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Identification of various factors associated with in-vitro fertilization treatment in infertility cases

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Abstract

Infertility is a worldwide problem affecting people of all communities. It is estimated that globally 60-80 million couples suffer from infertility every year of which between 15-20 million are in India alone. Infertility affects every eighth couple during reproductive life by reducing fertility rates due to various factors and excessive stress of fast moving work life, resulting in imbalanced hormone stimulation and diseases related to reproductive organs. These factor works as a barrier to the couples for a successful conception. In-vitro fertilization and Intracytoplasmic sperm injection are the most accepted treatment and frequent choice to overcome the infertility problem. The present study focuses on various factors affecting fertility in couples and possibilities of live birth through in-vitro fertilization and intracytoplasmic sperm injection treatment. In this study, 200 couples facing infertility problems were included. In-vitro fertilization and Intracytoplasmic sperm injection treatment were received by these couples to increase their chances of pregnancy and live birth. Clinical examination, diagnosis, analysis and procedure of In-vitro fertilization including embryo transfer technique and calculation of live births were carried out by following clinical guidelines of National Institute for Health Care and Excellence (NICE). 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 found 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 allow gynaecologists to inform the couples for possibilities of conception.

Key Words: Factors, Infertility, IVF treatment, Live birth rate, Clinical Pregnancy.

Introduction

Infertility is the inability of couples to achieve pregnancy within twelve months of unprotected intercourse (1, 2). In vitro fertilization is the most common infertility treatment used when the fallopian tubes are severely damaged, absent and endometriosis. Due to its high success rates in-vitro fertilization treatment is being used more frequently in recent years as a first line of therapy for all causes of infertility (3, 1). Couples seeking treatment for infertility are increasing regularly. World Health Organization proposed that the couples must be treated together as much as possible to overcome the problem of infertility (4, 5, 6, 7). There is a complex relation between fertility and psychological stress (8, 9). The work stress and low conception possibilities in women were reported by various studies (10-12, 13), 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" (14, 6). Failure to conceive following regular or frequent unprotected intercourse for a year or more has been defined as infertility (15, 16, and 17). 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 (18, 19, 7, 20, 21). 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 (22, 23, 24).The information about lifestyle, environmental and physical factors such as smoking, alcohol consumption every day, work load and diet of couples should be accessible to improve the fertility rate.

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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 in-vitro fertilization 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 cryopreserved semen failed to accomplish conception with Intrauterine insemination, ovarian malfunction due to cancer treatment where cryopreservation of eggs or embryos have been done (22,25, 26, 27, 28).

The cycle of in-vitro fertilization treatments following the stages, nevertheless depends on the protocol, used properly at every stage while treatment follow-up. Pre- treatment stimulate the in-vitro fertilization procedure by improving therapy of exogenous hormone response, reduces the risk of cyst formation in ovary and facilitate the in-vitro fertilization stimulation schedule to corroborate the time of oocyte recovery obtainable to laboratory and clinical staff (12, 29, 24). 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 (19, 30, 31).

After stimulation phase, an ovulation trigger stimulating hormones is used to mimic the function of the natural endogenous luteinizing 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 (11, 12, 32). 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 (5). The early phase of pregnancy is supported by drugs to mimic the natural conception process. The In-vitro fertilization cycle may stop or cancelled due to risk of ovarian hyperstimulation syndrome in women because of negative response to the part of In-vitro fertilization treatment (14, 33, 34). 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 (25, 26, 35, 36). Most of the studies on fertility show that the results of in-vitro fertilization 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 Reproductive Medicine and Mangalayatan Hospital and Research Center, Mangalayatan University, Aligarh, (UP), INDIA on 200 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 in-vitro fertilization being tubal pathology, unexplained sub-fertility, mild male, hormonal, cervical or immunological sub-fertility and endometriosis (15, 1, 3, 37, 34). The Patients having abnormal menstrual cycles history amenorrhea and oligomenorrhea endure ovarian stimulation. The menstrual cycles were regular. A hysteroscopy performed before starting any assisted reproductive technology procedure. During early follicular phase concentration of Follicle Stimulating Hormone 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 (18, 19, 6). 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 in vitro fertilization. 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

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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 in-vitro fertilization / intracytoplasmic sperm injection. On dropping out in-vitro fertilization 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 (38, 39, 40, 32). 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.

