BIOCHEMICAL PREGNANCY OUTCOME AMONG DAY 3 VERSUS DAY 5 EMBRYO TRANSFER IN THE CENTER FOR FERTILITY AND REPRODUCTIVE MEDICINE AT A TERTIARY HOSPITAL IN ADDIS ABABA, ETHIOPIA

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ABSTRACT

INTRODUCTION: The burden of infertility is the worst in sub-Saharan countries with the rare availability of assisted reproductive technology treatment. Even in areas where such treatment is available, low implantation and pregnancy rate is challenging health care providers which partially can be tackled by selecting the optimal day of embryo transfer. However, the effects of day of embryo transfer on pregnancy outcomes are an area of controversy even in countries with advanced fertility treatment.

OBJECTIVE: To see the effect of day of embryos transfer (day 3 vs. day 5) on biochemical pregnancy outcome in the center for fertility and reproductive medicine center at a tertiary hospital, Addis Ababa, Ethiopia.

METHOD: A retrospective record review conducted from January -December 2021 G.C.

RESULT: A total of 201 clients had undergone embryos transfer 107 were day 3 while 94 of them were day 5 embryos. Both groups are comparable in baseline characteristics like the mean age, the number and grade of embryo transferred. Biochemical pregnancy between day 3 and day 5 embryo transfer was similar in both groups 67 (62.6%) and 59 (62.8%) respectively (p=0.98).Although it is not statistically significant, the chance of having twin pregnancy was higher among day 5 embryo transfer (36%) compare to day 3 embryo transfer (17%) with p =0.053.

CONCLUSION: In women age less than 35, as long as good quality embryos available, transferring day 3 embryo is an alternative option over blastocyst transfer with comparable biochemical pregnancy without increasing the chance of multiple pregnancies.

KEY WORDS: day 5 and day 3 embryo transfer, Ethiopia, public fertility center, biochemical pregnancy

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INTRODUCTION

Although infertility is a global problem, it is more common in low income countries, particularly in sub-Saharan Africa where it has significant burden on every aspect of an individual's life[1]. So taking this in to consideration World Health Organization (WHO) has declared "infertility as public health problem, alleviation of infertility therefore becomes a necessity on many levels"[2]. The most common cause of infertility in this part of the world is infection-related tubal damage which can be prevented and treated easily and effectively by assisted reproductive technology (ART), unfortunately this is rarely available. Even in areas where ART is available, it carries challenges to health care providers as evidenced by the low implantation and pregnancy rate(PR) which can be tackled partially by the day of embryo[1,3].
In most part of the world, embryos can be transferred back to the uterine cavity either on day 3 (D3) (cleavage stage) or day 5(D5) (blastocyst stages) after ovum pick up. However studies, regarding the effects of day of embryo transfer (ET) on fertility outcomes has shown varied results and most are with low quality[4,5]. On top of this a large meta-analysis revealed inconclusive result regarding the pregnancy outcomes comparing the two and recommended to have more studies on this controversial issue[6]. Having answers for this relevant intervention will also help providers to give more accurate counseling for couples as to the optimal day of transfer[4–7].

With the advancement of invitro culture one can extend duration of embryo in the lab to D5 and in theory this gives the opportunity to select genetically normal embryos. This allows transfer of few embryos and decrease chance of multiple gestation and its undue complications[3,4,8,9]. Transferring a blastocyst improves uterine/ embryonic synchronicity that could imitate the natural transfer of embryo from fallopian tube to endometrium[5,10].

However D5 transfer has its own drawbacks as there is an increased possibility of some of the embryos failing to develop to blastocyst in vitro resulting in cancellation of embryo transfer; and this again decreases number of embryos available for freezing and missing the opportunity of future transfer. There are technical difficulties in the cryopreservation/ thawing process in such expanded embryos. It is also associated with increased risk of monozygotic twining[5,8,9,11]. This study was conducted in a public in vitro fertilization (IVF) center where the embryology lab is busy, crowded with incubation to D5 embryos using single step culture media, unlike most settings where they use two steps media. This could have a different effect on pregnancy outcomes[3,12].

