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Original Article

Role of Female Age in Determining Causes of Infertility and Predicting Outcome of in Vitro Fertilization

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Abstract

Objective: To look into various causes of infertility according to increasing age, differences in percentage of pregnancy rate after IVF in different age groups and role of various infertility parameters in determining pregnancy rate among these patients

Patients and methods: A total of 206 infertile couples were selected and divided into two groups according to female age: Group I (<35 years, n=108) and Group II (≥35 years, n=98). Diagnostic hysterosalpingography or laproscopy was done in order to rule out the tubal blockage and endometriosis. Ovarian dysfunction was excluded on the basis of hormonal analysis. IVF treatment following a long protocol for use of GnRH agonist was given to infertile couple. Analysis was done using SPSS 19 and MedCal software 12.

Results: Male causes were frequent in both groups without any significant difference (33%; p=0.882). Tubal factor was significantly raised in older group (33%; p=0.000), whereas ovarian dysfunction was significantly higher in younger patients (33%; p=0.000). Number of embryos transferred and pregnancy rate after IVF were significantly decreased in older couples (p≤0.05). Age of patient and serum FSH levels (p=0.02) have relatively better predictive capability of pregnancy outcome regarding IVF treatment as compared to duration of infertility (p=0.04).

Conclusion: Information of infertility factors can help clinician in estimating the chances of pregnancy rate before suggesting ART in old age couples.

Keywords: Infertility, advance reproductive age, IVF, Tubal factor, ovarian dysfunction

Introduction

Infertility is described as inability of a couple to conceive despite twelve months of regular sexual activity, in the absence of contraceptives.[1] Failure of a couple to have child is termed as primary infertility while secondary infertility is defined as inability of a parous women to have additional live births.[2]

It is a universally recognized dilemma. In more developed countries, about 3.5% to 16.7% prevalence of infertility is estimated, while in less developed nations estimated ratio is about 6.9% to 9.3% with an estimated overall median prevalence of 9%. As far as medical care is concerned approximately similar pattern is noted in less and more developed countries. The proportion of infertile couples not seeking medical treatment in all countries is about 45%, with a sensible range from 30% to 60%.[3] In Pakistan situation is worse due to combined effects of increase prevalence of infertility that is 27.9% and decreased awareness of proper treatment facilities.[4,5]

Etiology of this problem is recognized in both male and female at approximately equivalence rate.[6] Female age is most important parameter in case of fertility. Age 35 is considered as cut-off point. There is a general conception that beyond this age the chances of conception decrease.[7,8] Multiple factors are responsible for deliberate delay by women to get conceived. These factors are carrier oriented approach, increased desire to get higher education or late marriages.[9,10] It is recommended that women more than 35 years of age should consult doctor for further investigations and treatment.[11] Infertility evaluation is beneficial in women more than 35 years of age after 6 cycles of unprotected intercourse.[12]

There are multiple treatment options for infertile couples like intrauterine insemination (IUI), ovulation induction either alone or in combination and In vitro fertilization (IVF).[13] It is generally accepted that pregnancy outcome relatively decreases in women in their late 30's even after using these artificial reproductive techniques (ARTs).[8,14] However, some studies have shown that there are other factors beside female age that affects the pregnancy rate in infertile couples using these ARTs. These factors include type and duration of infertility, number of induced follicles

and spermatozoa count.[15] In these patients multiple markers like serum follicle stimulating hormone (FSH), Antimullerian hormone (AMH), Antral follicle count (AFC) and others can be used as predictive tools to assess pregnancy output of ARTs. Some researchers did not favor the idea of using these tests as part of routine diagnostic panel.[16] Contrary to this, few studies support this idea.[17]

In Pakistan some work has been performed regarding diagnostic approach to infertility.[18] Moreover pregnancy rate of ARTs and factors affecting the treatment outcome have also been studied.[19] But the comparative data that explain all of these factors in younger versus older age group is still lacking. Therefore, this study was conducted in order to compare different causes of infertility with respect to different age groups (younger and older) and type of infertility (primary and secondary). This study also evaluates the difference of IVF outcome (number of embryos transferred and pregnancy rate) in younger versus older ages. Besides this the capability of different parameters of infertility to predict pregnancy outcome regarding IVF in different age groups is also analyzed.