Results

The results obtained during this study are depicted in figure-1, 2 & 3 which clearly indicate about the characteristics that established the more chances of pregnancy and live birth. The study includes total 200 cycle of in-vitro fertilization undertaken. The outcomes revealed the upper and lower age limit for in-vitro fertilization 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 in-vitro fertilization. The data observation does not put forward any lower age limit for in-vitro fertilization treatment.

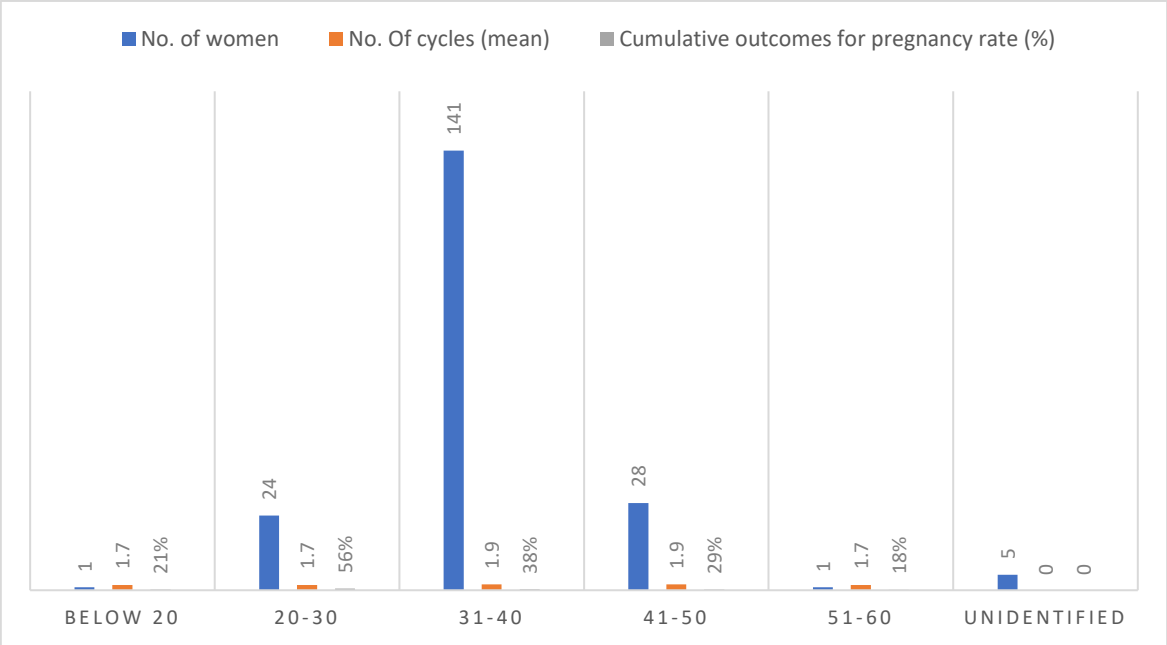

