Follicular fluid insulin like growth factor-1 (FF IGF-1) is a biochemical marker of embryo quality and implantation rates in *in vitro* fertilization cycles

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ABSTRACT

CONTEXT: Insulin-like growth factor-1 (IGF-1) has been reported to play a role in human follicular and embryonic development. However, earlier studies carried out mostly in animal models or in culture mediums supplemented with IGF-1 have been unable to directly link IGF-1 with embryo quality. Results correlating IGF-1 with pregnancy outcome have also been ambiguous so far. **AIM:** The aim of this study is to find if *in situ* follicular-fluid level of IGF-1 is predictive of embryo quality and implantation rates in *in vitro* fertilization (IVF) cycles. **SETTINGS AND DESIGN:** Prospective study involving 120 cycles of conventional IVF-embryo transfer in infertile women. **SUBJECTS AND METHODS:** IGF-1 concentrations were estimated in pooled follicular-fluid on the day of oocyte-pickup. Embryo quality was assessed daily at different developmental stages. Cycles were sorted into low and high follicular fluid insulin-like growth factor-1 (FF IGF-1) groups according to the median value of measurement. Embryo quality, clinical pregnancy and implantation rate were the main outcome measures. **STATISTICAL ANALYSIS:** Graph-pad Prism 5 statistical package. **RESULTS:** FF IGF-1 correlates with embryo quality (Pearson $r = 0.3894$, $r^2 = 0.1516$, $P < 0.0001$) and clinical pregnancy (Pearson $r = 0.5972$, $r^2 = 0.36$, $P < 0.0001$). High FF IGF-1 group shows significantly higher rates of fertilization, cleavage, blastocyst formation and top grade embryos compared with low FF IGF-1 group. Clinical pregnancy rates (38.33 vs. 20%, $P = 0.0272$) and embryo implantation rates (21.6 vs. 10.32%, $P = 0.0152$) are also significantly higher in the high versus low FF IGF-1 group. Threshold value of FF IGF-1 for clinical pregnancy is >58.50 ng/mg protein (receiver operating characteristics$_{AUC}$: 0.85 ± 0.03, 95% CI: 0.78-0.91). **CONCLUSION:** FF IGF-1 is a plausible biochemical marker of embryo quality and implantation rate and correlates with clinical pregnancy rates in conventional IVF cycles.

KEY WORDS: Clinical pregnancy, embryo quality, FF IGF-1, implantation rate, IVF cycles

INTRODUCTION

Selection of the best quality embryos for transfer may be a key factor in influencing implantation and pregnancy rates of *in vitro* fertilization (IVF) procedure. Several workers have employed various embryo grading systems which may be unreliable due to discrepancies in the morphological evaluation criteria used. Recently, pre-implantation genetic diagnosis (PGD) studies and use of embryoscope have augmented the possibilities for selection of good quality embryos. However, such expensive techniques may not be an economically viable option for all infertility centers. Furthermore, in stimulated cycles, where at least 2-3 embryos are selected for transfer, it is difficult to trace the fate of individual embryos of a particular morphological quality. Therefore, appraisal of embryo quality based solely on morphological examination may be incongruous and should be accompanied by biochemical evaluation. A robust biochemical marker predictive of embryo quality and its implantation potential remains elusive and hence warrants examination.

Insulin-like growth factor-1 (IGF-1), a member of the ovarian IGF system, has been shown

to serve as an intra-ovarian regulator of follicle function in rodents and exerts direct effects on human and rodent granulosa cell function.[1,2] IGF-I acts on granulosa cells in an autocrine fashion[3,4] and in conjunction with gonadotropins, appears to have a role in promoting follicle growth,[5] steroid secretion,[6,7] and as an anti-atretic hormone.[8] Few workers[9,10] have reported a major role of IGF-1 in regulation of human follicular and embryonic development through regulation of the cell cycle. It has also been implicated in mediating aromatase activity and estrogen production by the developing follicle.[11,12] Though the presence of IGF-1 has been reported in human follicular fluid (FF),[13-18] most studies on follicular fluid insulin-like growth factor-1 (FF IGF-1) have so far been carried out in animal models.[19-21] Human studies with IGF-1 have mostly employed *in vitro* cell culture techniques,[22-24] with exogenous supplementation instead of its estimation in *in situ* sources like FF.[25] A definitive role of IGF-1 in FF has not yet been established or is poorly understood with conflicting reports,[26-30] on its predictive values. Moreover, no approach has yet been made to directly correlate FF IGF-1 with embryo quality. This study contended that FF IGF-1 may be a plausible marker for assessment of embryo quality and hence implantation rates in IVF cycles.