On the other hand, significant number of studies done on the success of IVF measured by implantation, pregnancy and live birth rates had shown transferring D3 embryo to be not inferior compared to D5 embryo transfers especially if the numbers of embryo available for transfer are fewer than three with comparable complications including multiple gestations. Performing D3 transfer in patients with few available embryos also reduces the incidence of cycle cancellation, therefore, associated with greater number of embryos available for freezing[5,7,8,11].

So the objectives of this study is to determine whether transferring D5 could result in better biochemical pregnancy than transferring D3 embryos . In addition, no local data are available regarding the effect of transfer day on biochemical pregnancy outcome. As this research was conducted in a public center having catchment of large population where patients are referred from different corners of the country with different socio-demographic characteristics, it could have a better representation of the situation regarding the outcome of pregnancy from day of embryo transfer and this is also important for generalization.

METHOD AND MATERIALS

Study setting, period, and design

It was conducted at Center for Fertility and Reproductive medicine (CFRM), department of Obstetrics and Gynecology, St. Paul's Hospital Millennium Medical College (SPHMMC), Addis Ababa, Ethiopia. The center, the first and only public IVF center for the country, is governed by the federal minster of health Ethiopia. Study was done though record review of patient cards and computer registers of clients who have visited the center from January to December 2021 G.C.

The study populations were all female partners age less than 35 years who have undergone IVF/ intra cytoplasm sperm injection (ICSI) cycles with fresh embryo transferred having at least one grade 1 embryo available for transfer. Couples with 1 or more failed IVF cycle were excluded. The outcome variable was biochemical pregnancy determined 2 weeks after embryos transfer. This is taken as positive if serum ß HCG greater than 25 mIU based on previous study cut off point values.

Ovarian stimulation protocol

In this fertility center three major stimulation protocol including long protocol, antagonist protocol, and minimal stimulation protocols are used. The first, a long protocol, was usually applicable for patients younger than 35 years with good ovarian reserve (AFC more than 5). The patients are appointed on day 21 of menses to get depot Goserelin(Zoladex) injection 3.6 mg subcutaneous stat after checking for cyst or dominant follicles, and then reappointed for stimulation either on second day of menses or 14 days from Zoladex injection whichever comes first. If no contraindication to start stimulation (no ovarian cysts >10 mm), stimulation started with calculated dose (based on age and BMI) with Human menopausal gonadotropin (HMG) alone (Menopur) or in combination with recombinant FSH (Gonal-F) and the response followed with transvaginal ultrasound to see changes on the follicular size and endometrium and modification of doses made based on the response. Minimal stimulation protocol is applied for patient with poor ovarian reserve (AFC less 5), age more than 35 and who can't afford long protocol. With this protocol Letrozole 5mg PO is started on day 2 of the cycle and continued for 5 days and on the 4th day hMG SC 150 IU or 225 IU started . Once the leading follicle attained 14 mm in size, down-regulation with Cetrotide is instituted. Antagonist protocol is used rarely during the study period and usually used after failed minimal stimulation or for expected poor response. The only difference of antagonist from minimal stimulation protocol is that it is started directly with gonadotropin on second day rather than Letrozole and the doses of gonadotropin is not fixed and calculated based on age and BMI.

Triggering and ovum pick up (OPU)

Trigger for all three protocols is made with urinary HCG 10,000 IU or 5,000 IU SC, decided if 3 or more leading follicles reached 18 mm or more in size or greater or equal to 5 follicles reach/exceed 16mm in size. Ovum picks up procedure is done under transvaginal guidance after 36 h from HCG injection. The cycles either will be for IVF or ICSI depending on the semen analysis result. After OPU oral prophylactic antibiotic given and luteal support is started with injectable together with PO progesterone and estradiol valerate.