Figure 1: Age of women faced infertility

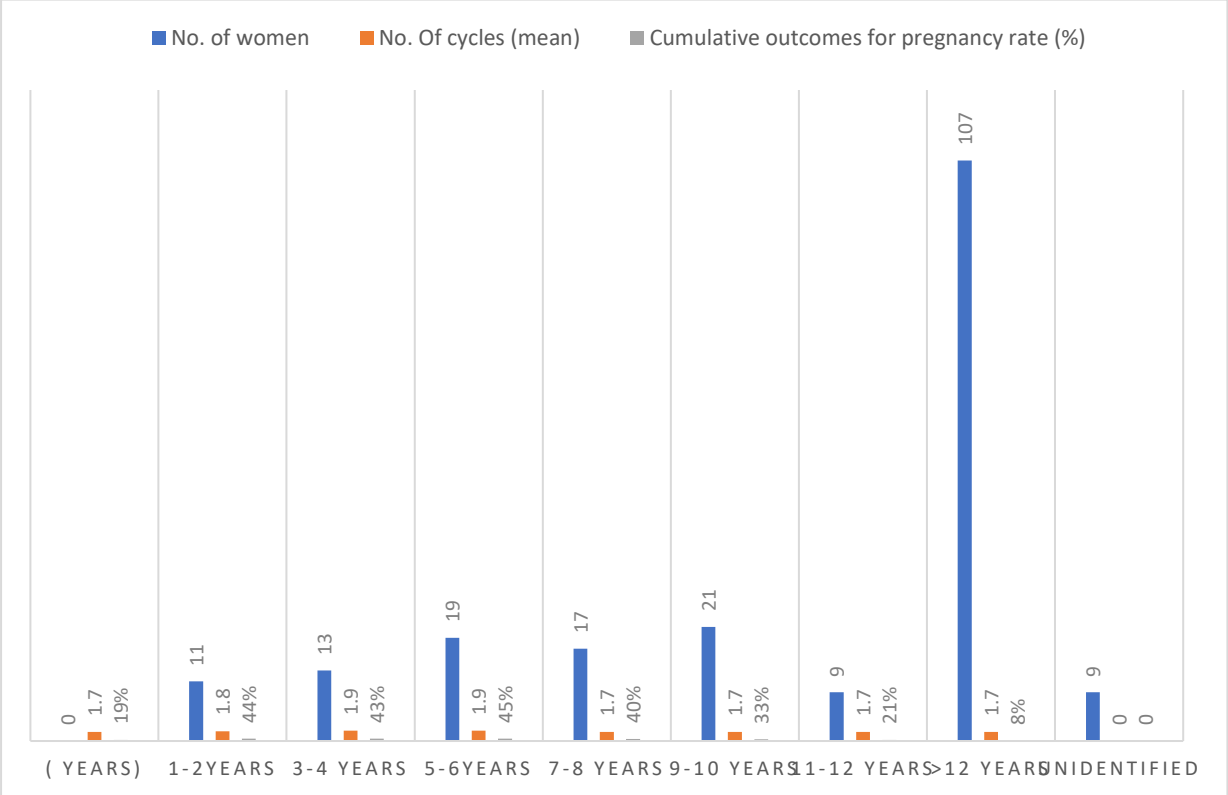

Figure 2: Duration of Infertility faced by couples

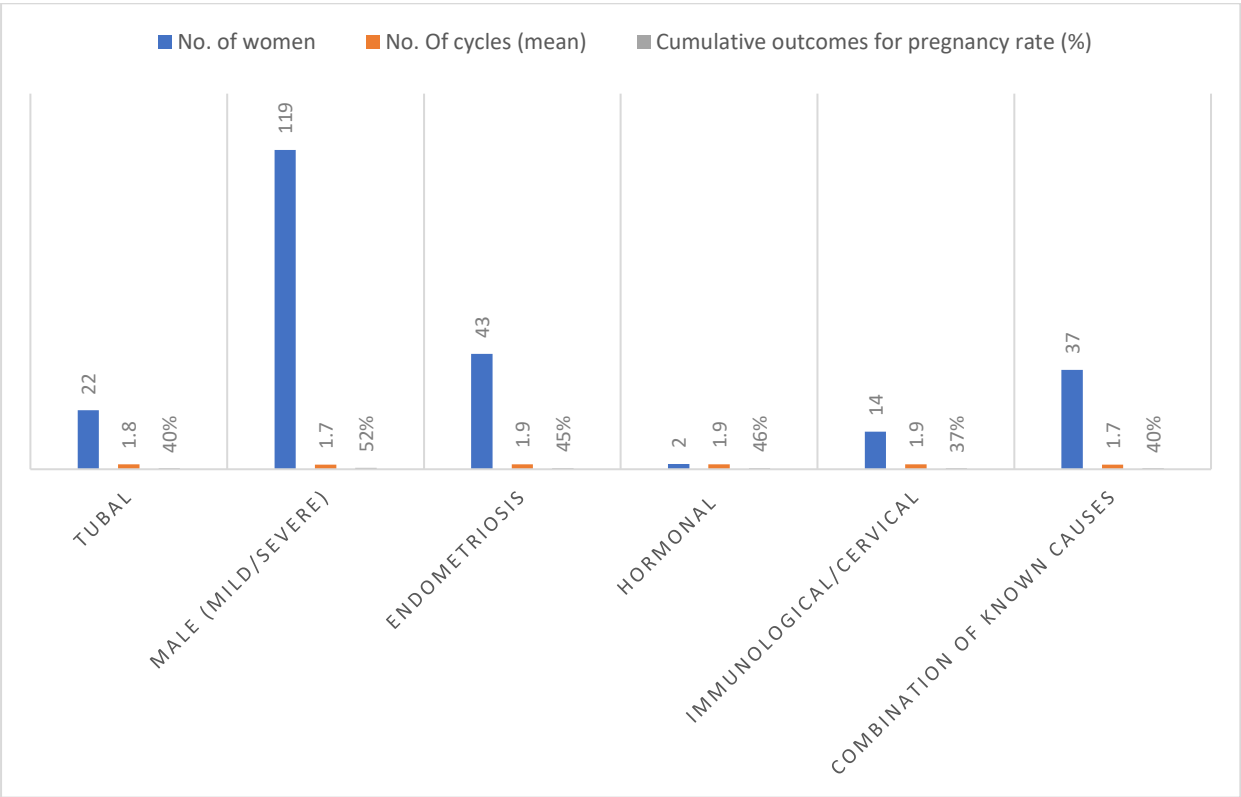

Figure 3: Causes of infertility faced by couples

The data demonstrated an increase in duration of infertility, was allied with a diminution in pregnancy rates in association with in-vitro fertilization 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

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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.

