Journal of Reproduction & Contraception
2013 Dec.; 24(4):221-227

doi: 10.7669/j.issn.1001-7844.2013.04.0221
E-mail: randc_journal@163.com

The Younger the Patients the Less Adverse Effect of Diminished Oocyte Reserve on Outcome Following *in vitro* Fertilization -embryo Transfer as Long as the Proper Ovarian Stimulation Protocol is Used

Jerome H. CHECK[1,2], Carrie WILSON[2]

1. Cooper Medical School of Rowan University, Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology & Infertility, Camden, New Jersey, USA

2. Cooper Institute For Reproductive Hormonal Disorders, P.C. Marlton, New Jersey, USA

Objective *To determine the confounding effect of oocyte reserve and age on pregnancy rates per oocyte retrieval and a comparison of the average number of babies born from a given oocyte harvest.*

Methods *In vitro fertilization-embryo transfer (IVF-ET) cycles were retrospectively reviewed over a 10-year period. They were stratified according to whether they had normal oocyte reserve (day 3 serum FSH <12 mIU/ml and serum estradiol <50 pg/ml) or diminished oocyte reserve. Mild stimulation was used if low oocyte reserve. The pregnancy rate per retrieval included all pregnancies (fresh or frozen embryo transfer) resulting from one retrieval. The average number of babies born was also compared according to reserve.*

Results *For the diminished oocyte reserve group, the live delivered pregnancy rates per transfer were 80% as good in women aged <35 years, 70% as good in women 36–39 years, but only 43% as good as women aged 40–42 years. However, because of more total embryos the pregnancy rate per given oocyte retrieval was only 55% as good for women with diminished oocyte reserve aged <39 years. The average number of babies born per retrieval was twice as high as those with normal reserve for this age group.*

Conclusion *The use of mild follicle stimulating hormone (FSH) stimulation allows a live delivered pregnancy rate per transfer that is 75% as good for women aged <39 years but because of less embryos a likelihood of pregnancy rate per retrieval only half as good as women with normal reserve.*

Key words: live delivered pregnancy rates; ovarian reserve; mild ovarian hyperstimulation

Corresponding author: Jerome H. Check; Tel: +215-635-4156; Fax: +215-635-2304; E-mail: laurie@ccivf.com

Many *in vitro* fertilization (IVF) centers have recorded very poor live delivered pregnancy rates in women with decreased oocyte reserve as manifested by increased day 3 serum follicle stimulating hormone (FSH) levels[1-3]. Since embryos appear normal, the conclusion is that oocyte quality is more comparable with older women with a similar paucity of oocytes rather than their age peers[1-3].

However, there is a claim that much improved live delivered pregnancy rates especially in younger women aged <39 years with diminished oocyte reserve can be achieved as long as mild not conventional or high dosage FSH stimulation is used[4].

One hypothesis to explain these disparate conclusions has been that the poor oocyte quality in younger women with diminished oocyte reserve reported in some other studies is iatrogenic, i.e., related to the high dosage of exogenous FSH which raises serum FSH levels which, in turn, down regulates FSH receptors to a key enzyme contributing to chromosome separation during meiosis[5].

The objective of this study was to present retrospectively a very large data set allowing comparison of pregnancy rates following *in vitro* fertilization-embryo transfer (IVF-ET) according to age and status of oocyte reserve where the ovarian stimulation regimen for those with diminished oocyte reserve followed the principles of mild ovarian stimulation.

Materials & Methods

A retrospective cohort comparison study evaluated all IVF-ET cycles over a 10-year period at a University based IVF center. The controlled ovarian hyperstimulation (COH) protocol for women deemed normal oocyte reserve (day 3 serum FSH<11 mIU/ml) was only a full stimulation protocol (225–300 IU FSH from day 3). The COH regimen for those with diminished oocyte reserve (day 3 serum FSH >12 mIU/ml) was always mild. Gonadotropin releasing hormone (GnRH) agonists or antagonists could be used. They were leuprolide acetate (Lupron®, Sandoz Inc., Princeton, NJ), cetrorelix (Cetrotide®, Serono Pharmaceuticals, Australia) and ganirelix (Organon, NJ).

Embryo selection and cryopreservation method

After fertilization 2–2.5 times, the embryos with intended number were allowed to cleave to day 3 and the rest were cryopreserved at the 2 pronuclear stage. The best of cleaved embryos with the designated number were transferred on day 3 and the others that progressed to day 3 were cryoprserved at the multi-cell stage on day 3. The cryopreservtion procedure was a simplified slow cool technique using a BioCool alcohol rate controlled freezer with a one step removal of the cryoprotectant 1,2 propanediol[6]. Similarly, when frozen ET was performed 2–2.5 times, the embryos were thawed and the ones with best morphology were transferred on day 3. Multi-cell day 3 embryos that survived but were not transferred were re-frozen[7,8].