Fertilization and embryo transfer

Once the oocyte cumulus complex identified in the standard media it is kept in the incubator for 2 hours and decided for IVF or ICSI. Then embryos transferred back to the incubator. Patients called for embryo transfer by embryologist either on D3 or D5 after OPU is based on grade and number of embryo available. If the grade of embryo is grade 1 and more than 2 embryos available culture is extended to D5 without changing embryo culture media otherwise D3 embryos will be the final choice. If transfer is successful luteal support continue and patient appointed after 2 weeks to check for serum pregnancy test. If this is found to be positive, luteal support continued and appointed after 2 weeks for evaluation using Trans vaginal ultrasound (TVUS) and to check the presence of clinical pregnancy.

Ethical considerations

Ethical approval was obtained from the institutional review board of the researchers' institution (SPHMMC research ethical team). The information was extracted using structural questionaire with kobocollect toolbox without mentioning patient name. Confidentiality was maintained during data collection, analysis, and interpretation by avoiding recording of names and returning client records to their place after completion of data collection.

Data processing and statistical analysis

The data were checked for completeness and then imported into Stata statistical software release 15 (StataCorp, College Station, TX, USA) for analysis. Bivariate analysis were performed using proportions and means (standard deviation) for normal distribution, or medians (interquartile range) when the distribution was not normal. The association of the biochemical pregnancy result and independent variables were assessed using Fisher's exact test for categorical variables. Independent t-test, or the non-parametric test of difference of means (Mann Whitney U test) used for continuous variable. Statistical significance was declared at p=0.05 and all tests were two-sided. A full model assessing the relationship between the pregnancy test result and predictor variables was constructed after which non-significant variables were removed by a backward procedure using the likelihood ratio test (p<0.05).

RESULT

Table 1 -Baseline characteristics of patients who have undergone embryo transfers

Factor		D5(N=94)	D3(N=107)	p-value
Age of the woman in years, median (IQR)		29.0 (27.0, 32.0)	30.0 (28.0, 32.0)	0.27
Duration of infertility in years, median (IQR)		7.0 (4.0, 8.0)	6.0 (4.0, 9.0)	0.58
****Type of infertility**	Primary	65 (73.0%)	84 (81.6%)	0.16
	Secondary	24 (27.0%)	19 (18.4%)	
****Cause of infertility**	Female factor	48 (53.3%)	54 (52.4%)	0.96
	Male factor	23 (25.6%)	26 (25.2%)	
	Unexplained	6 (6.7%)	9 (8.7%)	
	Both	13 (14.4%)	14 (13.6%)	
****Hysterosalpingography result**	Not done	22 (25%)	22 (22%)	0.12
	Normal	16 (18%)	31 (31%)	
	Abnormal	50 (57%)	46 (46%)	
Total number of AFC (value), median (IQR)		17.0 (11.0, 22.0)	10.0 (6.0, 14.0)	<0.001

AFC =Antral follicular count

IQR = Interquartile range *P-value was calculated for categorical variable using Fisher exact test, for continuous variables The Mann-Whitney U (non-parametric independent t-test),IQR = Interquartile range ,**there are missing data during data collection

During the study period there were a total of 201 clients who had undergone embryo transfer fulfilling the inclusion criteria, 94 of them were D5 embryos and 107 were D3.The median ages were comparable between the two groups 29.0 (27.0, 32.0) and 30.0 (28.0, 32.0) respectively with P=0.27. They are also comparable in the type and cause of infertility. In both group primary type of infertility is common and accounted for 73.0 %(65) of day 5 and 81.6% (84) of D3 embryo transfers. Female cause of infertility is the common cause both in D3 and D5 embryo transfer 54 (52.4%) and 48 (53.3%) respectively. However, the median of total number of AFC is higher in D5 embryo transfer group (17.0 (11.0, 22.0)) comparing D3 embryo transfers group (10.0 (6.0, 14.0)) with p value <0.001 (Table 1)

