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Research Article

DOES SEMINAL PLASMA EXPOSURE AT THE TIME OF START OF THE LUTEAL PHASE SUPPORT PRIOR TO EMBRYO TRANSFER HAS EFFECT ON THE EMBRYO IMPLANTATION?: A COHORT STUDY IN FROZEN EMBRYO TRANSFER CYCLES OVER PERIOD OF 9 MONTHS

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A B S T R A C T

Introduction: In Vitro Fertilization (IVF) has revolutionized the infertility management. Seminal plasma is a biologically active, protein rich, viscous fluid secreted by male accessory sex glands and it act as a medium of sperms transportation. It buffers acidic vaginal environment; contains antioxidants like superoxide dismutase and catalase, biologically active cytokines like TGF (tissue growth factor) beta, growth factors and PGE2. It impairs complement mediated antibody induced cell destruction, increase IL 10 expression and decrease expression of IL 2, making immunological environment favorable for acceptance of embryonic allograft.

Material and methods: A cohort study was performed over 328 subjects undergoing FET over a period of 9 months at a tertiary care centre with the aim to determine the effect of seminal plasma treatment on embryo implantation in frozen embryo transfer cycle.

Results: Serum Beta hCG levels was determined on day 15 post embryo transfer. There were 56 positive Beta hCG cases (Beta hCG >50mIU) on day 15 post embryo transfer in the (seminal plasma treatment group) first group and 78 positive Beta hCG cases in the second group (without seminal plasma treatment). The positive pregnancy rate was 56% in the study group whereas it was 39% in the control group however the difference was not statistically significant.

Conclusion: Results of our study support that exposure of female reproductive tract to seminal plasma improve implantation rates though the difference is not statistically significant.

INTRODUCTION

Embryo implantation rates are low in humans despite of improved embryo culture conditions and ovarian stimulation protocols. Exposure of female reproductive tract to seminal plasma is normal physiological event. Semen preparation for ART procedure removes the seminal plasma which contains many biologically active factors. Studies have shown beneficial effects of seminal plasma exposure on embryo implantation.

Frozen embryo transfer (FET) cycle is more controlled unlike fresh embryo transfer. It is better to study the effects of seminal exposure on embryo implantation in FET cycle. We conducted a cohort study over a period of nine months at a public sector tertiary care IVF center in 300 FET cycles. In Vitro Fertilization (IVF) has revolutionized the infertility management. After fertilization, blastocyst implants after hatching, if the endometrium is receptive. [1]

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Endometrial receptivity is a dynamic event and there exists a ' the window of receptivity'. [2,3] Asynchrony in embryo - endometrial chronology may cause implantation failure .[4]

IVF cycles have defective luteal phase. Luteal phase support with progesterone is added virtually in all embryo transfer cycles to improve pregnancy rates.[5] Blastomere and TE biosy, embryo growth pattern and morphological scoring, zona drilling or laser zona hatching, intrauterine GMCSF instillation and endometrial scratching prior to embryo transfer, endometrial gene profiling and receptivity assay; are some of the other strategies used to improve implantation rates.[6- 8] Supraphysiological estrogen elevations during ovarian stimulation may causes premature serum progesterone elevations despite of pituitary suppression resulting in embryo-endometrial asynchrony.[9] Optimization of the ovarian stimulation, serum progesterone estimations (withholding embryo transfer if >1.5ngm/ml) or freeze all-FET strategy can address this issue.[10-14]

Endometrium exhibit immune tolerance and accepts immunologically different conceptus. Seminal plasma has many cytokines, paternal antigens, growth factors and other biologically active factors which influence embryo viability,

immune tolerance and endometrial receptivity. [15-18] Barker postulated the intrauterine origin of the adult onset diseases .[19] Preembryos are more prone to the injury by oxidative stress, toxins, pH, radiation being deficient in protective mechanisms.[20-22]. Seminal plasma is a biologically active, protein rich, viscous fluid secreted by male accessory sex glands and it act as a medium of the sperms transportation. It buffers acidic vaginal environment; contains antioxidants like superoxide dismutase and catalase, biologically active cytokines like TGF(tissue growth factor) beta, growth factors and PGE2 .[23] It impairs complement mediated antibody induced cell destruction, increase IL 10 expression and decrease expression of IL 2, making immunological environment favorable for acceptance of embryonic allograft.[23]

The TGF beta produced by seminal vesicles induce GMSF & CSF (Granulocyte Monocyte and Colony Stimulating Factor) formation in endometrium which cause neutrophils induction and production of MMPs (Matrix Metalloproteinases), response similar to inflammation and favorable for implantation. MMP 9 is collagen modifying enzymes active during implantation. Seminal plasma contains PGE2 100000 times higher than inflammatory response.[24,25] Its exposure increase the expression of VEGF (Vasculo Endothelial Growth Factor), its receptors and angiogenesis.[26] Seminal plasma sensitizes maternal immune system to paternal antigens for accepting embryonic allograft. [27,28]. It also has TNF alpha, TGF alpha, IGF I&II, EGF, HBEGF which may influence implantation and embryonic development.[29-34]

Excision of seminal vesicles in mice impaired peri implantation embryonic development and reduced fertility. Male offspring's had deranged metabolic profile and fat accumulation. [35] Animal studies in sheep, hamster and rats has supported the role of seminal plasma in implantation. [36-37]. Some humans studies has also emphasized its importance on the implantation rates.[38-40]

ART procedures (IVF and IUI) require semen sample processing to separate healthy motile spermatozoa, however it separates the seminal plasma and devoid its potential benefits.

