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ORIGINAL ARTICLE **OPEN ACCESS**

Study on risk factors affecting *in-vivo* fertilization treatment and assessment of techniques in Embryo Transfer

Rakesh Kumar Sharma[1]; Renu Singh[1]; Soni Singh[1]; Deepa Agarwal[1]; Sukrit Srivasta[1], Devendra Kumar[1], Feroz Ahmad Shergojri[2]
[1]Department of Biotechnology & Life Sciences, Mangalayatan University, Aligarh, UP
[2]Department of Zoology, Himalayan University, Itanagar, Arunachal Pradesh
E.mail: rakesh.sharma_ibmer@mangalayatan.edu.in

ABSTRACT
Infertility affects every seventh couple during reproductive life by reducing fertility rates due to various physical factors and excessive stress of fast moving work life, resulting in imbalanced hormone stimulation and diseases related to reproductive organs. In vitro fertlization and Intracytoplasmic sperm Injection are the most accepted and frequent choice to overcome the infertility problem. The paper focuses towards various factors affecting fertility in couples and possibilities of live birth through IVF and ICSI treatment. In this study 200couples were included, facing infertility Problem. IVF and ICSI treatment were taken by these couples to increase their chances of pregnancy and live birth. Examination, diagnosis, analysis and procedure of IVF including embryo transfer technique and calculation of live births were done by using National Institute for Health Care and Excellence(NICE) clinical guidelines. To evaluate cumulative possibilities of ongoing pregnancy, Kaplan-Meier analysis was done. The highest pregnancy chances were given by oligospermia, however the immunological/cervical pathology gives the lowest chances. The upshots exposed that the chance of a live birth diminishes as the number of unsuccessful cycles raises. The age of women negatively influences the live birth rates and the cleavage transfer were less successful than blastocyst transfers. The single embryo transfer resulted only 2% to 3% of live birth and double embryo transfer resulting around 32% more multiple live births. In conclusion, the study supports the singleton live birth as a primary output. Clinical pregnancy and singleton live birth are significant results of this study and allow clinicians to inform the couples for possibilities of conception.
Keywords: Infertility, IVF, Embryo transfer, live birth rate

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INTRODUCTION

Couples seeking treatment for infertility are increasing drastically. World health organization (WHO) proposed that, the couples must be together for treatment as much as possible [1, 2]. There is a complex relation between fertility and psychological stress [3]. The work stress and low conception possibilities in women were reported by studies (4-6), however the relation with men is still under study. The United Nations depicts reproductive fitness as "a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity in all matters relating to the reproductive system and to its functions and processes"[7]. Failure to conceive following regular or frequent unprotected intercourse for a year or more has been defined as infertility [8, 9, and 10]. The infertility diagnosis is based on the failure of conceiving in 1 year has been disputed to embroider the infertility risk, seeing as about 50% of women who failed to conceive in the first year are possible to accomplish in the second year [11, 12]. The preliminary consultation should comprise an evaluation of history for fertility problems. Information concerning normal conception patterns will specific reassurance about good chance of conception conversely, there should also be a detailed enquiry on the medical, surgical, sexual, contraceptive and pregnancy history and a general physical examination to detect abnormalities, as well as measurement of height and weight to evaluate Body Mass Index that helps to identify couples who are probably experiencing delays in conception [13].The information about lifestyle such as smoking, alcohol consumption every day, work load and diet of couples should be accessible to improve the fertility rate.

In vitro fertilization (IVF) technique assists the outside fertilization of eggs and sperms usually preferred consequently to the failure of other treatments. The uses of IVF hold the following failed commencement; a phase of expectant management in patients amid unexplained infertility, therapy for ovulation induction, treatment for male factor infertility often in combination with intracytoplasmic sperm injection

(ICSI), treatment for endometriosis, Intrauterine Insemination (IUI) using partner or donor sperm, tubal disease treatment including severe tubal disease, severe male factor infertility, breakdown of spermatogenesis in patients going through the treatment of cancer wherever cryo-preserved semen failed to accomplish conception with Intrauterine Insemination, ovarian malfunction due to cancer treatment where cryo-preservation of eggs or embryos have been done [14-15, 13].