Patients and Methods

This retrospective study was conducted at Salma and Kafeel Hospital Islamabad from August to December 2011. Couples having history of more than one year of regular unprotected intercourse, presenting with primary or secondary infertility and were advised to take IVF treatment were included in the study. A total of 206 infertile couples were recruited. They were further divided into two age groups. Group I consisted of 108 infertile women < 35 years of age, while 98 women ≥35 years were included into group II. Investigations were performed in order to rule out the etiology of infertility. Diagnostic hysterosalpingography or laproscopy was performed in order to rule out the tubal blockage and to diagnose endometriosis. In order to exclude the ovarian dysfunction, hormonal analysis was performed. Sperm concentration > 20×10^6/ml, percentage of motile sperm > 50%, and normal sperm morphology (14%) were used to exclude the diagnosis of male infertility.[20] Mixed causes were labeled due to presence of more than one etiology in single couple. Unexplained infertility was diagnosed after exclusion of male causes, ovulatory dysfunction, tubal blockage and endometriosis.[21]

For hormonal analysis early follicular phase serum FSH, LH and estrogen were analyzed on day 3 of pre stimulation cycle in both young and old infertile women. These hormones were measured using a specific Immunometric assay kit (Immulite; Diagnostic Products Corporation, Los Angeles, CA, USA).

After the baseline analysis and determination of etiology of infertility, IVF treatment following a long protocol for use of gonadotropin releasing hormone (GnRH) agonist was given to infertile couple.[26] Good quality embryos were selected during the procedure according to criteria defined by Van Royen.[23]

Statistical analysis: In order to compare the mean between two age groups independent-t test was applied for parametric measurements. In case of non parametric data Mann-Whitney *U*- test was used. Percentage data was compared by continuity correction chi square test. Statistical Package for Social Sciences 19 (SPSS 19) was used in order to analyze the data. Predictive capability of different parameters regarding pregnancy outcome was measured by receiver operating curve (ROC). MedCalc software version 12 was used for this analysis. Comparison between areas under curve (AUC) in two age groups was made in order to estimate the relative ability of these infertility parameters. $p\leq0.05$ was considered significant.

Results

The infertile couples who participated in the study were segregated into group I (108 couples) and group II (98 couples) two groups. Mean ± SD age of females and males in group I was 27.38 ± 2.71 years and 33.39 ± 5.32 years respectively. In group II, mean ± SD age of female and male partners was 36.94 ± 2.27 and 41.05 ± 5.39 years, respectively. Duration of infertility in group I and II was 5.10 ± 2.67 and 7.91 ± 5.22 years. Base line FSH and LH levels in group I and II were 6.14 ± 2.26, 7.51 ± 2.74 IU/L and 8.10 ± 4.62, 8.81 ± 5.90 IU/L, respectively. Baseline estrogen level measured in group I and II was 84 (59.50-124.75) and 64.19 (50.36-89.75) pg/ml. There is statistically significant increase ($p\leq0.01$) in duration of infertility, serum FSH and estrogen levels in older age patients. The difference in LH levels in two groups was insignificant ($p=0.803$). The overall percentage of primary and secondary infertility in our patients was 61% and 39%, respectively. We further analyzed the date according to groups. In group I, 85 women presented with history of primary infertility while 23 female gave the history of secondary infertility. In group II number of women with history of primary and secondary infertility was 40 and 58 respectively. Analysis also revealed the statistically significant difference ($p\leq0.01$) in proportion of these two types of infertility in older versus younger age group.

Regarding etiology of infertility, over all prevalence of male factor among all patients (n=206) was the highest (33%); percentage of other causes was as follows: tubal factor 20%, ovarian dysfunction 22%, endometriosis 5%, mixed 09% and unexplained 11%. when we compare the etiology of infertility in two age groups (Table 1), difference in proportion of male factor was insignificant. But the overall percentage of male cause was very high in both groups. Tubal factor was significantly raised in older group in comparison with ovarian dysfunction that was significantly higher in younger group ($p\leq0.01$). Difference among other

etiologies like endometriosis, mixed causes and unexplained reasons in two groups was insignificant.

