

Follicular Fluid Activin A and Leptin Are Not Correlated With IVF Outcome Measures

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ABSTRACT

Objective: The study is designed to evaluate the relationship between follicular fluid (FF) Leptin, Activin A and IVF outcome.

Subjects & Methods:

Prospective observational study measuring FF Leptin and Activin A in 90 patients undergoing ICSI excluding women having PCOS. FF samples collected at oocyte retrieval from follicles > 18 mm in diameter were analyzed for Leptin and Activin A using ELISA and results were correlated to ICSI outcome.

Results: For the whole study population, mean FF Leptin level was 45.2 ng/ml and mean BMI was 25.3 kg/m². FF Leptin had a positive correlation with BMI. The mean FF Activin A was 880.8 pg/ml. 29/90 patients (32.2%) achieved pregnancy, 25 of them (86.2%) had more than 50% grade A embryos on Day 2 in contrast to 33 patients (54.1%) in the non-pregnant group (P < .02). FF Leptin and Activin A did not correlate to day 2 embryo quality or pregnancy outcome. FF total Activin A level did not relate to FF Leptin level or number of oocytes collected. Low or high FF Activin A or Leptin levels did not affect fertilization, pregnancy or embryo quality.

Conclusion: Neither FF Activin A nor FF Leptin levels can be used to predict IVF outcome measures.

Key Words: Activin A, Leptin, follicular fluid, intracytoplasmic sperm injection, pregnancy.

Introduction

Assisted reproduction is a complicated process involving multiple stages of ovarian stimulation, ovum pick up, fertilization, embryo cleavage and implantation. The ultimate goal of all these procedures is achievement of a viable intra uterine pregnancy as a step of achievement of a healthy baby. Good quality of all reproductive components and primarily embryos has a positive impact on success rates. Definitely, better selection of embryos is one of the greatest challenges in IVF [1]. A morphological approach of choosing good quality embryos at 2-8 cell stage based on number, equality of size and percent fragmentation has been the method of choice of day 2 or 3 embryo transfer [2].

Activins are disulphide-linked dimeric glycoproteins belonging to the TGF- β superfamily. Other members include inhibin and follistatin. Dimerization of β subunits alone gives rise to three forms of activin referred to as activin A ($\beta A-\beta A$), activin AB ($\beta A-\beta B$) and activin B ($\beta B-\beta B$) [3]. While inhibin and activin exert their actions mainly through a negative feedback effect on pituitary FSH, activins, mainly activin A exert their effects through local autocrine and paracrine effects on granulosa cells through action on specific receptors [4]. Evidence from in vitro and animal studies suggests that activin effects are mainly stimulant to granulosa cell proliferation and maturation, steroidogenesis and oocyte maturation [5]. Activin can promote FSH receptor expression on undifferentiated rat granulosa cells, evidence that can explain the transformation of follicle from late pre-antral to early antral stage [6]. Once granulosa cells have acquired functional FSH receptors, their proliferation and differentiation would be driven mainly by FSH, but modulated by other extrinsic and locally produced factors including insulin, growth hormone and leptin [7]. Activin A was shown to be higher in follicular fluid of follicles containing high quality oocytes of IVF cycles [8].

Leptin, a protein secreted from adipose and many other tissues of mammals was found to a greater extent in people with a high body mass index [9]. Leptin was shown to be involved in various reproductive aspects including initiation of puberty, fertility and preg-

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nancy. Oestrogen was shown to induce leptin secretion. Obesity per se was found to have detrimental effects on conception whether in natural or assisted reproduction cycles [10]. Serum leptin at various stages of IVF cycles, in addition to follicular fluid leptin were suggested as predictors for IVF outcome with some conflicting results. Serum and follicular fluid leptin were consistently rising during IVF cycles with consistent correlation between serum leptin level on day of oocyte retrieval and follicular fluid leptin. Anifandis et al. reported that elevated follicular fluid leptin concentration was associated with reduced ovarian response, follicle maturation, embryo quality and response [11]; however, others failed to prove those associations [12, 13]. The aim of this work is to study a correlation between follicular fluid activin A and leptin and the various IVF outcome measures.

Subjects & Methods

The study was carried out in a private IVF center setting in Cairo, Egypt. Patients less than 42 years with day2-3 FSH <10IU/L were included in the study excluding patients with polycystic ovarian syndrome to nullify its effect on follicular development and IVF outcome. Ninety women were included in the study after signing initial relevant consents and after obtaining the ethical approval from Suez Canal University ethical committee. All women had long protocol ICSI cycles as per the center protocol. They received GnRH α in the form of 0.1 mg triptorelin acetate as daily subcutaneous injection starting on day 21 to 23 of the menstrual cycle. Triptorelin acetate administration was continued until loss of follicular activity by transvaginal ultrasonography. At this stage exogenous gonadotropins were initiated and triptorelin acetate was decreased to half. When ≥ 3 follicles reached 18 mm diameter or more, a single 10,000 IU intramuscular dose of human chorionic gonadotropins (hCG) was administered. Transvaginal follicular aspiration took place 35-36 hours later under sedation/general anesthesia.