The cycle of IVF treatments following the stages, nevertheless depends on the protocol, used properly at every stage while treatment follow-up. Pre- treatment stimulate the IVF procedure by improving therapy of exogenous hormone response, reduces the risk of cyst formation in Ovary and facilitate the IVF stimulation schedule to corroborate the time of Oocyte recovery obtainable to laboratory or clinical staff (6). Down regulation temporarily stops the function of the pituitary gland to reduce the risk of cancellation of cycle from early exposure to luteinizing hormone that could disrupt the development of normal follicle and Oocyte or stimulate the release of an egg at pre-maturation before retrieved. Controlled ovarian stimulation aims to produce numerous mature egg to get retrieved surgically before fertilization in laboratory [12].

After stimulation phase, an ovulation trigger/stimulating hormones is used to mimic the function of the natural endogenous Leutinizing Hormone surge to initiate ovulation process. Oocyte and sperm get fertilized in-vivo and embryo is transplanted into the uterus of women after 2-3 days, basically at the cleavage stage of developing embryo (5,6). The good quality eggs are used for longer laboratory culture with intra-uterine replacement done after 5 days to 6 days, basically at blastocyst stage [2]. The early phase of pregnancy is supported by drugs to mimic the natural conception process. The IVF cycle may stop or cancelled due to risk of ovarian hyperstimulation syndrome in women because of negative response to the part of IVF treatment [7]. This is mostly occurring before retrieval of Oocyte or during ovarian stimulation. However, Oocyte may be collected and frozen for future transfer. Ovulation triggering combined with intrauterine insemination is the most common and first choice of couples facing infertility problems because of their ease and affordability [14-16]. Most of the studies on fertility shows that the result of IVF success depend on per cycle that is going through treatment; however the outcome of complete treatment is the matter for a patient.

MATERIAL AND METHODS

The study was conducted at Department of Biotechnology &Experimental Molecular Medicine of Institute of Biomedical Education and Research, Mangalayatan University, Aligarh;& Mangalayatan Hospital and Research Center, Aligarh, (UP), INDIA on200 patients experienced infertility problem. The study includes females with bilateral tubal patency, minimum one follicle with a diameter of about ≥18 nm on triggering day and male with total motile sperm of about five million beyond preparation. The study excludes the patients lost to follow-up.

Each patients incorporated in this study was subjected to analyses prognostic variables, including women age, duration of sub-fertility, pregnancy history, (defined as primary or secondary sub-fertility of the woman treated) and all diagnostic categories of IVF, being tubal pathology, unexplained sub-fertility, mild male, hormonal, cervical or immunological sub-fertility and endometriosis [8)].The Patients having abnormal menstrual cycle's history (amenorrhea and/or oligomenorrhea) endure ovarian stimulation. The menstrual cycles were regular. A hysteroscopy performed before starting any assisted reproductive technology procedure. During early follicular phase Follicle Stimulating Hormone concentration in the cycle preceding the treatment was 11 IU/ml. The semen analyses in the male partners were conducted. The patients underwent ovarian stimulation with human menopausal gonadotrophin (HMG), 450 IU daily. An ultrasound performed after 5 days to identify follicular development and serum 17β-oestradiol concentration. Daily dose of HMG was increased up to 750 IU for three additional days. The cycle was cancelled if no ovarian response was achieved. On cycle cancellation, a second treatment was performed with clomiphene citrate 150 mg daily from day 2 to day 6 of the cycle. On day 14 the patients had one follicle of 18 mm in diameter, one of 14 mm and three of 10 mm, and a trilaminar endometrial stripe measuring 9 mm [11-12]. Final maturation was then triggered with human chorionic gonadotrophin,5000 IU. Three oocytes were retrieved transvaginally under ultrasound guidance 36 h later, and were inseminated by conventional IVF. One oocyte fertilized and one 2-cell embryo was replaced in the uterine cavity via a transcervical route two days after oocyte retrieval. Luteal phase was sustained with natural progesterone in oil 50 mg Intramuscular daily from day 1 after oocyte retrieval. Fifteen days after embryo transfer, blood β-human chorionic gonadotrophin (β-HCG) test was positive. Ultrasound scans performed 4 weeks after embryo transfer confirmed the presence of one intrauterine gestational sac with cardiac activity. To estimate the cumulative probability of ongoing pregnancy Kaplan–Meier analysis was done after IVF/ICSI. On dropping out IVF procedure by couples within 12