This study aimed at evaluating IGF-1 in FF pooled from follicles from which oocytes had been retrieved in each IVF cycle. Per cycle pooled FF was used to get a comprehensive replicate of granulosa cell function since it has recently been reported that FF specimens collected from single dominant follicles might not truly reflect granulosa or thecal cell production.[30] The objective was to establish *in situ* FF IGF-1 as a plausible biochemical marker influencing embryo development; thus, affecting its quality and implantation potential. This is arguably the first ever study, which attempts to correlate FF IGF-1 with embryo quality and implantation rate in IVF cycles.

SUBJECTS AND METHODS

Subjects
Prospective study involved 146 normoovulatory women (mean age 32.22 ± 4.25 years, body mass index 23.97 ± 4.53, W/H ratio 0.88 ± 0.06, menstrual cycle length range 25-32 days) undergoing their first conventional IVF cycle. 26 cycles were abandoned due to either no oocytes retrieved (12 cycles) or fertilization failure (14 cycles). Finally, 120 cycles were considered for assessment of embryo quality, clinical pregnancy and implantation rates. Informed consent was sought from all patients for participation in this study. Study protocol was approved by the local Hospital Ethical Committee.

Exclusion criteria
1. Women older than 42 years of age
2. Women with polycystic ovary syndrome (as defined according to the Rotterdam consensus)
3. Oocyte pickup failure and fertilization failure cycles
4. Women laparoscopically diagnosed with endometriosis
5. Intracytoplasmic sperm injection cycles were excluded in order to remove any male factor bias.

Main outcome measure
1. Embryo quality
2. Clinical pregnancy rate: Gestational sac with positive cardiac activity observed at ultrasound at around 6th week of amenorrhea was defined as confirmation of clinical pregnancy
3. Implantation rate:

$$\frac{\text{Total no. of gestational sacs}}{\text{Total number of embryos transferred}} \times 100$$

Cycle monitoring
Pituitary desensitization involving treatment with gonadotropin-releasing hormone (GnRH) agonists (500 µg/day of Luprolide acetate) was started in the mid-luteal phase of the menstrual cycle 7 days prior to the earliest expected date of menstruation. Comprehensive down-regulation was confirmed by measurement of serum follicle stimulating hormone (FSH) and estradiol (E2) levels below 1.0 mIU/mL and <20 pg/ml respectively, either on the day of onset of menstruation or 1 or 2 days at the most, after onset.

After confirmation of comprehensive down-regulation, standard long protocol followed for controlled ovarian hyperstimulation involved daily administration of recombinant FSH (Recagon 200 IU/day). Transvaginal ultrasound scan was performed on days 8 and 10 of ovarian stimulation and every 1 or 2 days thereafter as required. Final oocyte maturation and trigger for ovulation was induced by administering human chorionic gonadotropin (hCG) 5000 IU, when there was at least one leading follicle reaching a mean diameter of 18 mm and at least two-four other follicles reaching mean diameter of 16 mm.

Transvaginal ultrasound-guided oocyte retrieval under patient sedation was done between 34 h and 36 h after hCG administration. FF was aspirated from follicles (≥16 mm) using a double lumen needle and maintained at steady 37°C temperature conditions. Only the original follicular aspirate was collected in the few instances wherein oocyte was retrieved in the flush. In every cycle, FF from each follicle was collected separately and equal volume of FF from individual follicles from which an oocyte had been obtained, was pooled together. FF was then centrifuged at 3,000 g for 15 min at 4°C to eliminate cellular elements. Clear supernatant was used for estimations.

Hormonal estimations

FF obtained on the day of oocyte retrieval was estimated for IGF-1 levels by enzyme-linked immunosorbent assay technique using diagnostic kits [Diagnostic Systems Laboratories, Texas, USA (DSL-10-2800)]. Protocol was followed as per manufacturer's instructions. Theoretical sensitivity or lowest detection limit was 0.01 ŋg/ml with no detectable cross reactivity. Since extraction method has been reported to involve overestimations due to interference by binding proteins,[31] we followed the non-extraction method of estimation. The intra-assay precision determined from a mean of 10 replicates each with three human FF samples (mean ± standard deviation [SD]) was 12 ± 3.6, 87 ± 9.6, 295 ± 44.8 ng/ml (coefficient of variation 3.4, 6.4 and 7.2% respectively), whereas the inter-assay precision was 14 ± 4.1, 79 ± 8.1, 347 ± 59.8 ng/ml (coefficient of variation 2.9, 7.4 and 10.8% respectively).

