups.

Materials and methods

We prospectively collect and store data of all patients undergoing IVF/ICSI in our unit in a Medical System for IVF (MedicalSys, London, UK). We analysed data on all women aged 25 to 43 years who underwent IVF/ICSI treatment cycles with known basal FSH level in the two years between January 2005 and December 2006. The basal FSH level had been checked prior to commencing treatment. The women were divided into two groups: those with a high basal FSH (≥10 IU/L) and women with a low basal FSH (<10 IU/L). The level of 10 IU/L was found to be the level above which there was a significant change in the pregnancy rate in our previous study [4]. We then analysed data to show treatment outcome in the two groups, following embryo transfer on day 2 or 3 and following extended embryo culture and transfer on day 5. Outcome measures include, number of oocytes retrieved, number of embryos available, implantation rate, pregnancy and live birth rate.

FSH assay

Serum FSH was measured in early follicular phase (days 2 to 4) in the cycle preceding treatment. FSH concentration was measured using a two-step chemiluminescent microparticle immunoassay (CMIA) and analysed by Abbott Architect System (Abbott Laboratories IL). The analytical sensitivity of the assay was calculated to be better than 0.05 mlU/ml (n = 36 runs). Analytical sensitivity is defined is defined as the concentration at 2 SDs from the ARCHITECT FSH Master-Check Level 0 (0.00 mlU/ml), and represent the lowest measurable concentration of FSH that can be distinguished from zero. The specificity of the assay was determined by studying the cross-reactivity of LH, thyroid-stimulating hormone (TSH), and HCG. The percentage cross-reactivity was calculated and was shown to be 0.002% for LH, 0.043% for TSH, and 0.001% for HCG. The inter-assay coefficients of variation were 2.9 and 3.8% respectively.

IVF stimulation and blastocyst culture

In brief, the IVF treatment protocol includes ovarian stimulation, with either recombinant FSH, human menopausal gonadotrophin or urinary FSH. A trans-vaginal scan was performed prior to ovarian stimulation to ensure the ovaries were quiescent.

Patients were down regulated with either Nafarelin or Buserelin at mid luteal phase. When follicles reached pre-ovulatory size (18 to 22 mm), 10,000 IU of hCG was administrated. Oocytes were aspirated using trans-vaginal ultrasound guidance 34 to 36 h after hCG administration. For fertilisation, standard insemination or ICSI was performed as clinically appropriate. Embryo culture was performed using a sequential micro-drop system at an atmosphere of 5–6% CO_2 at 37°C. SAGE sequential cleavage media (SAGE In-vitro Fertilization Inc. Trumbull, Connecticut) was used for embryos on day 1–3, and patients who met the criteria for extended culture continued to the blastocyst stage. To qualify for extended culture patients should have at least 6 embryos on day 3, with at least 3 at 8-cell stage and at top quality [5]. If this condition was met, all embryos were allowed to progress to the blastocyst stage irrespective of their cell number or quality. Quinn's Advantage Fertilization medium (SAGE In-vitro Fertilization Inc. Trumbull, Connecticut) was used for day 3–5 embryos. Using the Gardner and Schoolcraft scoring system [5], the best quality blastocysts were selected on day 5 for transfer. 400 mg cyclogest pessary was administered to all the patients for luteal support. A pregnancy test was performed 10 days following blastocyst transfer and a transvaginal ultrasound scan at 5–6 weeks to determine the number of gestation. In this study a pregnancy was defined as a positive serum or urine HCG test and a sac on ultrasound scan, or an ectopic pregnancy. A live birth was defined as a pregnancy resulting in a viable infant. Twins were counted as one live birth. Fertilisation rate was defined as number of two pronuclear (2PN) embryos per number of oocytes collected x 100 for each treatment cycle including ICSI cycles.