months, the follow-up was continued till 12 months, believing that they had a negligible chance of pregnancy, consequently no censoring was concerned (17). Additionally, we evaluate the 'cumulative probability of ongoing pregnancy' aligned with a number of cycles. The statistical tools were used in this study to identify the mean value and standard deviation for the data observed.

RESULT

The results obtained during this are depicted in figure-1; 2 & 3that characteristics and have been established the chances of pregnancy and live birth. The study includes total 200cycle of IVF undertaken between two year before Covid Pandemic. The outcomes reveled the upper and a lower age limit for IVF treatment. On the other hand, the lower age limit was basically depends on robust data rather than ineffective evidence. The consequences illustrate that increases in female age escort the decrease in pregnancy rates as well as substantiate the association between age and probable accomplishment of IVF. The data observation does not put forward any lower age limit for IVF treatment.

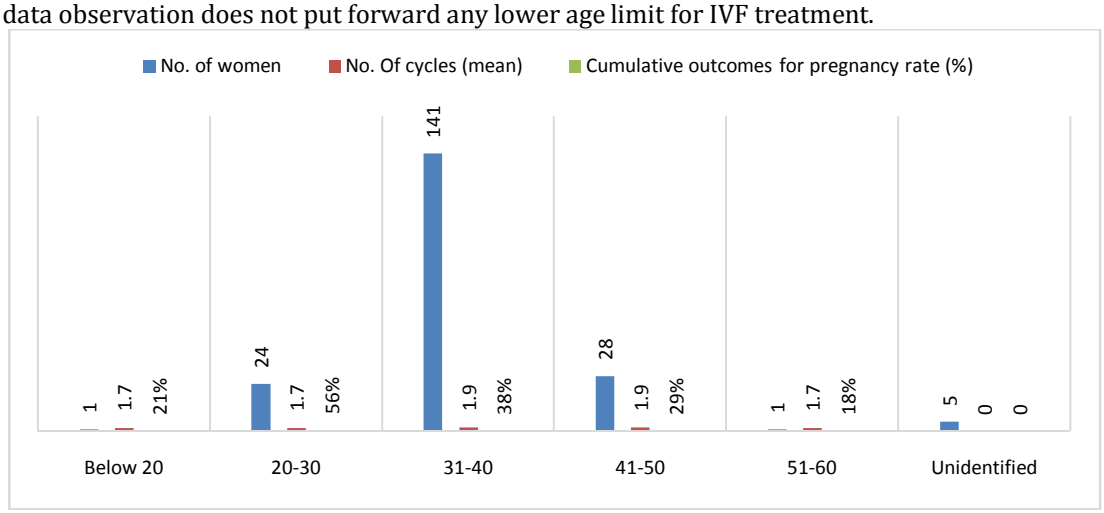

Figure 1: Age of women faced infertility

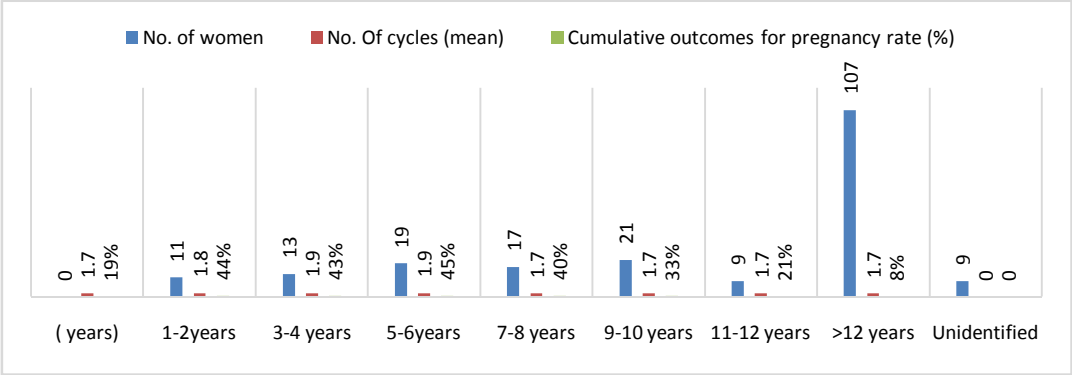

Figure 2: Duration of Infertility faced by couples

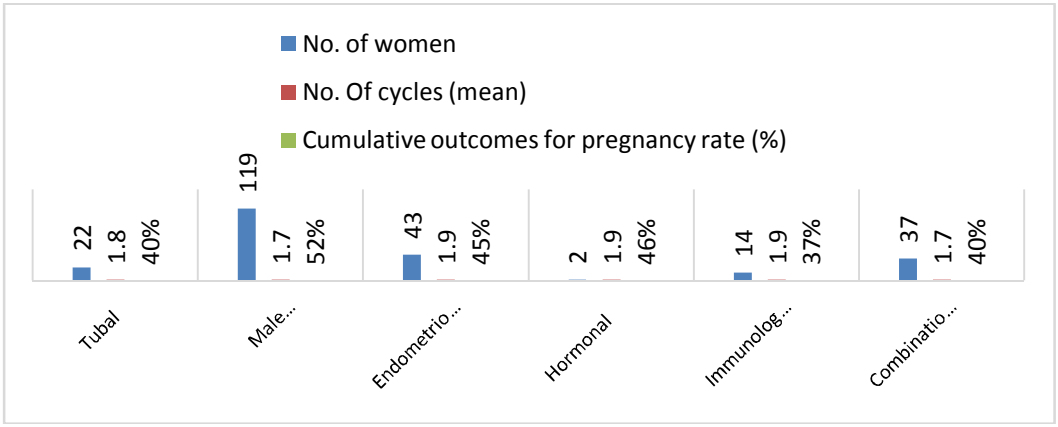

Figure 3: Causes of infertility faced by couples

The data demonstrated an increase in duration of infertility, was allied with a diminution in pregnancy rates in association with IVF treatment even subsequent to adjusted women age. The effect was different for women of age around 20 to 22 years and below. There was no linear relationship between age and chances of pregnancy, however the women of age around 30 to 32 years shows maximum changes. These chances were contently decreased after age of 35 years. Duration of infertility was opposite to the rate of pregnancy, the pregnancy ratio decreases with increasing duration of infertility. The highest pregnancy chances were given by oligospermia, however the immunological/cervical pathology gives the lowest chances.