All large mature follicles (>18 mm) were aspirated into empty sterile tubes and oocytes noted carefully to follow them up after fertilization and embryo development. Samples with blood contamination or with flushing fluid were excluded. The fluid was used only if it contains good quality (grade I) oocyte and follicular fluid was centrifuged at 1500 r.p.m. for 15 minutes and the supernatants were frozen at -70 C for future analysis [11]. ELISA tests were used for measuring leptin and activin A as described elsewhere [12, 14]. Body mass index was evaluated on day of oocyte recovery. Standard ICSI procedure for all cases as per the IVF center protocol was carried out only to metaphase II (M II) oocytes with daily evaluation of fertilization and embryo grading. Embryos were graded on day 2 and 3 according to the number of blastomeres (<5 or >5), equality of size and degree of fragmentation, giving a score of 1 or 2 for each item. Embryos were scored 'A' for those achieving a score of 5-6 and 'B' for those scoring less.

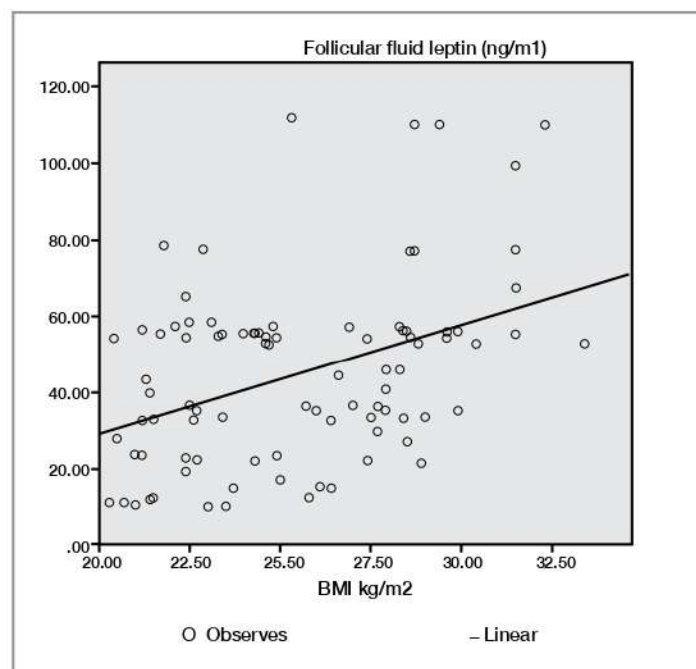
For statistical purposes, Day 2 embryo quality was considered good when the majority of embryos were grade A and poor when the majority was grade B. Two embryos (or 3 embryos in women over 40 years) were transferred in early (day 2 or 3) and late (day 5) ET respectively. ET was performed using labotect catheter (Germany) under ultrasound guidance. Luteal phase support was achieved by 400 mg vaginal progesterone. Pregnancy was confirmed by quantitative β -hCG after 2 weeks of oocyte retrieval followed by sonographic confirmation of cardiac activity 2 weeks later.

SPSS 19 package (SPSS, Chicago, IL, USA) was used for statistical analysis. Data was expressed as means \pm SD. Student t, chi square and Pearson correlation test were used when appropriate. Significant values were set at $p < 0.05$ level.

Results

A total of 90 women were counseled, consented, and enrolled into the study from October 2010 till April 2011. There were no patient drop-outs or cycle cancellation. The indications for treatment were: Male factor: 34 patients (37.7%), tubal factor: 14 patients (15.5%), endometriosis: 12 patients (13.3%), unexplained infertility: 18 patients (20%) and combined factors: 12 patients (13.3%). The whole study group age ranged between 23 to 41 years with a mean of 29.6 ± 5.1 years. All women were nulliparous. No difference in the total dose of gonadotropins was found between the pregnant and the non-pregnant groups.

For the whole group, mean follicular fluid (FF) leptin level was 45.2 ± 26.7 ng/ml and BMI was 25.3 ± 3.3 kg/m². FF leptin had a positive correlation with body mass index ($r=0.4$, $p < 0.005$) (Fig 1). Mean follicular fluid activin was 880.8 ± 954 pg/ml. No correlation between follicular fluid activin and oocyte number (Pearson correlation: 0.089 $p=0.4$) or follicular fluid leptin (Pearson correlation: 0.15 $p=0.15$).