Patient selection

Comparisons were made between 3 age groups: <35 years, 36–39 years and 40–42 years. Not only were comparisons made for fresh ET but also live delivered pregnancies per retrieval which allowed a pregnancy if the fresh ET failed but a pregnancy resulted from a subsequent frozen ET from embryos solely derived from that retrieval.

If an oocyte was retrieved but failed to progress to an embryo for transfer on day 3, the cycle was not included in this study. This study was intended to simply compare the quality of embryos formed as evidenced by live delivered pregnancy rates in women with normal *vs* diminished oocyte reserve and thus an ET was needed.

Mild stimulation method

Mild stimulation could range anywhere from completely natural, to a small boost of 75–150 IU FSH(either follistim from Merck, Whitehouse Station, NJ or bravelle or menopur from Ferring, Incorporated, Parsippany, NJ) from the late follicular phase, or to 150 IU FSH from day 3 to day 5 with an increase to 225 IU when the GnRH antagonist (cetrorelix or ganirelix) was used[4].

Menstrual cycles could vary from short intervals of 21 d to regular menses of 28–30 d, to oligomenorrhea, or to amenorrhea with normal estrogen [as evidenced by withdrawal menses because of progesterone (P) withdrawal], to amenorrhea with estradiol (E_2) deficiency. Some women with E_2 deficiency failed to respond with a rise in E_2 despite previous exposure in other IVF centers to high-dose gonadotropin therapy, yet they developed a mature follicle(s) and were able to undergo oocyte retrieval by lowering the elevated serum FSH with ethinyl E_2 (theoretically by possibly restoring down-regulated FSH receptors in granulosa and theca cells)[9,10]. The technique of using ethinyl E_2 either alone, or with a small boost of gonadotropins once a dominant follicle has been recruited, was previously described[11-14]. Ethinyl E_2 does not provide any increase to serum E_2 and thus allows appropriate monitoring of follicular maturation[14]. Ovulation can also be induced in woman in apparent ovarian failure by also lowering FSH by the use of GnRH agonists or antagonists[15-17].

If a woman was being observed in a natural cycle but did not attain a minimum average 17 mm diameter follicle with a serum E_2 >200 pg/ml, oocyte retrieval was not attempted, but in the next cycle, 75 IU of exogenous FSH would be started once the follicle was about 10 mm in diameter with a serum E_2 >80 pg/ml, or else would be started on day 5 if the FSH was not increased. Sometimes high FSH would be reduced by treating the women with 20 μg of ethinyl E_2 (compounded by pharmacist-raw material from Schering, Germany)(which is not recorded in the serum E_2 assay), and gonadotropins would be started once the serum FSH approached normal, i.e., <10 mIU/ml. A woman showing three antral follicles on day 3 might be started with 75 IU gonadotropin by days 5–7 if the E_2 was spontaneously rising and the FSH dropped to <10 mIU/ml.

Some cycles did not use a GnRH antagonist, but if the LH doubled from baseline,

frequently either cetrorelix or ganirelix was added SC at 250 μg/d. If a GnRH antagonist, e.g., cetrorelix or ganirelix was given at 250 μg/d SC daily when a follicle attained an average diameter of 14 mm, generally an extra 75 IU of gonadotropins were added once the GnRH antagonist was started.

If the baseline serum FSH was increased and remained increased despite the serum E_2 rising as the follicle matured, frequently the retrieval would be performed completely naturally without gonadotropins. When evaluating a natural cycle, if the follicle never reached maturity, the retrieval would be cancelled, but for the next cycle, a minimal boost of gonadotropin would be given. If a woman was started on ethinyl E_2, to lower FSH, and the FSH dropped to <6 mIU/ml, 75 IU gonadotropin would usually be started even if there had been no endogenous rise in serum E_2.

For the decreased oocyte reserve group there were no cancellations for inadequate number of dominant follicles. If there was one follicle, a retrieval was performed[4]. Even women in apparent menopause with serum FSH levels >100 mIU/ml had oocyte retrievals as long as FSH down regulation led to a dominant follicle[9,10,17].

Statistical analysis

Statistical analysis used chi-square to compare pregnancy rate per fresh ET and oocyte retrieval for each age group for those with normal egg reserve and similarly for those with diminished egg reserve group comparing those with normal versus diminished egg reserve. Furthermore chi-analysis was used to compare differences in the live delivered pregnancy rate per fresh ET and per oocyte retrieval within each age group. $P<0.01$ was considered significant.