Table 2 -Clinical data for D3 embryo transfer versus D5 transfer

Factor		D5 N=94	D3 N=107	p-value
Number of metaphase 2 oocytes (value), median (IQR)	Median	12.0 (9.0, 16.0)	5.0 (3.0, 8.0)	<0.001
Number of embryo developed available for transfer (value), median (IQR)	Median	6.5 (5.0, 7.0)	3.0 (2.0, 5.0)	<0.001
Number of embryo transferred(value), median (IQR)	Median	2.0 (2.0, 2.0)	2.0 (2.0, 2.0)	0.16
Method of sperm insemination for fertilization	IVF	43 (45.7%)	56 (52.3%)	0.35
	ICSI	51 (54.3%)	51 (47.7%)	
Level of physician who has transferred the embryo	Second-year Fellow	18 (19.1%)	15 (14.0%)	0.33
	Subspecialist	76 (80.9%)	92 (86.0%)	

*P-value was calculated for categorical variable using Fisher exact test, for continuous variables The Mann-Whitney U (non-parametric independent t-test),

In D5 embryo transfer group the median number of metaphase 2 oocytes and number of developed embryo available were 12.0 (9.0, 16.0), 6.5 (5.0, 7.0) respectively, all were significantly higher relative to D3 embryo transfer, 5.0 (3.0, 8.0), 3.0 (2.0, 5.0) respectively with p value in all cases<0.001. However in both groups the number of embryos transferred was two with at least one grade one embryo. Otherwise method of sperm insemination and level of physician doing the embryo transfer were comparable (Table 2).

Table 3 Predictors of biochemical pregnancy

Factors		Serum ß HCG		
		Negative (N=75)	Positive (N=126)	p-value*
Age cut at 30	<30 years	23 (30.7%)	65 (51.6%)	0.004
	>=30 years	52 (69.3%)	61 (48.4%)	
Duration of infertility in years, median (IQR)		7.0 (5.0, 10.0)	6.0 (4.0, 8.0)	0.007
Total number of AFC (value), median (IQR)		12.0 (6.0, 17.0)	13.0 (9.0, 21.0)	0.038
****Type of protocol used**	Long	33 (44.0%)	72 (57.6%)	0.13
	Minimal /mild	37 (49.3%)	49 (39.2%)	
	Antagonist	5 (6.7%)	4 (3.2%)	
Method of sperm insemination for fertilization	IVF	42 (56.0%)	57 (45.2%)	0.14
	ICSI	33 (44.0%)	69 (54.8%)	
Day of embryo transfer	D5	35 (46.7%)	59 (46.8%)	0.98
	D3	40 (53.3%)	67 (53.2%)	

*P-value was calculated for categorical variable using Fisher exact test, for continuous variables The Mann-Whitney U (non-parametric independent t-test), **there are missing data during data collection

Out of 201 patients 126 of them had positive serum ß HCG after 2 weeks of embryo transfer which accounted for 62.7 % of patients who have under gone embryo transfer who fulfill inclusion criteria. Looking at predictors of biochemical pregnancy age less than 30 significantly increase biochemical pregnancy compared to age greater than 30,65 (51.6%) and 61 (48.4%) respectively with p value = 0.004.Similarly a short duration of infertility with median of 6.0 (4.0, 8.0) has high chance of pregnancy relative to 7.0 (5.0, 10.0) together with this median of total number of AFC 13.0 (9.0, 21.0) has a better chance of biochemical pregnancy compare to 12.0 (6.0, 17.0) p=0.038.On the other hand type of protocol used, method of sperm insemination to oocyte and day of embryo transfer has no significant effect on the biochemical pregnancy (Table 3).

Table 4 Clinical outcomes for day 3 embryo transfer versus day 5 transfer.