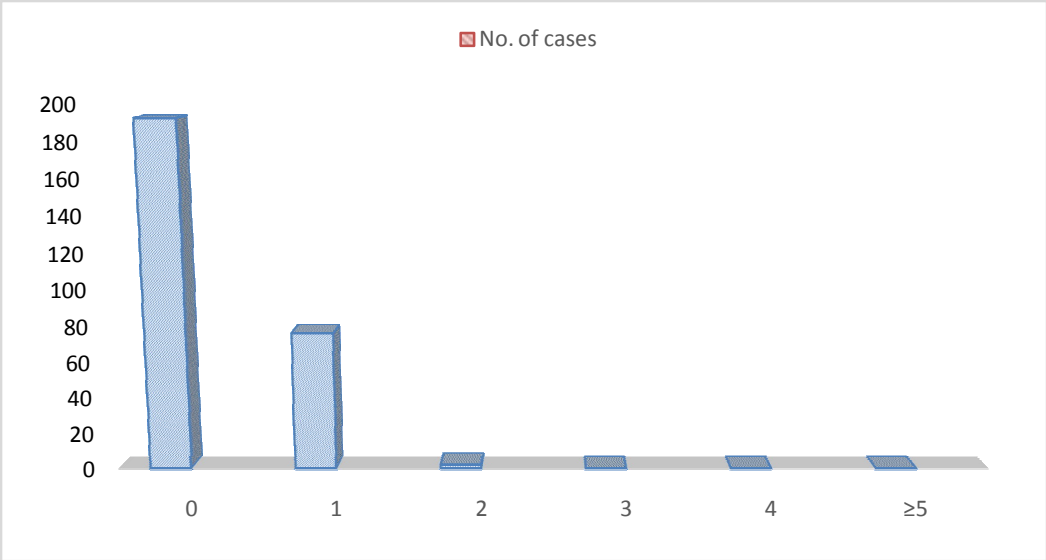

Figure 4: Previous Unsuccesful IVF

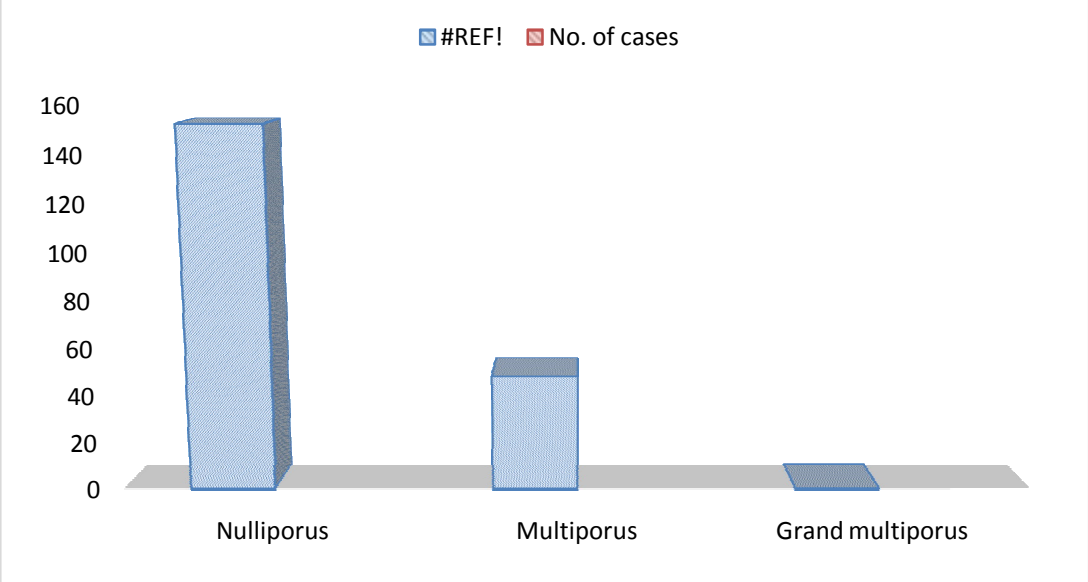

Figure 5: Distribution of parity among IVF cases

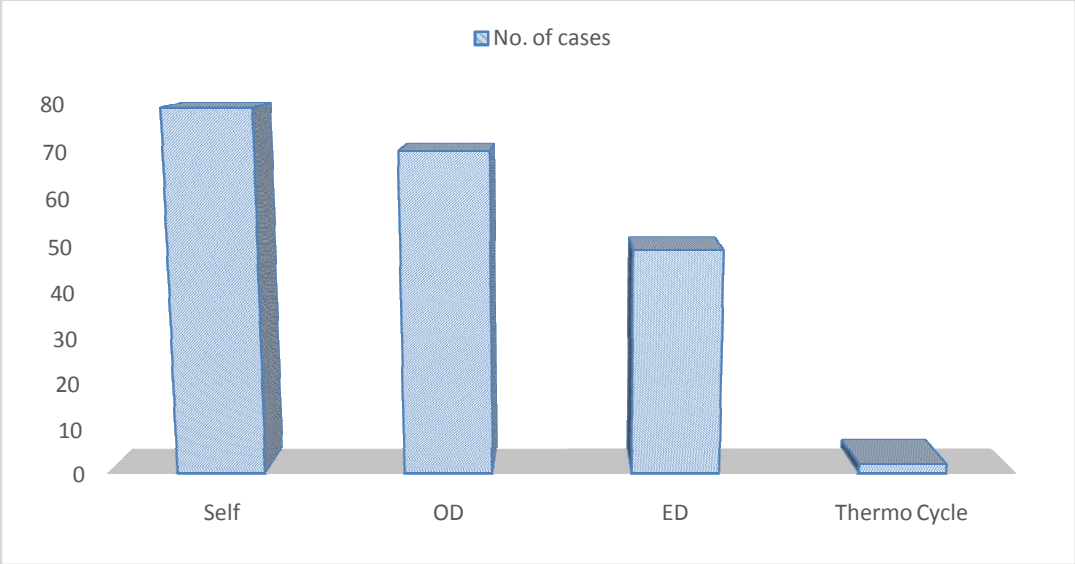

Figure 6: Female Procedures used in this study

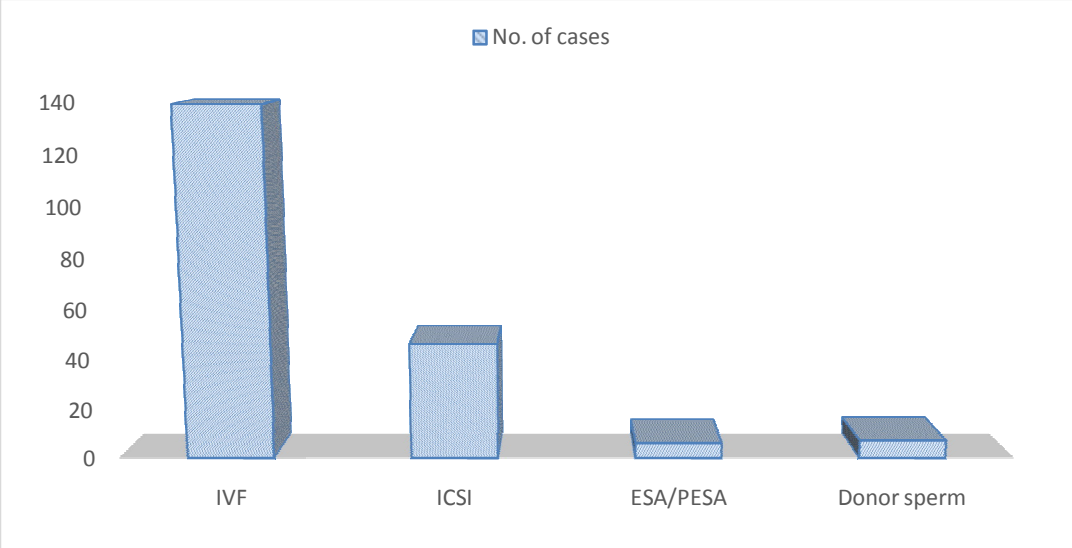

Figure 7: Female Procedures used in this study

Table **1**:Procedure for In-Vitro fertilization used in this study

Characteristics	Category	No. of cases
Procedure for IVF Agonist/Antagonist + hMG/rFSH + hCG/luperide	Antagonist+ hMG+ hCG	9
	Lupride	19
	hMG+Lupride	2
	hMG+ hCG	4
	Agonist+ hMG	1
	Agonist+lupride	94
	Antagonist+ hMG+ lupride	9
	Antagonist+lupride	3
	Antagonist+ hMG	49
	Antagonist+rFSH	1
	Antagonist+FSH+fbFSH+HMG+luprid	2
	Antagonist+FSH+fbFSH+HMG+hCG	1
	Antagonist+hMG+hMG+FSH+hCG	3
	Antagonist +hMG+DP	1
	Antagonist+FSH+DP	1
	Antagonist+FSH+Matuna	1

The information from the study, regarding previous IVF treatment is shown in Figure 4, Figure 5, Figure 6, Figure 7 &Tables1. The examinations illustrate that there is a reduced likelihood of a live birth subsequent to IVF, for patients who have had earlier IVF cycles in the 4th cycle compared to the first cycle. The data showing that the chance of a live birth diminishes as the number of unsuccessful cycles raises and begins to drop quickly following 4 previous unsuccessful cycles.