Pregnancy rate for the whole group was 32.2% (29/90). Table 1 shows patient characteristics in pregnant and non-pregnant groups. No significant differences were shown regarding age, duration of infertility. Body mass index, follicular fluid leptin or activin as well as fertilization rate were not significantly different in the two groups. The only significant difference was in the pregnant group having significantly better quality embryos on day 2 ($p=0.02$).

Table (1): The demographic parameters of the patients before Table 1: patient characteristics according to pregnancy state

	Pregnant (N=29)	Not pregnant (N=61)	All patients (N=90)	*P value
Age (years)**	28.9 ± 4.6	30.3 ± 5.3	29.6 ± 5.1	0.61
Months of Infertility **	54.6 ± 23.6	57.8 ± 29.5	56.7 ± 27.6	0.64
BMI (kg/m ²)**	24.9 ± 3.6	25.7 ± 3.1	25.5 ± 3.3	0.25
FF leptin (ng/ml)**	43.5 ± 24.8	45.7 ± 23.1	45.2 ± 26.7	0.62
Activin A (pg/ml)**	782.2 ± 368.2	925.2 ± 338.3	880.8 ± 354.6	0.53
Day 2 good emb. quality	25 (86.2%)	33 (54.1%)	58 (64.4%)	0.02 (S)
Fertilization rate **	65.7 ± 12.7	65.8 ± 22.2	65.7 ± 19.7	0.90

** mean ± SD S: Significant at 0.05 level BMI = Body Mass Index; FF = Follicular Fluid

Looking at the embryo quality, follicular fluid leptin and activin were not significantly different in the two groups of 'good' or 'bad' embryo quality on day 2; (34.4 ± 21.5 vs. 37.2 ± 20.7 ng/ml for leptin and 825.2 ± 725.4 and 895.4 ± 912.3 for activin A, P = 0.5 and 0.62 respectively).

The data were classified according to follicular fluid leptin of ≤ and >60 ng/ml into low and high FF leptin respectively (table 2). There were no significant differences between the two subgroups as regards to patients' age or duration of infertility, number of oocytes retrieved, fertilization rate, good quality embryo rate and pregnancy rate. Also, mean FF activin was not significantly different between the two subgroups.

Similarly, the data were classified according to follicular fluid activin of ≤ and >1000 pg/ml into low and high FF activin respectively (table 2). There were no significant differences between the two subgroups as regards to patients' age or duration of infertility, number of oocytes retrieved, fertilization rate, good quality embryo rate and pregnancy rate. Also, mean FF Leptin was not significantly different between the two subgroups.

Table 2: Low and high leptin and activin A in the study group.

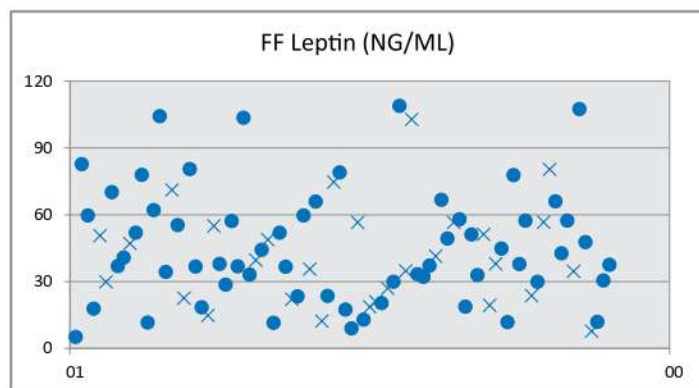
	Leptin (ng/ml)			Total Activin A (pg/ml)		
	Low (no = 74)	High (no = 16)	*P value	Low (no = 65)	High (no = 25)	*P value
Age (years)**	30.3 ± 5.7	29.6 ± 5.1	0.7	31.6 ± 5.7	28.9 ± 5.1	0.5
Months of Infertility **	50.2 ± 22.3	55.4 ± 24.3	0.4	52.2 ± 23.6	54.8 ± 21.3	0.4
No. of oocytes collected **	10.09 ± 5.5	11.88 ± 6.5	0.9	12.95 ± 5.3	11.8 ± 7.2	0.67
Fertilization rate **	65.6 ± 20.7	62.9 ± 23.5	0.6	64.6 ± 21.7	65.7 ± 16	0.83
Day 2 good emb. quality	50 (67.5%)	8 (50%)	0.1	41 (63%)	17 (68%)	0.63
Pregnancy	25 (33.8%)	4 (25%)	0.1	23 (35.3%)	6 (24%)	0.09
FF level **	FF Activin 904.3 ± 442	FF Activin 796 ± 491	0.2	FF Leptin 36.9 ± 28.5	FF Leptin 27.3 ± 14.1	0.1

* Significant at 0.05 level

** mean ± SD

Figures 2 and 3 show the distribution of pregnancy among patients with different levels of Leptin and Activin A respectively. The occurrence of pregnancy did not correlate to the level of the hormones.