Levels in FF were expressed as the ratio of corresponding total protein content to remove bias due to volume variability. Protein estimation was performed by Folin-phenol reagent method described by Lowry *et al*. (1951).[32] The original method was scaled down to accommodate micro-quantities of sample and reagents.

Estradiol levels were measured in FF by radio-immuno-assay kits [Diagnostic Systems Laboratories, Texas, USA (DSL-4400)]. Estimations were performed as per manufacturer's protocol. Values were expressed as pg/ml with theoretical sensitivity or lowest detection limit 4.7 pg/ml.

Assessment of embryo quality

Embryos were evaluated on the basis of following parameters:

I. Fertilization: Assessed 16-18 h after insemination, was characterized by the presence of two pronuclei and two polar bodies.
II. Cleavage: Main morphologic characteristics assessed were:
   - Evenness of blastomeres
   - Lack of multinucleation
   - 4-5 blastomeres on day 2
   - >6 blastomeres on day 3
   - Multicellular (morula) stage with compaction on day 4
   - Embryo fragmentation %.

   Taking into consideration above characteristics, cleavage stage embryos were graded as per Veeck's criteria[33] as grade 1 (top), grade 2 (average) and grade 3 (poor).
III. Blastocyst: Timing of blastocyst formation, expansion and hatching was evaluated alongwith following criteria:
   - Day 5: Formation of blastocoel cavity, orientation of the inner cell mass and thinning of zona
   - Day 6: Formation of hypoblast and point of hatching/spontaneous hatching.

Blastocyst stage embryos were graded as per Gardner grading system,[34] as:

1. Early blastocyst: Blastocele < half the volume of embryo
2. Blastocyst: Blastocele ≥ half the volume of embryo
3. Full blastocyst: Blastocele completely filling the embryo
4. Expanded blastocyst: Blastocele volume larger than that of the early embryo and thinning zona-pellucica
5. Hatching blastocyst: Brophectoderm has started to herniate through the zona pellucida
6. Hatched blastocyst: Blastocyst has completely escaped from zona.

In addition, grades A, B or C were assigned on the basis of composition of inner cell mass (ICM) and number and texture of trophectodermal (TE) cells. Depending on cumulative gradation, embryos were designated top, average or poor quality.

For example, 4AA (top/grade 1): Represents fully expanded blastocyst with distinct round or oval shape, compact inner cell mass and trophectoderm consisting of a high number of flat epithelium-like cells without dark granulation.

5BB (average/grade 2): Represents hatching blastocyst with slightly dispersed ICM and consisting of few number of TE cells.

4CC (poor/grade 3): Represents fully expanded blastocyst characterized by flat, irregularly shaped or fragmented ICM and trophectoderm formed by very few cells with poor cell-to-cell attachment.

ET

Quality of embryos influenced the decision on whether the transfer was performed at cleavage stage or at the blastocyst stage. If there were at least three morphologically good quality embryos available on day 3, culture was extended to blastocyst stage. If number of good quality embryos was <3 or if quality was average/poor, cleavage stage transfer was done. Also, if patients were unwilling for blastocyst transfer due to financial constraints, transfer was done at cleavage stage even if >3 good quality embryos were available.

Micronized progesterone 200 mg twice daily was administered to support luteal phase starting from the evening of day ET until day 14 of ET. On d14 ET, serum β-hCG >50 mIU/ml was considered as a positive indicator of pregnancy. Regular trans-vaginal ultrasound scan was done and presence of gestational sac/s with positive cardiac activity at around 6th week of amenorrhea confirmed clinical pregnancy.

Statistical analysis

Data was analyzed for statistical significance using Graph

Pad Prism 5.0 statistical package. Student's *t*-test was used to assess the difference between means. Comparisons between continuous variables from more than two groups were performed using one-way analysis of variance (ANOVA). Receiver operating characteristics (ROC) analysis was done to obtain cutoff values. Correlation was obtained and expressed as Pearson correlation coefficient (*r*). All values are expressed as mean ± SD. In all cases, $P < 0.05$ was considered to be statistically significant.

RESULTS

Cycles were sorted into pregnant and non-pregnant groups depending on clinical pregnancy and then into low (≤59.25 ng/mg protein) and high (>59.25 ng/mg protein) groups according to FF IGF-1 concentrations. Cutoffs for defining low and high concentrations corresponded to round value of median (50th centile) of each measurement.