Table 1: Etiology of infertility in two age groups (n=206) participating in study

Diagnostic category	Age <35 yrs n=108 (%)	Age ≥35 yrs n=98 (%)	p value
Male factor	36 (33)	32 (33)	0.882[a]
Tubal factor	09 (08)	32 (33)	0.000[ab]
Ovarian dysfunction	36 (33)	10 (10)	0.000[ab]
Endometriosis	08 (07)	03 (03)	0.215[a]
Mixed	12 (11)	06 (06)	0.217[a]
Unexplained	07 (06)	15 (15)	0.114[a]

[a] continuity correction Chi square test, [b] p≤0.05 considered significant

Table 2: Diagnostic category according to primary and secondary infertility

Etiological causes	Primary infertility (n=83)			Secondary infertility (n=55)		
	Age <35 years n=57 (%)	Age≥ 35 years n=26 (%)	p value	Age < 35 years n=15 (%)	Age ≥ 35 years n=40 (%)	p value
Tubal factor	07(12)	09(35)	0.05[a]	02(13)	23(58)	0.02[ab]
Ovarian dysfunction	29(51)	05(19)	0.008[a]	07(47)	05(13)	0.006[ab]
Endometriosis	07(12)	2(08)	0.72[a]	01(07)	01(05)	0.97[a]
Mixed	08(14)	02(08)	0.57[a]	04(27)	04(10)	0.15[a]
Unexplained	06(11)	8(31)	0.07[a]	01(07)	07(18)	0.70[a]

[a]continuity correction Chi-square test, [b] p≤0.05 considered significant

How ever, the trend of unexplained infertility was increasing in older age group. While high percentage of endometriosis and mixed causes were found in females less than 35 years of age. When we analyze the data according to type of infertility (primary and secondary), similar pattern was noted (Table 2). Percentage of tubal causes was significantly higher in secondary infertility (p≤0.05). In case of ovarian dysfunction, significantly increased proportion was noted in case of primary infertility (p≤0.01).

Results of IVF treatment were also analyzed according to younger and older groups (Table 3). Total number of transferred embryos and number of good embryos transferred were significantly decreased in older age groups as compare to younger ones (p≤0.01). Pregnancy rate after IVF treatment was also significantly higher in younger age group (p≤0.05).

Predictive capability of different factors of infertility regarding pregnancy rate after IVF treatment was analyzed with the help of ROC curves (Table 4). Increased area covered by Age of patients, duration of infertility and serum FSH levels revealed significantly high predictive power of these parameters in older ones (p≤0.05). On comparison between younger and older age groups, it was observed that females' age and serum FSH levels had significantly better predictive capability of pregnancy rate than duration of infertility after IVF treatment (p≤0.05).

Table 3: Outcome of IVF treatment in patients of two age groups (n=206)

	Age<35 years (n=108)	Age ≥ 35 years (n=98)	(p value)
Number of total embryos transferred (mean±SD)	3.39±0.61	2.64±0.75	0.000[ac]
Number of good embryos transferred (mean±SD)	2.47±0.62	2.15±0.60	0.000[ac]
Pregnancy rate (%)	30.1	17.48	0.05[bc]

[a] independent t test, [b] continuity correction Chi-square test, [c] p≤0.05 considered significant

Table 4: Comparison between areas under ROC curves of different parameters of infertility to pregnancy rate in two age groups

parameters	Age<35 years (n=108)		Age ≥35 year (n=98)		P value*
	AUC (CI)	p- value	AUC(CI)	p-value	
Age	0.504 (0.404-0.604)	0.9485	0.715 (0.618-0.800)	0.000[a]	0.02[a]
Duration of infertility	0.523 (0.422-0.622)	0.7083	0.713 (0.615-0.798)	0.003[a]	0.04[a]
FSH	0.502 (0.402-0.603)	0.9688	0.723 (0.626-0.806)	0.003[a]	0.02[a]
Estrogen	0.526 (0.425-0.625)	0.6852	0.550 (0.449-0.648)	0.500	0.80