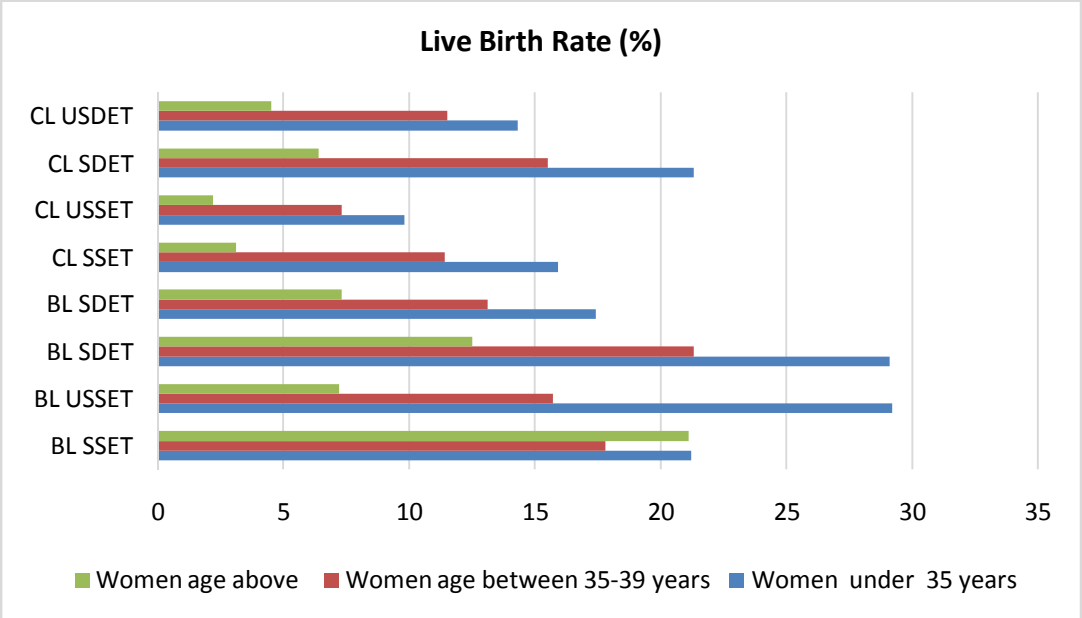

Figure 8 :Live birth ratio in respect of women's, age and embryo transfer at different stage(BL blastocyst, CL cleavage, DET double transfer, S selective, SET single embryo transfer, US unselected)

Figure 8 summarises the observation of live birth rate by employing various embryo transfer strategies. The data helps to identify the risk factors related to Women age, embryo development and number of embryos available, and has been transferred. The observation concludes the variation in live birth rate on applying different embryo transfer strategies and recognized different conditions for availability of enough embryos for transfer and for selective frozen. The observed data revealed that age of women negatively influences the live birth rates and the cleavage transfer were less successful than blastocyst transfers. The data revealed the live birth rates that were assorted with age of females and the number of embryos transferred by elective SET or DET procedure. The quality of the study was low because there are no differences between the blastocyst and cleavage embryos through addressing predisposition, inconsistency is absent and no indirectness with low impression. The observations revealed that rate of live birth with eDET/DET are higher then eSET/SET among all age women. The SET resulted only 2% to 3% of live birth and DET resulting around 32% more multiple live births then SET cycles.

DISCUSSION

The success ratio of treatment of any health problem and disease depends on the individual's characteristics and lifestyle who desire to receive IVF treatment. This study outlines the factors likely to influence the IVF procedure. The Age of female, available embryo numbers thawed of fresh embryo, previous successful treatments, history of pregnancy, lifestyle and body mass index affect the treatment prediction. The duration of infertility and infertility type, influences the rate of live birth and pregnancy.

Since 2004, the data are available, supporting age has influenced over the outcome of IVF. The outcome of a meta-analysis of the few studies illustrate that the increasing age of females overcome the pregnancy rates (18), and this prediction was also supported by HFEA data (19,23). However, lower age limits for IVF treatment is not supported by any study. On analysing the database"HFEA", it was revealed that previous pregnancies and live birth are allied with high probability of treatment success (20).Nevertheless, the secondary infertility rate is most frequent in the common population rather than in referrals of IVF clinic (21, 23).