Results

The live delivery rates per transfer and retrieval according to ovarian oocyte reserve status and age are shown in Table 1. For the youngest group (<35 years) there was a significant difference in the live delivered pregnancy rate per transfer (42.2% normal oocyte reserve *vs* 33.5% diminished oocyte reserve) (P=0.001 4) and per retrieval and a significantly higher pregnancy rate per retrieval in those with normal *vs* diminished oocyte reserve (66.9% *vs* 36.5%) (P<0.000 1). Similarly there were significantly higher pregnancy rates per transfer and per retrieval in those women aged 36–39 years and 40–42 years in the normal *vs* diminished oocyte reserve group (P<0.000 1).

The live delivery rate per transfer in women with diminished oocyte reserve in women aged <35 years (33.5%) was significantly higher than that in women with normal oocyte reserve who were aged 40–42 years (20.9%) (P<0.000 1). Even when one considers more embryos produced with normal oocyte reserve in the group aged 40–42 years and thus more ET per retrieval than the group aged <35 years with diminished ovarian reserve, the live

Table 1 Live delivery and implantation rates per retrieval according to ovarian oocyte reserve status and age

Item	Age (year)		
	<35	36–39	40–42
With diminished ovarian reserve			
No. of retrievals	304	485	433
No. of transfers	331	519	440
No. of deliveries	111	115	40
Live delivered pregnancy rate per fresh embryo transfer (%)	33.5*#	22.2#	9.1*#
Live delivered pregnancy rate per retrieval (fresh and frozen) (%)	36.5*#	23.7#	9.2*#
No. of babies delivered	142	130	45
Average No. of babies per retrieval oocyte cycle	0.47	0.27	0.10
Normal oocyte reserve			
No. of retrievals	1 349	708	406
No. of transfers	2 138	916	473
No. of deliveries	902	302	99
Live delivered pregnancy rate per fresh embryo transfer (%)	42.2*	33.0	20.9*
Live delivered pregnancy rate per retrieval (fresh and frozen) (%)	66.9*	42.7	24.4*
No. of babies delivered	1 234	390	114
Average No. of babies per retrieval	0.91	0.55	0.28

*: $P<0.01$, compared with 36–39-year group at the same ovarian oocyte reserve status

#: $P<0.01$, compared with those with normal oocyte reserve for each age group

delivery rate per oocyte retrieval (36.5%) was still significantly higher than that in the group aged 40–42 years with normal oocyte reserve (24.4%) ($P<0.000\ 1$).

Discussion

The data on number of babies born per retrieval reveals the following: women aged <35 years with normal oocyte reserve will average about 1 baby per retrieval which would be about twice as high as women aged <35 years with diminished oocyte reserve and about twice as high as women aged 36–39 years with normal oocyte reserve.

Similarly women aged 36–39 years with normal oocyte reserve are approximately twice as likely to produce a live baby per retrieval than a woman of comparable age with diminished reserve and also twice as much as a woman aged 40–42 years with normal oocyte reserve.

The data used for this manuscript were presented at ther 2013 meeting at the American Society for Reproductive Medicine in Boston, Massachusetts. At the same meeting we presented data on a subset of the group reported here—women in overt ovarian failure[18]. There were only 20 transfers but there was a 20% pregnancy rate per transfer. However, the group was so small it did not alter the pregnancy rate for the diminished oocyte group by much reported in this study[18].

The live delivered pregnancy rate per transfer was 80% as good for women <35 years with diminished oocyte reserve compared to age peers with normal reserve. These relatively good results seems to conflict with conclusions made by some other very fine centers for IVF[1-3]. We suspect but cannot prove that the reason for our better success was the use of mild stimulation[19]. We have hypothesized that high dose FSH protocols may down regulate a key FSH directed enzyme which helps chromosome separation during meiosis and thus high dosage FSH may lead to an increased risk of aneuploidy[20].

One should not extrapolate from this study that mild stimulation should be used for all women even those with normal oocyte reserve. At the same 2013 meeting of the ASRM, we presented the data from another study comparing outcome following mild *vs* normal FSH stimulation for women with normal oocyte reserve[21]. Full stimulation did produce slightly higher live delivered pregnancy rates per transfer and the difference was even more increased if one considers the pregnancy rate per retrieval[21]. There are pros and cons of using mild *vs* full stimulation for women with normal reserve. The present data merely speculates that mild stimulation should be exclusively used for women with diminished reserve but these same conclusions should not necessarily be applied to women with normal oocyte reserve.

Since women aged <35 years were almost twice as likely to have a live baby from an oocyte retrieval than women aged 40–42 years with normal reserve, it is clear that although both age and diminished oocyte reserve independently reduce the chance of a live baby from IVF-ET, age exerts the greater adverse effect.

Pregnancy rates vary amongst different IVF centers. However, if principles of mild ovarian stimulation are followed, it is likely that any given IVF center would produce the same relative effect with diminished reserve compared to their pregnancy rates in women of different age groups with normal reserve. Couples should be presented these data so that women of advanced reproductive age of 40–42 years or women of any age with diminished ovarian reserve who need IVF-ET to achieve a pregnancy can make their own decision to attempt IVF-ET with their own eggs or switch to the use of donor oocytes.