120 cycles of conventional IVF led to an overall clinical pregnancy rate of 29.17% (35/120) with 5 (14.29%) twin pregnancies. A total of 251 embryos were transferred giving an implantation rate of 15.94% (40 × 100/251). Table 1 depicts a comparison of FF IGF-1 levels and embryological data between pregnant and non-pregnant groups. FF IGF-1 levels, fertilization and blastocyst formation rates were significantly higher in pregnant group than in non-pregnant group. However, cleavage rates showed no statistically significant difference between the two groups. One-way ANOVA test for fertilization, cleavage and blastocyst formation rates between the two groups was highly significant ($P \leq 0.0001$). Embryo grades (top, average and poor) differed significantly (one-way ANOVA $P < 0.0001$) between pregnant and non-pregnant groups.

Table 2 represents a comparison of embryological data between high and low FF IGF-1 groups. Not only was

the difference in fertilization and blastocyst formation rate more significantly pronounced; but cleavage rate also differed significantly between high versus low FF IGF-1 groups. Difference in embryo grades was also more significantly demarcated when cycles were divided into high and low IGF-1 groups (one-way ANOVA for embryo grades $P < 0.0001$). Notably, clinical pregnancy and embryo implantation rates showed considerable improvement, above the overall rates, when cycles were grouped on the basis of FF IGF-1 levels [Table 2]. Age of the women did not seem to have any impact on the FF IGF-1 levels in the selected group of patients [Tables 1 and 2].

An interesting finding was the significant difference in FF IGF-1 levels between cycles involving day 5/6 blastocyst transfers (36 cycles, 75 blastocysts), day 3 ET (73 cycles, 153 embryos) and "forced" (due to few number of embryos available or due to patient's economic status) day 3 ET (11 cycles, 23 embryos). Cycles involving day 3 ET showed significantly lower FF IGF-1 compared with both: "forced" day 3 ET cycles (44.99 ± 14.69 vs. 124.0 ± 49.76, $P < 0.0001$) and blastocyst transfer cycles (44.99 ± 14.69 vs. 151.1 ± 71.20, $P < 0.0001$).

FF IGF-1 shared a very strong correlation with embryo quality (Pearson $r = 0.3894$) as well as clinical pregnancy (Pearson $r = 0.5972$) [Table 3]. Table 4 shows ROC curve data with a threshold value of FF IGF-1 for clinical pregnancy (>58.5 ng/mg protein, sensitivity 89.58%, specificity 60.24%).

DISCUSSION

This is the first ever study that has successfully investigated the significance of *in situ* FF IGF-1 in effecting embryo quality and its efficacy in influencing implantation rates. The presence of IGF-1 has been reported in FF. FF

Table 1: Embryological data in pregnant versus non-pregnant groups

Parameter	Pregnant (*n*=35)	Non-pregnant (*n*=85)	*P* value
Mean age (years)	31.92±3.83	31.86±4.35	0.72 NS
FF IGF-1 (ng/mg prot.)	130.5±50.6	62.64±14.61	0.0002***
FF E2 (pg/ml)	315300±146900	192600±71300	0.0229*
Total number of eggs retrieved	321	429	<0.0001***
Fertilization (no.) %	(278) 86.6±14.08	(327) 76.22±22.12	0.0403*
Cleavage (no.) %	(249) 77.57±20.68	(307) 71.56±24.89	0.3302 NS
Blastocyst formation (no.) %	(122) 38.00±18.98	(117) 27.27±16.82	0.0388*
Top grade (1) embryos %	53.33±24.76	30.73±19.24	0.0084**
Average grade (2) embryos %	33.33±18.39	40.22±21.18	0.0452*
Poor grade (3) embryos %	13.33±7.624	29.05±16.61	0.0247*
Total no. of embryos transferred (D3+D5/6)	61 (20+41)	190 (156+34)	-
Mean no. of embryos transferred	1.74	2.23	<0.0001

All values are represented as mean±SD. Statistically significant difference was obtained by student's *t* test. *P*<0.05*=Significant;<0.01**=Highly significant; *P*>0.05=Non-significant (ns); Pregnant and non-pregnant groups are with respect to clinical pregnancy; *n*=Number of patients; E2=Total estradiol levels. D3 represents cleavage stage embryos which were graded by Veeck's criteria on the basis of blastomere size/shape/even-ness and % fragmentation. D5/6 represents blastocyst stage embryos that were graded as per Gardner grading system. Embryos meeting the maximum positive criteria of morphological evaluation were considered top grade (1) followed by average grade (2) and poor grade (3). FF IGF-1=Follicular fluid insulin like growth factor-1; FF=Follicular fluid