Data analysis

Data was collected in Medical System for IVF (MedicalSys, London, UK) and analysed by Statistics Package for Social

Table 1 Treatment outcome in high or low basal FSH groups for women age≤43 following day2 or 3 embryo transfer

	FSH≤10IU/L	FSH>10IU/L	*P*-value
Number of patients	1368	494	NA
Basal FSH levels IU/L ± SD	6.82±1.7	15.79±6.1	NA
Mean age ± SD	37.1±4.5	38.9±3.8	NS
Days of taking Gonadotrophins (mean ± SD)	11.5±3.1	11.9±3.8	NS
No of ampoules[a] consumed (mean ± SD)	38.6±15.6	47.4±18.7	0.011
Average no. of oocytes collected ± SD	10.12±5.6	6.18±3.9	0.019
Average no of normal fertilized embryos ± SD	6.18±3.9	3.40±2.5	0.001
Average no of embryos transferred ± SD	1.92±0.58	1.88±0.62	NS
Pregnancy rate (%)	**43.3%** (594/1368)	**27.9%** (138/494)	0.021
Live birth rate (%)	**30.8%** (421/1368)	**17.6%** (87/494)	0.028

NS difference not statistically significant (P>0.05); *NA* not applicable; *a* each ampoules contain 75 iu of gonadotrophins

Sciences (SPSS, Surrey, UK). Descriptive statistical analysis was performed initially to examine the normal distribution of all continuous variances for parametric statistical tests. Chi-square Cross Tabulation test was used to analyse the significant difference in pregnancy rates, live birth rates and twin rate between the groups. Statistical significant was set at $P<0.05$.

Results

Two thousand two hundred fifty six cycles of IVF/ICSI in women with known basal FSH levels during study period were identified. 1,858 women had day2/3 embryo transfer, while 398 women had extended embryo culture to blastocysts; transferred on day5.

Day2 or 3 embryo transfer (n=1858)

Among the 1858 women who under-went a day2 or 3 transfer, 1368 had a basal FSH≤10 IU/L, and in 492 women the basal FSH was above 10 IU/L. The average number of oocyte retrieved was significantly lower among women with elevated FSH (10.12±5.6 Vs 6.16±3.9) compared to women with FSH below 10 IU/L. In the low FSH group, 594 of 1368 (43.3%) women got pregnant and 421 had a live birth (30.8%) compared to 138 of 494 (27.9%) and 87 (17.6%) respectively among women with elevated basal FSH (Table 1).

Day5 embryo (blastocyst) transfer (n=398)

Among the women who under went blastocyst transfer, 366 women had a basal FSH≤10 IU/L and in 32 women the basal FSH was greater than 10 IU/L. In this group, 246 of 366 (67.2%) women with low FSH got pregnant and 190 (51.9%) had a live birth. 21 of 32 (65.6%) women with elevated FSH got pregnant and 14 (43.8%) had a live birth. (Table 2).

Table 3 shows the outcome when women with similar basal FSH level (and who generated enough embryos for blastocyst culture) were sub-divided according to age. Among the 366 women with low FSH, 240 were age <38, and 126 were≥38 years old. 149 of 240 (70.8%) women age <38 with low FSH got pregnant. In this sub-group, the implantation rate was 57.5% and 57.1% (137/240) had a live birth. The results were better than that obtained by their older counter parts with similar FSH levels (Table 3). In this older group of women age≥38, with low FSH, 61 of 126 (48.4%) got pregnant. The implanta-

Table 2 Treatment outcome in high or low basal FSH groups for women age≤43 following blastocyst transfer

	FSH≤10IU/L	FSH>10IU/L	*P*-value
Number of patients	366	32	NA
Basal FSH levels IU/L ± SD	7.11±2.1	13.21±3.8	NA
Mean age ± SD	35.4±4.7	36.8±5.9	NS
Days of taking Gonadotrophins (mean ± SD)	10.9±2.8	11.8±3.9	NS
No of ampoules[a] consumed (mean ± SD)	39.2±11.7	49.2±19.7	0.019
Average no. of oocytes collected ± SD	12.7±5.2	9.87±3.1	0.046
Average no of normal fertilized embryos ± SD	8.8±3.8	7.5±2.9	NS
Average no of embryos transferred ± SD	1.62±0.21	1.66±0.29	NS
Pregnancy rate (%)	**67.2%** (246/366)	**65.6%** (21/32)	NS
Live birth rate (%)	**51.9%** (190/366)	**43.8%** (14/32)	NS