Clinical outcome		D5 (N=94)	D3 (N=107)	p-value
Serum ß HCG positive	Yes	59 (62.8%)	67 (62.6%)	0.98
	No	35 (37.2%)	40 (37.4%)	
**Number of intrauterine pregnancies	Singleton	34 (64%)	48 (81%)	
	Twins	19 (36%)	10 (17%)	0.053
	Triplet	0 (0%)	1 (2%)	

*P-value was calculated for categorical variable using Fisher exact test, for continuous variables The Mann-Whitney U (non-parametric independent t-test), **there are missing data during data collection

Although the biochemical pregnancy between D3 and D5 embryo transfer was not statistically significant 67 (62.6%) and59 (62.8%) respectively p=0.98, the chance of having twins pregnancy was higher among D5 embryo transfer, 19 set of twin pregnancies (36%), compared to D3 embryo transfer 10 set of twins (17%) with p =0.053. there were 34 (64%) singlton pregnancies among D5 whereas 48 (81%) among day 3 embryo transfers. One triplet set of pregnancy was seen in D3 embryo transfers (Table 4).

DISCUSSION

To our knowledge this is the first type of study in Ethiopia to see the effect of day of embryo transfer on biochemical pregnancy. Although the comparison of the D5 vs D3 group was a retrospective and cross-sectional study, we believe that the two groups were closely matched. We selected 201 patients from both groups with age less than 35,having at least one good quality embryo (grade 1) and in both groups, 2(two) embryos have been transferred. Furthermore, the median age, duration of infertility, causes of infertility and type of infertility, the patients' parameters (SA, HSG results) and stimulation protocols all were comparable in both groups.

In this study although the number of oocytes retrieved ,number of mature oocytes and embryo available for transfer is significantly high in D5 relative to D3 ,the number of embryo transferred were equal(two) in both groups with similar quality of embryo. The chance of biochemical pregnancy was not significantly different in D5 embryo compared to D3, 59 (46.8%) and 67 (53.2%), respectively, with P=0.98.This result implied as long as we have good selection of patients in terms of age, quality of embryo and number of embryo transfer, the day of embryo transfer doesn't affect pregnancy outcome as it has been seen also in other studies[5–8,11].

The result of this study contradicted with the finding of other studies that demonstrate D5 embryos have a better pregnancy outcome[8,13–18].One of the attributing factors mentioned for the increased chance of pregnancy in D5 is the use of sequential culture which is believed to imitate the natural transfer of embryo from fallopian tube to the endomterium[13,14,16].However, in our study setting, the type of the culture media used is a one- step which might have partially contributed to the difference in the result of our study, as it has been seen in other parts of the world before the era of sequential culture media use, in which pregnancy outcome of D5 embryo was disappointing[11,17]. Prolongation of culture to day 5 may allow chromosomally competent embryos to develop to the blastocyst stage, thereby promoting intact embryos[16]. Despite the potential benefits of blastocyst-stage embryo transfer, it is not clear whether extension of embryo culture toward blastocyst production is a good option for all patients or all embryos in the same cohort[11].

The number of multiple significantly seen in this study was higher among D5 comparing to cleavage stage similar to the finding by Peter Schwarzler et al retrospective cohort study with increased risk of preterm delivery[16].However, in modern IVF practice it is encouraged to have single embryo transfer (SET) with singleton pregnancy to decrease the chance of having multiple pregnancy related complications[17]. So this study indirectly demonstrated that a day 5 embryo transfer predispose patients to multiple pregnancy that obviously will lead to increased risk of all complications of multiple pregnancy. A number of studies succeeded in decreasing chance of these complications by transferring single D5 embryo[14]. In view of the high incidence of multiple pregnancy in our study, in which all patient have received two blastocysts, we strongly recommend limiting the number of blastocysts transferred to one should be consider.

The limitations of this study are being retrospective study, and that it is only applicable in age group less than 35. The center is also using single step culture media for D5, unlike most settings where they use two step or sequential step for D5 culture, this might change the pregnancy outcome of D5 sage embryo. So in the future a prospective study involving all age groups after using D5 sequential culture media is recommended.

In conclusion, in women age less than 35 , as long as we have available good quality embryos, transferring day 3 embryo is an alternative option over blastocyst transfer with comparable biochemical pregnancy without increasing the chance of multiple pregnancies which in turn decreases the risk of complications related to multiple pregnancies. In addition, cleavage stage embryo transfer carries fewer burdens and cost in a set up with low resources and busy embryology labs. It has also less cost and stress for the patient.

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