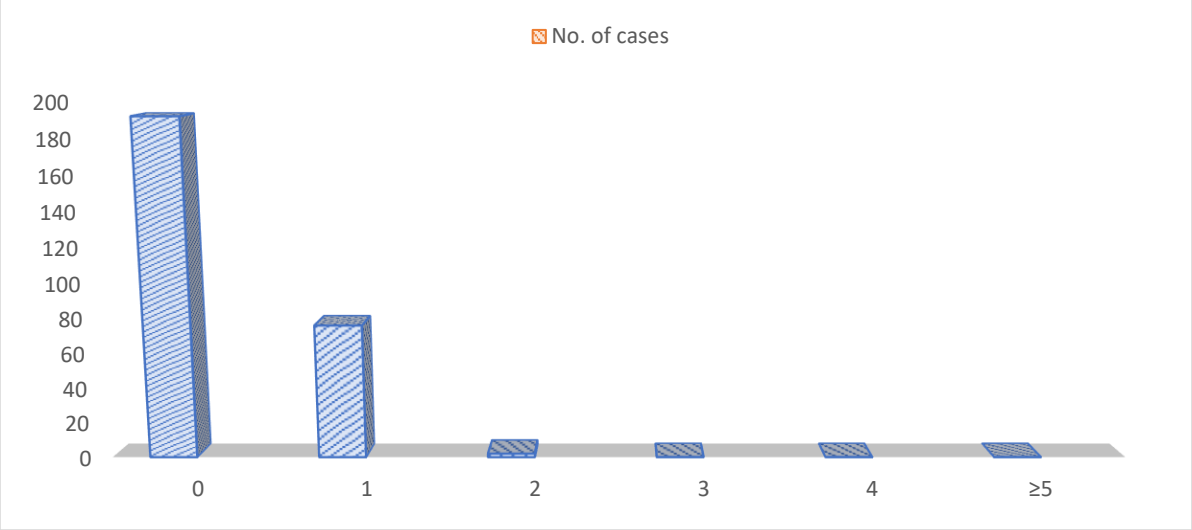

Figure 4: Previous Unsuccessful in vitro fertilization

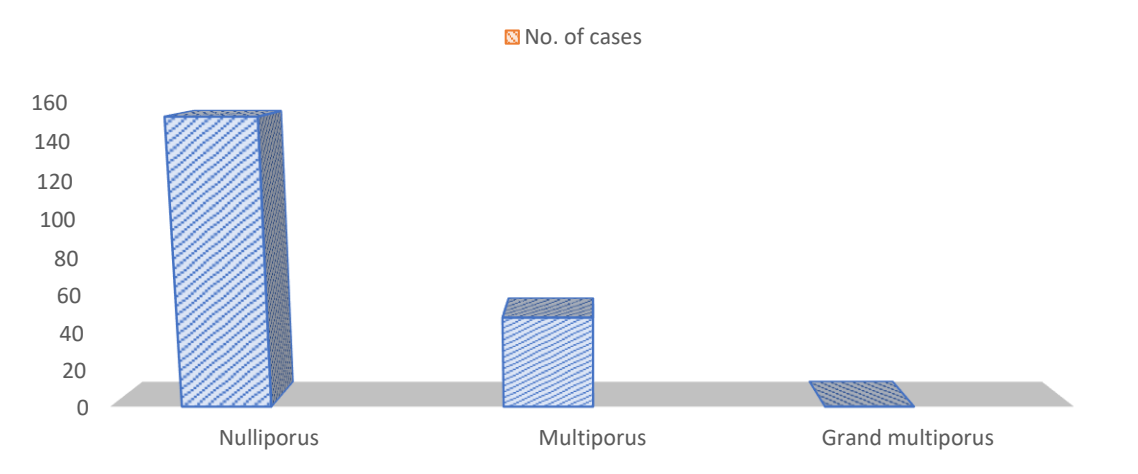

Figure 5: Distribution of parity among in vitro fertilization cases

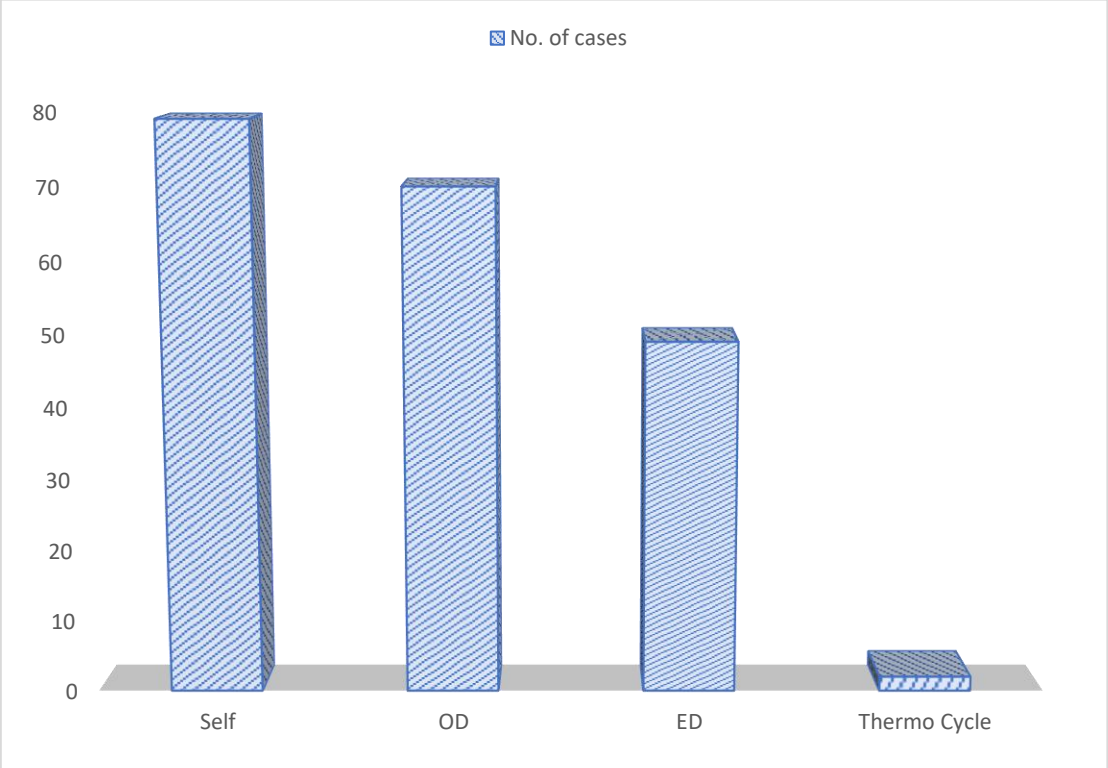

Figure 6: Female Procedures used in this study

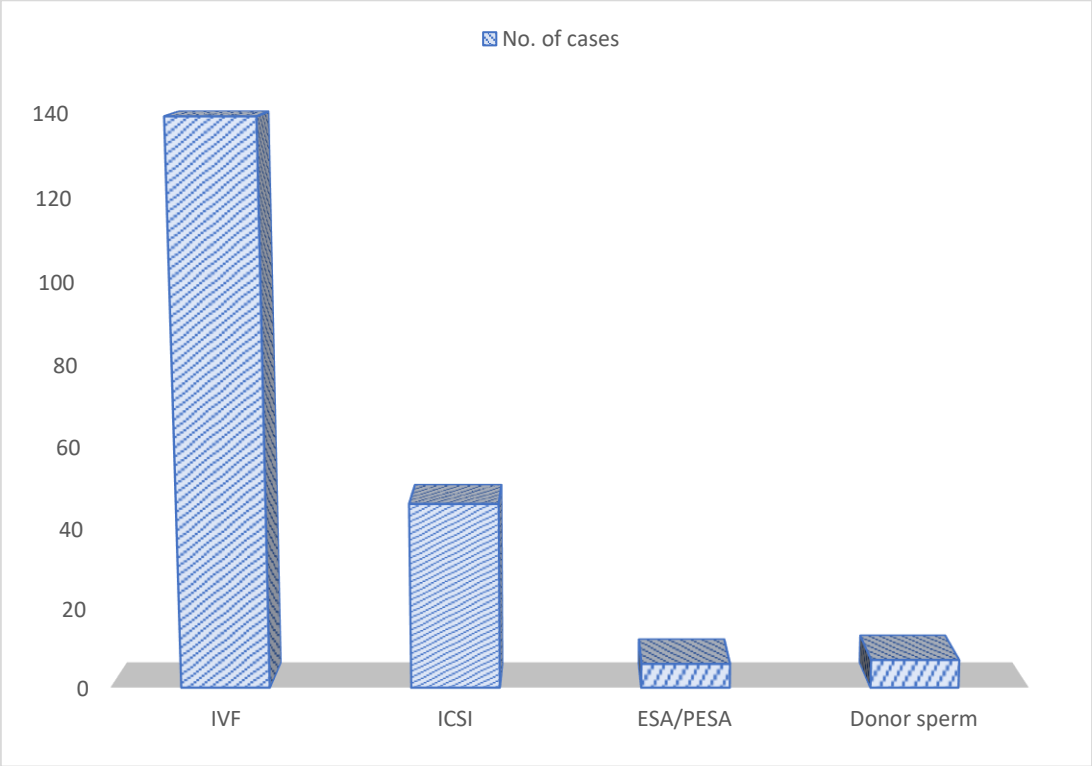

Figure 7: Female Procedures used in this study

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

Characteristics	Category	No. of cases
Procedure for IVF	Antagonist+ hMG+ hCG	09

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Agonist/Antagonist + hMG/rFSH + hCG/luperide	Lupride hMG+Lupride hMG+ hCG Agonist+ hMG Agonist+lupride	19 02 04 01 94
	Antagonist+ hMG+ lupride	09
	Antagonist+lupride	03
	Antagonist+ hMG	49
	Antagonist+rFSH	01
	Antagonist+FSH+fbFSH+HMG+luprid	02
	Antagonist+FSH+fbFSH+HMG+hCG	01
	Antagonist+hMG+hMG+FSH+hCG	03
	Antagonist +hMG+DP	01
	Antagonist+FSH+DP	01
	Antagonist+FSH+Matuna	01

The information from the study, regarding previous in-vitro fertilization treatment is shown in Figure 4, Figure 5, Figure 6, Figure 7 & Table 1. The examinations illustrate that there is a reduced likelihood of a live birth subsequent to in-vitro fertilization, for patients who have had earlier in-vitro fertilization 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.