The study given by Nelson et.al and Robert et al concluded that the factor was correlated with pregnancy rate. As the duration of infertility increases, the rate of pregnancy reduced during IVF treatment [19].The number of Oocyte retrieved and embryos available for IVF has been exposed to predict pregnancy and

live birth rate. A meta-analysis of the few studies revealed that pregnancy rate and Oocyte retrieved depends on each other. Both of them increase equivalently. A report summarized that the ovulation and pregnancy may improve with weight loss program in overweight women for every type of infertility treatment. The obesity articulated risk factor for spontaneous abortion after IVF or ICSI [24]. Obesity also associated with reduced pregnancy ratio in comparison to women having a normal BMI (25kg/m^2). Alcohol consumption by male or female in daily routine affects the success rate of IVF [25]. Smoking habit of both or either any one of them has been significantly related to the low success rate of IVF/ICSI (26-29). Generally the caffeine consumption was supposed to be safe for, natural fertilization rate under limited conditions, however the women undergoing IVF were advised to ignore caffeine consumption to increase success rate of treatment. In other words, the consumption of caffeine decreases the age, if infant gestation [8].

Women receiving IVF program without Pre-treatment or with- treatment show similar number of singleton live birth. The number of clinical pregnancies significantly more with progesterone used for pre-treatment while allowing agonist protocol [31]. There were notable similarities in the proportion of resulting adverse pregnancy on comparing pre-treatment with in patients with no-treatment in patients. The evidences of congenital abnormalities were not reported through the study. No significant variation was reported in the number of singleton live birth within women having low response from pre-treatment and/or no pre-treatment as a follow-up on Antagonist protocol [32].

The treatment principally scheduled the initiation of IVF procedure and not involve in increasing pregnancy and live birth rate. Pre- treatment scheduled the GnRH antagonist cycle as well as long GnRH agonist cycle [34]. Pre-treatment were used as a part of GnRH antagonist cycle to make it more convenient for patients to navigate necessity of down - regulation periods, required beyond GnRH agonist treatment [34].

The Outcomes of Various studies for adverse pregnancies revealed that comparatively 100 IU rFSH have very less chances of miscarriages than that of 200 IU r FSH per pregnancy [31]. However 100IU rFSH assist the frequent ectopic pregnancy and miscarriages in comparison to 200 IU rFSH [31]. One research data reported that there were noticeably high abortion or extrauterine and/or biochemical pregnancies while using doses of 150 IU rFSH that was pre-determined. No significant variations were observed in a number of miscarriages and pregnancy on using various FSH/rFSH doses [31, 35]. The number of live births was significantly more when hCG trigger the ovulation rather than GnRH agonist.

A study explain that the cumulative rate of live birth from fresh single embryo transfer subsequent the frozen embryo transfer were similar to the two fresh embryo transfer succeeding the frozen embryo transfer [35,36,37, 28]. The single embryo transfer shows the higher number of singleton live birth in comparison to double embryo transfe[33]. This was shown that transfer of blastocyst was associated with high live singleton birth rate in comparison to transfer of the embryo at the cleavage stage [32,33,25]. Nevertheless, on extending embryo culture from stage 'cleavage' to stage 'blastocyst', only a few embryos being present for transfer and resulting a condition where only few numbers of embryos at the cleavage stage were available. This may recommend for transfer at this particular stage. The previous studies support the findings of our study. The study discussed the incidence and factor influencing the success of embryo transfer technique and live birth ratio.

CONCLUSION

The study concluded that the chances of success of IVF decrease with the unsuccessful number of cycles. The IVF is effective in women with a previous pregnancy history. Pre-treatment with an Oral contraceptive pill or progesterone will not affect the outcomes of IVF treatment. The Clinical pregnancy and singleton live birth are significant results, and allow clinicians to inform the couples for possibilities of conception. The study accomplished that stimulated cycles ensuing more clinical pregnancy in comparison to natural cycles. The study supports the singleton live birth as a primary output. Rate of Multiple live births was considered as a proxy itself for various unfavourableoutcomes, including disability, prematurity and prenatal mortality. In conclusion, the study discussed the influence of a range of dynamic/factor affecting the success ratio of live birth through IVF and/or ICSI.

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