Table 2: Embryological data in high versus low FF IGF-1 groups

Parameter (rate %)	High FF IGF-1 (*n*=60) (>59.25 ng/mg protein)	Low FF IGF-1 (*n*=60) (≤59.25 ng/mg protein)	*P* value
Mean age (years)	32.81±3.83	31.77±4.51	0.14 NS
FF E2 (pg/ml)	284631±15486	189972±67400	0.0411*
Total number of eggs retrieved	378	372	NS
Fertilization (no.) %	(313) 82.80±17.65	(292) 78.49±19.49	0.0320*
Cleavage (no.) %	(297) 78.57±17.82	(259) 69.62±19.77	0.0010**
Blastocyst formation (no.) %	(169) 44.71±15.13	(70) 18.82±2.75	<0.0001***
Top/grade 1 embryos (%)	61.19±23.08	25.19±10.29	<0.0001***
Average/Grade 2 embryos (%)	26.19±18.67	40.00±19.54	0.0060**
Poor/Grade 3 embryos (%)	12.69±9.14	34.81±23.76	0.0007***
Total no. of embryos transferred (D3+D5/6)	125 (68+57)	126 (108+18)	NS
Mean no. of embryos transferred	2.08	2.1	NS
Clinical pregnancy rate (no.) %	(23/60) 38.33	(12/60) 20.00	0.0272*
Twin pregnancies (no.) %	(4/23) 17.39	(1/12) 8.33	0.4819 NS
Implantation rate (no.) %	(27/125) 21.6	(13/126) 10.32	0.0152*

All values are represented as mean±SD statistical significance was obtained by student's *t* test. *P*<0.05*=Significant;<0.01**=Highly significant;<0.0001***=Extremely significant; NS=Non-significant; n=Number of patients; E2=Total estradiol levels. D3 represents cleavage stage embryos which were graded by Veeck's criteria on the basis of blastomere size/shape/ eveness and % fragmentation. D5/6 represents blastocyst stage embryos that were graded as per Gardner grading system. Embryos meeting the maximum positive criteria of morphological evaluation were considered top/grade 1 followed by average/grade 2 and poor/grade 3. Implantation rate=Total no. of gestational sacs×100/total no. of embryos transferred. IGF-1=Follicular fluid insulin like growth factor-1; FF=Follicular fluid

Table 3: Correlation of FF IGF-1 with embryo quality and clinical pregnancy

FF IGF-1 (ng/mg protein)	Embryo quality	Clinical pregnancy
Pearson *r* (95% CI)	0.3894 (0.23-0.53)	0.5972 (0.48-0.70)
P value	<0.0001***	<0.0001***
R^2	0.1516	0.36

Pearson *r* correlation coefficient was obtained. *P*<0.0001***extremely significant; FF IGF-1=Follicular fluid insulin like growth factor-1; CI=Confidence interval

Table 4: ROC analysis of FF IGF-1

ROC analysis	FF IGF-1
Area (95% CI)	0.85±0.03 (0.78-0.91)
Threshold value for pregnancy	>58.50 ng/mg protein
Sensitivity %	89.58
Specificity %	60.24
Likelihood ratio	2.25
P value	<0.0001***

Threshold cutoff values were obtained from tabular results of the ROC analysis. CI=Confidence interval; ROC=Receiver operating characteristic; FF IGF-1=Follicular fluid insulin like growth factor-1; *P*<0.0001***extremely significant

microenvironment may thus be considered to be a dynamic milieu with its rich source of growth factors and their binding proteins facilitating follicular growth and embryonic development. In recent years, several components have been assayed in monofollicular fluids (fluid obtained from each individual follicle) to study oocyte and embryo quality. However, studies in monofollicular fluids are cumbersome and have their limitations.[35] Though few studies have also focused on monodominant follicles (fluid obtained from single lead follicle), such estimations may not truly reflect granulosa or thecal cell production.[30] Therefore, in each cycle, we estimated IGF-1 levels in fluid pooled from follicles from which oocytes had been retrieved. It

may also be pertinent to note that this study followed the recommended non-extraction method for estimation of IGF-1 as the extraction method has been reported to involve interference due to binding proteins.[31]

Our study was based on the hypothesis that apropos role of IGF-1 in follicular and embryonic development,[36] FF IGF-1 may be a potential biochemical marker of embryo quality and its implantation potential in IVF cycles. This contention is amply supported by our findings of significantly higher rates of fertilization, cleavage, blastocyst formation and top quality embryos in high FF IGF-1 group than in low FF IGF-1 group.