*ROC curve: Receiver operating curve, AUC(CI): Area under curve (95% confidence interval), *p-value by comparison of areas under respective ROC curves in two age groups, [a] p≤0.05 was considered significant.*

Discussion

Age is considered as an important factor regarding infertility. Our study demonstrated that male factor remains an important cause in all age groups. Regarding females, etiology of infertility varies according to increasing age. In younger group ovarian dysfunction plays an important role while in older patients tubal factor is mainly responsible to cause infertility. Among other etiologies, percentage of endometriosis and mixed causes of infertility are slightly more frequent in younger group. On the other hand, unexplained infertility is found slightly increase in older patients. When we analyzed the data according to primary and secondary infertility similar trend was noted. Our results are in accordance with study carried out by Maheshwari et al.[24] They conducted study on large sample size and

concluded that with age the chances of tubal factor and unexplained infertility decreases by two fold, while one third rise in ovulatory dysfunction was noted in female more than 35 years of age. This study also explains that although all these changes are more marked at the age of 35 years, but the trend can be visualized after 30 years of age. That is why the cut off age of 35 years regarding infertility should be reassessed. Reason of diagnosing increase ovarian dysfunction in younger patients was perhaps due to increase query of younger ones about irregular cycles. However, high chances of pelvic infections with increasing age enhance the percentage of tubal factors in older age group.[25]

Our data revealed that infertile couples more than 35 years of age have increased duration of infertility as compared to younger ones. Secondary infertility was also significantly raised in these older couples. This can be explained in this way that couples having one child with them have potential to wait for a longer time as compared to couples having no child with them. This is the reason that these couples came late in clinics for their infertility evaluation.[24]

Ovarian aging can be assessed by different ovarian reserve markers. Our data revealed that serum FSH and estrogen levels are significantly affected with aging. In women more than 35 years of age these markers are significantly raised as compare to younger age groups. These results are in accordance with study conducted by Kim et al. They conducted the study in normal ovulatory women of younger and older age groups. Elevated FSH levels in older age group prove this marker as best predictor of age. This trend was also noted in case of estrogen levels.[26] In 2009 similar pattern of FSH was analyzed in infertile couples of two age groups.[17]

After analysis of data related to IVF treatment given, we demonstrated that outcome of ART also affects by increasing age of infertile couple. The outcome variables like number of transferred embryos and good embryos transferred were significantly decreased in group more than 35 years of age. This explains the decrease pregnancy rates even after using ART in women with increasing age. In 2004 Rizvi demonstrated that IVF/ICSI treatment shows better results in women with good quality of embryos transferred.[19] Similar results were demonstrated in two age groups by Lee.[17] There was significant difference in number of total embryos and good quality embryos transferred in women more than 35 years of age.

As we have demonstrated that age is a basic parameter that affects the outcome of IVF. Other factors like duration of infertility, serum FSH and estrogen levels are also significantly varied in older age group as compare to younger ones. In order to confirm our data we perform ROC analysis and determine the predictive power of these parameters regarding pregnancy outcome. Increased area was covered by age of patients, duration of infertility and serum FSH levels in older age group. Data revealed that these factors have significant predictive power of pregnancy rate after ARTs only in patients more than 35 years of age. Comparison among ROC analysis done in two age groups showed that age of patients and serum FSH levels have relatively better predictive capability as compare to duration of infertility. Our results are in accordance with discussion done by Lee. He concluded that different ovarian reserve markers have better predictive capability in females with increasing age.[17]

Conclusion

According to age of patient a clinician can make a differential diagnosis of infertility. Moreover, information of other factors like duration of infertility and levels of different hormones can help clinician to estimate the chances of pregnancy rate before suggesting ART in old age couples.

Limitation of study

We conducted study on small sample size, and did not analyze the some factors like role of AMH and AFC etc in ART regarding different age groups. That's why more studies are needed in Pakistan to further elaborate factors affecting outcome of ART in different age groups.

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