References

1. Fenichel P, Grimaldi M, Olivero JF, et al. Predictive value of hormonal profiles before stimulation for *in vitro* fertilization. Fertil Steril, 1989, **51**(5):845-9.
2. Scott RT, Toner JP, Muasher SJ, et al. Follicle-stimulating hormone levels on cycle day 3 are predictive of *in vitro* fertilization outcome. Fertil Steril, 1989, **51**(4):651-4.
3. Roberts JE, Spandorfer S, Fasouliotis SJ, et al. Taking a basal follicle-stimulating hormone history is essential before initiating *in vitro* fertilization. Fertil Steril, 2005, **83**(1):37-41.
4. Check JH, Summers-Chase D, Yuan W, et al. Effect of embryo quality on pregnancy outcome following single embryo transfer in women with a diminished egg reserve. Fertil Steril, 2007, **87**(4):749-56.
5. Check JH. The importance of up and down regulation of receptors for FSH, progesterone and the gonadotropin

releasing hormone (GnRH) in the ovary, endometrium and lymphocytes in correcting various ovulatory disorders and achieving live deliveries//De Lange K, Dequire E (eds.). Ovulation Detection Signs/Symptoms and Outcomes. New York: Nova Biomedical, 2012:1.

6. Baker AF, Check JH, Hourani CL. Survival and pregnancy rates of pronuclear stage human embryos cryopreserved and thawed using a single step addition and removal of cryoprotectants. Hum Reprod Update, 1996, **2**:271(CD-ROM), Item 12.
7. Check JH, Brittingham D, Swenson K, et al. Transfer of re-frozen twice-thawed embryos do not decrease the implantation rate. Clin Exp Obstet Gynecol, 2001, **28**(1):14-6.
8. Check JH. Refreezing of embryos//Sharif K, Coomarasamy A (eds). Assisted Reproduction Techniques. Challenges and Management Options. Hobokon, NJ: Wiley-Blackwell, 2012, **74**:317-9.
9. Check JH, Summers D, Nazari A, et al. Successful pregnancy following *in vitro* fertilization-embryo transfer despite imminent ovarian failure. Clin Exp Obstet Gynecol, 2000, **27**(2):97-9.
10. Check ML, Check JH, Choe JK, et al. Successful pregnancy in a 42-year-old woman with imminent ovarian failure following ovulation induction with ethinyl estradiol without gonadotropins and *in vitro* fertilization. Clin Exp Obstet Gynecol, 2002, **29**(1):11-4.
11. Check JH, Nowroozi K, Chase JS, et al. Ovulation induction and pregnancies in 100 consecutive women with hypergonadotropic amenorrhea. Fertil Steril, 1990, **53**(5):811-6.
12. Check JH, Check ML, Katsoff D. Three pregnancies despite elevated serum FSH and advanced age: case report. Hum Reprod, 2000, **15**(8):1709-12.
13. Check ML, Check JH, Kaplan H. Pregnancy despite imminent ovarian failure and extremely high endogenous gonadotropins and therapeutic strategies: Case report and review. Clin Exp Obstet Gynecol, 2004, **31**(4):299-301.
14. Check JH. The multiple uses of ethinyl estradiol for treating infertility. Clin Exp Obstet Gynecol, 2010, **37** (4):249-51.
15. Check JH, Wu CH, Check M. The effect of leuprolide acetate in aiding induction of ovulation in hypergonadotropic hypogonadism: a case report. Fertil Steril, 1988, **49**(3):542-3.
16. Check JH, Katsoff B. Ovulation induction and pregnancy in a woman with premature menopause following gonadotropin suppression with the gonadotropin releasing hormone antagonist, cetrorelix–a case report. Clin Exp Obstet Gynecol, 2008, **35**(1):10-2.
17. Check JH. The concept and treatment methodology for inducing ovulation in women in apparent premature menopause. Clin Exp Obstet Gynecol, 2009, **36**(2):70-3.
18. Check JH, Wilson C, Citrino-DiAntonio G, et al. *In vitro* fertilization (IVF) outcome in women in overt menopause attempting to induce follicular maturation by follicle stimulating hormone (FSH) receptor down-regulation. Fertil Steril, 2013, **100**(3):S150.
19. Check JH. Mild ovarian stimulation. J Assist Reprod Genet, 2007, **24**(12):621-7.
20. Slovis BH, Check JH. 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. Clin Exp Obstet Gynecol, 2013, **40**(1):29-32.
21. Check JH, Choe JK, Cohen R, et al. The effect of conventional *vs* mild ovarian hyperstimulation on the total number of live babies born from a given oocyte retrieval. Fertil Steril, 2013, **100**(3):S269.

(Received on November 5, 2013)