Two earlier studies,[26,27] had linked FF IGF-1 with follicular development and oocyte maturation but found no significant correlation with embryo cleavage rates. Bencomo *et al.* (2006)[28] reported a dose dependent decrease in the rate of apoptosis of human granulosa lutein (GL) cells and embryo fragmentation rates. However, their study was carried out with GL cells cultured in a medium exogenously supplemented by IGF-1 and could not correlate response to exogenous IGF-1 with IVF outcome. They also failed to establish a direct correlation between IGF-1 and embryo quality. Pertinently, in our study, cleavage rate was significantly much higher in high FF IGF-1 group compared with low FF IGF-1 group [Table 2] as opposed to the non-significant difference in cleavage rates observed between pregnant and non-pregnant groups [Table 1]. This finding emphasizes the role of IGF-1 as a cell cycle regulator through promotion of cell division. It is also evident from Table 1 that the fertilization and blastocyst formation rates remained significantly lower in non-pregnant group compared with pregnant group.

We also obtained a strong correlation of FF IGF-1 with embryo quality. Further, significant differences in FF IGF-1 levels observed between cycles involving day 5/6 blastocyst transfers, day 3 ET and "forced" day 3 ET indicates that the good quality embryos that were transferred on day 3 itself owing to the fewer number available or due to patient's economic considerations, also carried the potential to develop to blastocyst stage. This remarkable finding further underlines the competence of FF IGF-1 in regulating embryonic development, influencing its quality.

Another important finding of this study was the significant difference in estrogen levels observed not only between pregnant and non-pregnant groups [Table 1] but also between high and low FF IGF-1 groups [Table 2]. Our result corroborates with earlier observations implicating IGF-1 in mediating aromatase activity and estrogen production.[11,12,36]

The role of FF IGF-1 in predicting pregnancy outcome seemed ambiguous from previous studies. Though Dorn *et al.* (2003),[29] reported higher levels of IGF-1 in conception versus non-conception cycles in serum on the day of oocyte retrieval; no such association was observed in FF samples. Another study[30] found no significant difference in levels of FF IGF-1 or pregnancy rates in women undergoing IVF using agonist versus antagonist stimulation protocols. Yet another study,[37] indirectly indicated an IGF-1 mediated influence of embryo on the endometrial milieu during early implantation. However, our study not only reports significantly higher levels of FF IGF-1 in conception versus non-conception cycles but has also found a strong, direct correlation of FF IGF-1 with clinical pregnancy. In our study, when cycles were grouped on the basis of FF IGF-1 levels, high FF IGF-1 group showed significantly demarcated differences in embryo quality despite comparable number of eggs retrieved between low and high groups. Furthermore, a considerable improvement in clinical pregnancy and embryo implantation rates above the overall rates was obtained inspite of comparable number of embryos transferred, suggesting that FF IGF-1 is not only an add-on to morphological evaluation as an indicator of embryo quality but also influences clinical pregnancy and embryo implantation rates.

It was our conjecture that the FF micro-environmental milieu with its rich source of growth factors, cytokines and hormones may provide the trigger for embryonic development and may dictate the course of events leading to successful implantation of ensuing embryo. Therefore, evaluating levels of biochemical molecules in *in situ* sources like FF may be a more feasible approach than extrapolating data from animal models or cell-culture studies involving GL cells/endometrial tissue in culture exogenously supplemented by the molecule of interest.

Moreover, as mentioned earlier, in each cycle, we used pooled FF obtained from a follicular cohort from which oocytes had been retrieved, to get a comprehensive replicate of granulosa cell function and assessment of embryo quality. Our stance stands vindicated by a direct correlation obtained in our study between FF IGF-1 levels and embryo quality (especially cleavage rates) as well as pregnancy outcome, both of which have not yet been established together for IGF-1 in IVF cycles by any of the earlier studies. However, supplementation of clinical findings and biochemical data with genetic studies like PGD could offer a more promising recourse in future studies on embryo quality. Another remarkable finding of higher fertilization rates in high FF IGF-1 and pregnant groups in our study may prompt future research into the significance of IGF-1 in effecting fertilization.

In conclusion, it may be said that this study establishes FF IGF-1 as a plausible biochemical marker of embryo quality and implantation rate. It also successfully correlates FF IGF-1 with clinical pregnancy rates in conventional IVF cycles.

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