NS difference not statistically significant (P>0.05); *NA* not applicable; *a* each ampoules contain 75 iu of gonadotrophins

Table 3 Treatment outcome for women with blastocyst transfer in cycles with high or low basal FSH level stratified according to age

	Low FSH			High FSH		
	Age<38	Age≥38	*P*	Age<38	Age≥38	*P*
Number of patients	240	126	NA	18	14	NA
Mean age ± SD	33.1±3.3	39.9±4.1	NA	34.8±4.2	39.5±4.4	NA
Basal FSH levels IU/L ± SD	6.8±2.3	7.7±2.8	NA	12.4±2.6	14.3±2.6	NA
No. of oocytes collected ± SD	12.7±6.6	12.6±5.1	NS	10.5±2.6	9.1±3.4	NS
No of embryos transferred ± SD	1.52±0.2	1.81±0.6	NS	1.72±0.33	1.57±0.69	NS
Pregnancy rate (%)	170/240 (70.8%)	76/126 (60.3%)	0.028	13/18 (72.2%)	8/14 (57.1%)	0.032
Implantation rate (%)	210/365 (57.5%)	94/228 (41.2%)	0.009	14/31 (45.2%)	5/22 (22.7%)	0.005
Live birth rate (%)	137/240 (57.1%)	53/126 (42.1%)	0.007	11/18 (61.1%)	3/14 (21.4%)	0.028

NS difference not statistically significant (P>0.05); *NA* not applicable

tion rate in this subgroup was 41.2% and 42.1% (53/126) had a live birth (Table 3).

Of the 32 women with elevated basal FSH, 18 were age <38, and 14 were age≥38. 12 of 18 (66.7%) women <38 got pregnant. The implantation rate in this subgroup was 45.2% and 61.1% (11/18) had a live birth. Among the 14 women≥38 who had elevated FSH, 3 got pregnant (21.4%). The implantation rate was 22.7% and 21.4% had a live birth (3/14) (Table 3).

Table 4 shows the results when women of similar ages where categorized according to basal FSH levels. Of the 258 women age<38, 240 had a basal FSH≤10 IU/L and in 18 women the basal FSH was>10. The implantation rate, pregnancy rate and live-birth rate was similar between women with normal and those with elevated basal FSH (57.5 vs 45.2%, 70.8 vs 72.2%, and 57.1 vs 61.1% respectively).

Of the 140 age≥38, 126 had a basal FSH≤10 IU/L and in 14, the FSH was >10 IU/L. Again the implantation rate, pregnancy and live-birth rates was not statistically different between women with normal FSH and those with elevated FSH (41.2 vs 22.7%, 60.3 vs 57.1% and 42.1 vs 21.4% respectively) (Table 4).

Discussion

The assumption that an elevated basal FSH is associated with adverse IVF outcome has been used to counsel women against proceeding with IVF treatment using their own gametes. This practice has significant implications, not only in this era of donor scarcity, but also as it deprives these women of the opportunity of having their own biological off-springs. This assumption has also been instrumental in the formulation of health authority criteria for state funded IVF treatment. Some Primary care Trusts in the UK, would not fund treatment for women with basal FSH over 10 IU/L since the outcome is expected to be poor.

We have shown that elevated basal FSH is not necessarily associated with poor IVF outcome. It is generally agreed that elevated FSH is associated with diminished ovarian reserve [6]. Our data is in keeping with

Table 4 Treatment outcome for women with blastocyst transfer in cycle with young or older age women and high or low basal FSH groups

	Age<38			Age≥38		
	Low FSH	High FSH	*P*	Low FSH	High FSH	*P*
Number of patients	240	18	NA	126	14	NA
Mean age ± SD	33.1±3.3	34.8±4.2	NA	39.9±4.1	39.5±4.4	NA
Basal FSH levels IU/L ± SD	6.8±2.3	12.4±2.6	NA	7.7±2.8	14.3±2.6	NA
No. of oocytes collected ± SD	12.7±6.6	10.5±2.6	NS	12.6±5.1	9.1±3.4	NS
No. of embryos transferred ± D	1.52±0.2	1.72±0.33	NS	1.81±0.6	1.57±0.69	NS
Implantation rate (%)	210/365 (57.5%)	14/31 (45.2)	0.126	94/228 (41.2%)	5/22 (22.7%)	0.065
Pregnancy rate (%)	**70.8%** (170/240)	**72.2%** (13/18)	0.57	**60.3%** (76/126)	**57.1%** (8/14)	0.516
Live birth rate (%)	**57.1%** (137/240)	**61.1%** (11/18)	0.47	**42.1%** (53/126)	**21.4%** (3/14)	0.101