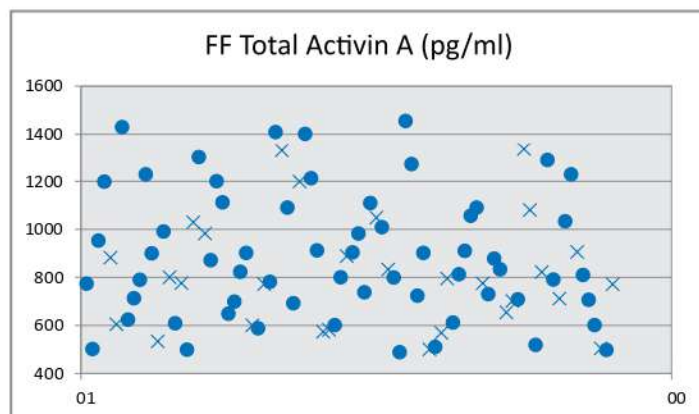
Figure 2: Low and high follicular fluid Leptin and pregnancy



Leptin level of ≤ 60 ng/ml is considered low

• = Non pregnant X = pregnant

Figure 3: Low and high follicular fluid Activin A and pregnancy



Activin A level of ≤ 1000 pg/ml is considered low

• = Non pregnant X = pregnant

Discussion

Leptin and activin are two hormones that do have some role in folliculogenesis and the local regulations of follicular development and therefore were studied to try to prove or not any association between them and the ART parameters and outcome. Ninety patients were enrolled, of them 29 (32.2%) achieved pregnancy. Patients' characteristics were similar in the pregnant and non-pregnant groups as regards to age, duration of infertility and body mass index. The only significant difference was in the pregnant group having significantly better quality embryos on day 2 which was in accordance with other studies [15, 16]. We defined good embryo quality at day 2 as those patients having 50% or more of their embryos of grade A.

Consistent with other studies [17, 18], follicular fluid leptin was positively correlated with body mass index. Obesity was defined by WHO as having a BMI of more than 30 kg/m² and obese

women are almost three times more likely than non-obese women to have some degree of infertility [19]. This could be either related to obesity itself or due to associated polycystic ovarian syndrome that is associated with obesity in more than 50% of its population [20]. For that reason women with PCOS were excluded from the study.

Follicular fluid leptin was not found to be correlated with pregnancy or indeed any other ART outcome measures. This result was observed in other studies [21, 21] although other investigators reported an association between FF leptin and IVF/ICSI outcome [10, 11]. Leptin can affect follicular development through a central (hypothalamic-pituitary) and end organ (ovary and endometrium) effects [22].

When the group was further divided to those with high and low FF leptin (\leq and >60 ng/ml) – this cut of point was based on the observation by Anifandis et al, 2005 [10] that below this level poor embryo quality and IVF failure are expected – there was no significant differences between the two subgroups as regards to the outcome measures namely fertilization, pregnancy or day 2 good embryo quality rates. This was consistent with other reports [12, 13].

Lau et al (1999) found that level of Activin A was higher in follicles containing good quality oocytes, however this increase did not reach significant difference and failed to show an association with the fertilizing ability of the oocytes [8]. We tested this hypothesis by measuring the level of Activin A in large follicles containing good quality oocytes. There was no significant difference in Activin A levels in pregnant and non-pregnant groups.

The group was further classified into low and high Activin A level subgroups for those having FF Activin A \leq and > 1000 pg/ml respectively – the choice of the cut of point 1000 pg/ml was arbitrary -, again, there were no significant differences in any of the outcome measures namely fertilization, pregnancy or day 2 good embryo quality rate. This could be explained by the fact that available Activin A is tightly bound to follistatin, a cysteine rich glycoprotein locally secreted by theca cells that was found to have a high binding affinity to Activin A [7] and was suggested to neutralize its biological activity in distant target tissues [23]. Our results suggest that this binding affinity is also functioning in local follicular fluid medium.

A hypothesis that follicular fluid Activin A affects follicular fluid Leptin synthesis was tested. We failed to find a correlation between FF Activin A and Leptin levels. Whether there was actual no relation or an effect is neutralized by follistatin binding requires further validation by testing the effect of purified FF Activin A on FF Leptin.

In conclusion, the local hormonal milieu in the ovary can only be examined by measurement of follicular fluid hormones. This can give us an idea about the hormonal influence on the developing follicles subjected to the controlled ovarian stimulation. In our study, there was no correlation between FF Activin A, Leptin and the IVF outcome measures as well as no correlation between FF Activin A and Leptin.

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