MATERIALS AND METHODS

This cohort study was performed over period of 09 months at a Tertiary care IVF centre. The study populationwere patients undergoing FET cycle over nine months.

328 patients were selected for study. Sample size was calculated on the basis of sample size for hypothesis testing with following parameters; the level of significance (α) is 0.05. The Power of study (1-β) is 0.80. Anticipated value of RR is 1.5 and condition of interest among non exposed population is (P2) is 0.40.

Exposure to seminal plasma was the independent or exposure variable and beta hCG level >50 mIU on day 15, after day three cleavage stage embryo transfer was the dependent or outcome variable.

All patients undergoing FET cycle over period of nine months were included. Endometrial preparation was done with tablet estrogen valerate 2mg (Zydus cadila) thrice a day starting from day two or three of the menstrual cycle. Endometrial response was evaluated by measuring the endometrial thickness on day twelve by transvaginal ultrasonography. Progestins (Inj Micronised progesterone 100mg im once daily and Tab dydrogesterone 10 mg twice daily) were added if endometrial thickness was more than or equal to 7.5mm. When less than 7.5mm, estrogens continued for two more days and response was reevaluated. Luteal support started if the endometrial thickness was more than 7.5mm. If it was less than 7.5 mm, individualized decision was taken after analyzing previous endometrial response and in poor response, luteal support was started even if thickness was > 7 mm, however these cases were excluded. Estrogens were continued in all the cases along with luteal support.

On the day of starting luteal support, seminal plasma treatment was given to all patients. Husband's fresh semen sample was taken. After liquefaction, microscopic examination was performed for excessive pus cells as indicator of infection. It was centrifuged at 1000 RPM for 8 minutes. One ml of the supernatant was taken and instilled over external cervical os and posterior vaginal fornix. Patient was allowed to lie supine for minimum of 15 minutes. Patients continued luteal support and day three embryo transfer was done for all.

Embryo transfer

Thawing

Embryos had been vitrified on Mcgrill cryoleaf device using SAGE embryo vitrification media kit. Thawing of vitrified embryos done using SAGE embryo thawing kit as per standard thawing protocol.

Two or three; day three, morphologically grade I, eight cell stage, embryo transfer was done with double lumen soft inner catheter using sandwich method (cleavage medium ,air, embryos in cleavage medium, air, and cleavage medium sequentially) under transabdominal ultrasonography guidance. Cervical mucus at external os was cleaned with warm buffer media prior to catheter placement. Embryo placement was done 1- 1.5 cms below fundus.

Primary Objective

To determine the effect of seminal plasma treatment on embryo implantation in frozen embryo transfer cycle.

Inclusion criteria

1. Frozen embryo transfer
2. Day three, eight cell stage embryo transfer
3. At least one grade 1 eight cell embryo , maximum two grade 1 eight cell embryos for age <35yrs and maximum of three grade 1 eight cell embryos for age >/=35yrs
4. Grade I transfer
5. Endometrial thickness more than or equal to 7.5 mm

Exclusion criteria

1. Fresh embryo transfer
2. Natural cycle FET
3. Day two or blastocyst transfers
4. All grade 2 embryos
5. Difficult transfers
6. Semen with excessive pus cells
7. Unhealthy cervix
8. Inability to give semen sample
9. Husband seropositive for HIV/HCV/HBV or syphilis
10. Structural uterine anomalies
11. Intrauterine adhesions

12. Medical disorders (DM, HTN, Thyroid disorder, Thrombophilias, APLA positive status, immunological disorders)
13. Coitus without barrier protection within two weeks prior and five days after embryo transfer.

Written informed consent was obtained from the entire study subject. Data was collected collated, and analyzed using SPSS, relevant statistical tests were applied as per study need. Ethical clearance was taken from the institutional ethic committee.

RESULTS

Total 328 patients were studied over nine months. 25 patients were excluded from study. In two patients embryo transfer was not done for inability to SPSS cervical canal and another had fever.

Seminal plasma treatment on the day of starting of luteal support to all patients undergoing frozen embryo transfer was adapted as a new protocol in the IVF center. 200 patients who underwent FET cycle and met the inclusion criteria prior to change in protocol (ie seminal plasma exposure) were studied as control group which lack the exposure. 100 patients who underwent FET after seminal plasma exposure were studied as study group. Day three embryo transfer was done and luteal support was given in both groups as per protocol.

Baseline characteristics of both groups are given in Table 1. There is no statistically significant difference between the two groups in mean age female & male, infertility duration, endometrial thickness achieved and number of embryos transferred.