NS difference not statistically significant (P>0.05); *NA* not applicable

this observation, as women with elevated FSH were shown to have a lower egg yield compared to those with a normal FSH (Table 1). This reduced number of oocytes translates to fewer numbers of embryos available for transfer and this we believe is responsible for the lower pregnancy and live birth rates in women with elevated FSH.

Our results on women who responded well to controlled ovarian hyperstimulation despite an elevated basal FSH clearly show that treatment outcome is similar to their counterparts with normal basal FSH (Table 2). Age is clearly the most important factor that determines outcome of treatment and this reflects on the results on Table 3, where among women with similar FSH levels, younger women consistently did better than their older counter-part. When we nullified the effect of age by analyzing women in similar age groups, the results for women with elevated FSH was comparable to their peers with normal FSH. We have shown that some women with elevated FSH can still respond well to stimulation and make blastocysts. And when this happens, their chances of taking home a baby is similar to those of women of their own age, with a similar number of embryos generated. Our results are in keeping with others [7-9] who also showed that women with elevated basal FSH levels can still achieve reasonable pregnancy rates with ART. Women with elevated FSH may be a heterogenous group. Some may have true reduced ovarian reserve, and therefore respond poorly to ovarian stimulation. Other cases of elevated FSH may be due to the presence of heterophylic antibodies. Furthermore FSH receptor polymorphism could also result in an elevated value in women with otherwise normal ovaries [10]. This latter group of women are likely to respond well to stimulation if given the opportunity to under go IVF.

In our series, women with elevated basal FSH who made enough oocytes and embryos to meet our criteria for blastocyst culture had a pregnancy rate of 65.6% and a live birth rate of 43.8% which was comparable to 67.2% and 51.9% respectively achieved by their counterparts with normal FSH. As it is currently not possible to identify this subset of women with 'high egg /embryo yield' despite elevated FSH, denying some women the opportunity to under go IVF on the sole basis of FSH levels, may be difficult to justify.

This is the first paper to discuss blastocyst culture among women with elevated FSH and to establish that women with elevated basal FSH also have the potential to develop good quality blastocysts. Embryo quality is generally reflected by implantation rates. That women with elevated basal FSH had the same implantation rates as their age-matched peers implies that embryo quality is not significantly affected by FSH level. It seems that maternal age rather than basal FSH is a better determinant of embryonic quality. The lower implantation rate, clinical pregnancy and live birth rates among the older women in both the low and high FSH groups may reflect age associated qualitative decline of ovarian reserve, rather than an FSH effect as has been suggested by some authors. [11, 12]. The negative effect of advance maternal age rather than basal FSH on embryo quality has been assessed in a recent paper from our unit. In that paper [13], we showed that although the proportion of aneuploid embryos increased with advanced maternal age, the percentage of aneuploid embryos was not significantly different between women with high basal FSH and those with normal FSH. Our results clearly confirms the hypothesis that women with elevated basal FSH, who make adequate number of embryos, have a chance of having a live-birth similar to their peers with normal basal FSH.

Conclusion

While women with elevated FSH may be counselled to expect a lower pregnancy rate following IVF, we have shown that this is dependent on how well they respond to ovarian stimulation. Women with elevated FSH who get to the stage of egg collection and embryo transfer, have the same chances of becoming pregnant and having a live birth similar to that of women of their own age, with a similar number of embryos generated. Pre-treatment management of patients' expectation is however important since outcome is poorer among those with poor response to controlled ovarian hyperstimulation. Women with elevated basal FSH should however not be denied the benefits of IVF based solely on FSH levels, as a subgroup of them have a good chance of conceiving.

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