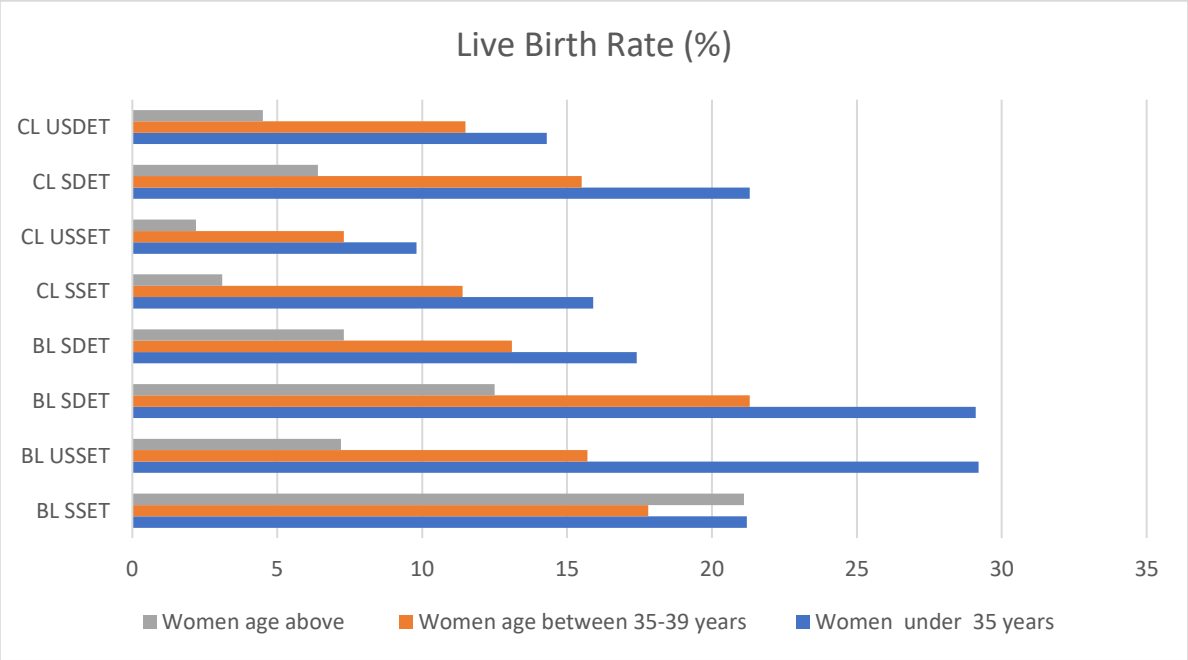

Figure 8: Live birth ratio in respect of women's age and embryo transfer at different stages-BL: Blastocyst, CL: Cleavage, DET: Double embryo transfer, S: Selective, SET: Single embryo transfer, US: Unselected.

Figure 8 summarizes 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 which 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 transfers 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 single embryo transfer

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or double embryo transfer 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 e- double embryo transfer / double embryo transfer are higher than e-single embryo transfer / single embryo transfer among all age women. The single embryo transfer resulted only 2% to 3% of live birth and double embryo transfer resulting around 32% more multiple live births then single embryo transfer cycles.

Discussion

The success ratio of treatment of any health problem and disease depends on the individual's characteristics and lifestyles who desires to receive an in-vitro fertilization treatment. This study outlines the various factors likely to influence the in-vitro fertilization procedure that is the age of female, available embryo numbers thawed of fresh embryo, previous successful treatments, history of pregnancy, lifestyle and body mass index which affects the treatment prediction. The duration of infertility and infertility type, influences the rate of live birth and pregnancy.

It is well documented in the available literature that supporting age influenced over the outcome of in-vitro fertilization. The outcome of a meta-analysis of the few studies also illustrates that the increasing age of females overcome the pregnancy rates (41, 9), and this prediction was also supported by Human Fertilization and Embryo Authority data (42, 43). However lower age limits for in-vitro fertilization treatment is not supported by any study. On analyzing the database of Human Fertilization and Embryo Authority, it was revealed that previous pregnancies and live birth are allied with high probability of treatment success (44).Nevertheless, the secondary infertility rate is most frequent in the common population rather than in referrals of in-vitro fertilization clinic (45, 43, 24).

The study given by Nelson et.al and Robert et al concluded that the factors are correlated with pregnancy rate. As the duration of infertility increases, the rate of pregnancy reduced during in-vitro fertilization treatment (42, 1).The number of Oocyte retrieved and embryos available for in-vitro fertilization 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 in- vitro fertilization or Intracytoplasmic sperm injection (46, 3). 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 in-vitro fertilization (47, 6). Smoking habit of both or either any one of them has been significantly related to the low success rate of in-vitro fertilization / Intracytoplasmic sperm injection (48-51). Generally the caffeine consumption was supposed to be safe for, natural fertilization rate under limited conditions; however the women undergoing in-vitro fertilization 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 (15, 7).

Women receiving in-vitro fertilization 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 (52). 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 (53).

The treatment principally scheduled the initiation of in-vitro fertilization 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 (54). 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 (54).

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 (52). 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 (52, 55). 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 (55,56,57, 50). The single embryo transfer shows the higher number of singleton live birth in comparison to double embryo transfer (58). 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 (53,58,47). 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 in-vitro fertilization decrease with the unsuccessful number of cycles. The in-vitro fertilization is effective in women with a previous pregnancy history. Pre-treatment with an Oral contraceptive pill or progesterone will not affect the outcomes of in-vitro fertilization 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 unfavorable outcomes, including disability, prematurity and prenatal mortality. In conclusion the study clearly indicates the influence of a range of dynamic factors affecting the success ratio of live birth through in-vitro fertilization and Intracytoplasmic sperm injection in infertility cases.

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