Serum Beta hCG levels was determined on day 15 post embryo transfer. There were 56 positive Beta hCG cases (Beta hCG >50mIU) on day 15 post embryo transfer in the (seminal plasma treatment group) first group and 78 positive Beta hCG cases in the second group (without seminal plasma treatment) (table 2). The positive pregnancy rate was 56% in the study group whereas it was 39% in the control group however the difference was not statistically significant (56% versus 39%: p= 0.467). Fig 2 shows the probability of implantation without plasma treatment.

Table 1 Baseline characteristics of study subjects

	Study Group	Control Group	Remarks (statistical difference)
Average age(wife) in years	29.8	27.5	Not Significant
Average age(Husband) in years	33.8	32.1	Not Significant
Primary infertility	35	32	Not Significant
Secondary infertility	26	29	Not Significant
Duration of infertility in years	6.4	6.1	Not Significant
Mean Endometrial Thickness	8.01	8.0	Not Significant
Cause of infertility			
Tubal factor	20 (20%)	33 (16.5%)	Not Significant
Ovulatory factor	16 (16%)	36 (18%)	Not Significant
Endometriosis	13 (13%)	20 (10%)	Not Significant
Male factor	23 (23%)	49 (24.5%)	Not Significant
unexplained	28 (28%)	62 (31%)	Not Significant

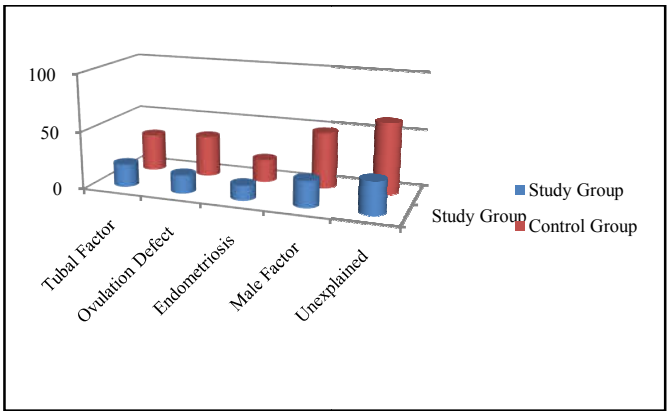

Fig 1 Cause of infertility

Table 2 Comparison of B hCG in study and control group

Group	Study Group (Seminal Plasma+)	Control Group (Seminal Plasma-)	Total	Remarks
Beta hCG positive	56	78	134	P value=0.400
Beta hCG negative	44	122	166	**Not Significant**
Total	100	200	300	

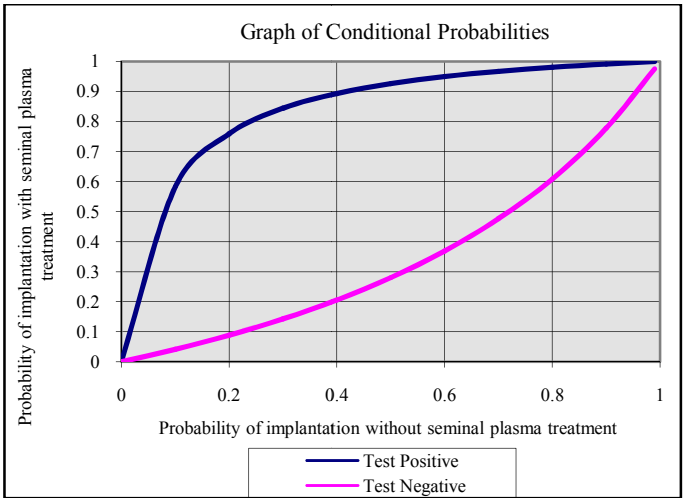

Fig 2 Probability of implantation without plasma treatment

DISCUSSION

Our study support that seminal plasma treatment on the day of start of luteal phase support in frozen embryo transfer cycles may improve the implantation rates of embryos.

In a multicenter RCT Tremellen KP el in 2000 concluded that there is no significant difference in pregnancy rates in seminal plasma exposed group (11.01 versus 7.69, p=0.036) [39]

Our study is in concordance with the result of another meta analysis by Crawford G *et al* which has concluded that clinical pregnancy rates are significantly higher in group exposed to seminal plasma. [40]

Saccone G *et al* in a meta analysis of seven RCTs with 2128 patients concluded that intravaginal application of seminal plasma has higher clinical pregnancy rates (30.0 versus 25.1%; RR 1.20; 95% CI, 1.04-1.39).[41]

CONCLUSION

Results of our study support that exposure of female reproductive tract to seminal plasma improve implantation rates though the difference is not statistically significant an RCT with adequate sample size is needed to determine the statistical significance, however considering the safety of procedure it can be routinely adopted to improve the implantation rates.

Strength and limitations

Adequate sample size and study in frozen embryo cycles are strengths of the study.

There is limitations of information bias regarding history of coitus without barrier protection two weeks prior and five days after the embryo transfer. Though morphologically best graded embryos were transferred but there genetic growth potentials were different. The composition of seminal plasma may vary in different individuals. Many patients had unexplained infertility who might have unknown seminal factors contributing to infertility, however efforts have been made to equally distribute the confounders among different groups.

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