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ORIGINAL ARTICLE

Comparison between steroid expression in serum and follicular fluid in polycystic ovary patients and unexplained infertility patients undergoing assisted reproductive techniques

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KEYWORDS

Serum steroids;
Follicular fluid steroids;
PCO

Abstract *Objective:* Comparison of the steroid content in the pre-ovulatory serum and follicular fluid between patients with unexplained infertility and PCOS patients undergoing ICSI and its relation to the outcome.

Methodology: The study was a prospective randomized control study conducted on ninety infertile female patients from the Agial IVF fertility center, divided equally into 45 patients with unexplained infertility and 45 PCOS patients undergoing ICSI. Outcome measures included the relation between steroid content in the pre ovulatory serum and follicular fluid and the number of oocytes retrieved, maturity of oocytes, grading of embryos, coasting, cancelation rate, number of embryos transferred and pregnancy rates.

Results: It was noted from the current study that the PCO group had higher serum estradiol levels (7495.33 ± 3726.982), higher coasting rate (40%), more oocytes retrieved (23.84 ± 10.68), more M2 oocytes (19.56 ± 11.42), and more grade A embryos, 4A (14.89 ± 8.873) but no difference in pregnancy outcome in comparison with the unexplained group. As regards the correlations done in the unexplained group it was noted that there was no significant correlation between serum

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progesterone, follicular fluid steroids and all parameters including pregnancy outcome. As regards the correlations done in the PCOS group, there was a positive correlation between serum estradiol ($P = .001$), serum progesterone ($P = .002$) and class 4A embryos. There was no significant correlation between follicular fluid steroids and all other parameters including pregnancy outcome.

Conclusion: From the current study the pre ovulatory serum steroids as well as the follicular fluid steroids could not be used as an accurate marker for predicting the outcome of ICSI in both groups.

1. Introduction

The main goal of ICSI is to obtain developmentally competent embryos so as to achieve the highest chances of live birth. However, the identification of the best embryos is not a simple task (1). Follicular fluid constitutes an important microenvironment, which influences oocyte growth (2). The endocrine microenvironment of individual antral follicles varies in relation to follicular developmental stage and health status (growing or atretic) of the follicle, and to exposure to varying concentrations of circulating hormones (3).

Several studies have analyzed pre-ovulatory follicular fluid aspirates from women undergoing assisted reproduction in an attempt to identify a relationship between the composition of hormones in the follicular fluid and the quality of the oocyte, its potential to be fertilized and to develop into a viable embryo and outcome after embryo transfer (4–7). The results of these studies, however, are not consistent, their discrepancies may relate to the diversity of protocols used for ovarian stimulation in different studies, since circulating hormone concentrations during stimulation, and presumably the composition of hormones in pre-ovulatory follicular fluid, vary significantly in relation to the type of stimulation protocol (8).

The role of steroids and gonadotrophins during ovarian stimulation and especially whether or not Lutenizing Hormone (LH) activity should be added to the stimulation protocol to improve the reproductive outcome of assisted reproduction, has been debated and this focused on the importance of changes in circulating concentrations of LH and estradiol induced by different stimulation protocols, whereas effects regarding the composition of hormones in follicular fluid have not been studied in detail yet (9). The successful conversion to estrogen-determined follicle marks the selection of a follicle destined to ovulate (10). If the follicle fails to achieve an estrogen microenvironment, decreasing Follicular Stimulating Hormone (FSH) support would interrupt granulosa cells proliferation and function, promote conversion to an androgenic microenvironment, and thereby induce irreversible atretic changes (11).

The loss of follicles through atresia is a response to changes in many factors. Certainly gonadotrophin stimulation and withdrawal are important, but ovarian steroids are also involved (12).

Polycystic ovary syndrome (PCO) is a major cause of anovulatory infertility (13), but factors other than failure to ovulate may also contribute to reproductive impairment. The classic endocrine abnormalities of PCO are hypersecretion of LH with normal concentration of FSH and increased secretion of androgens (principally of ovarian origin) (14). Granulosa cell function is abnormal, particularly in anovulatory women with PCO. Follicles are more heterogeneous than those from normal ovaries and include a significant hypersecretion of both estradiol and progesterone (15), these follicles appear to be prematurely responsive to LH. Hyperinsulinemia may contribute to the abnormal response of these follicles to LH (16).

Some evidence suggests that obesity is an important pathogenic factor; it is thought to be involved in the development of hyperinsulinemia and contributes to hyperandrogenism and anovulation (17,18).

Hypersecretion of LH, another common endocrine abnormality in PCO, also interferes with folliculogenesis, resulting in anovulation and poor quality oocytes (19,20).

In this study, we will attempt to compare the differential expression of steroids (estradiol, progesterone, and androstenedione) in pre-ovulatory follicular fluid and (estradiol, progesterone) in the serum of both unexplained and PCO patients undergoing ICSI and their relation to the outcome.

2. Materials and methods

2.1. Patients

The study was conducted on 90 female patients recruited from Agial IVF/ICSI center.

2.1.1. Inclusion criteria

Age group between 20–40 years, patients with unexplained infertility and patients suffering from polycystic ovary syndrome.

2.1.2. Exclusion criteria

Hypogonadotrophic patients (class I ovulatory dysfunctions), hypergonadotrophic patients (class III ovulatory dysfunction), uterine factor infertility and endometriosis.

The follicular fluid will be collected on the day of oocyte retrieval and the serum will be collected on the day of HCG administration from all the patients. The patients will be subdivided into two groups:

Group A: 45 patients with unexplained infertility.
Group B: 45 patients with polycystic ovary syndrome.

2.2. Methods

After approval of the medical ethics committee and signing a written informed consent all patients will be subjected to: full history taking, complete general examination, complete gynecologic examination and infertility workup including: Husband's semen analysis, hystrosalpingography and transvaginal ultrasound:

1. Detection of antral follicles number and size in the early follicular phase (day 3).

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2. Size, direction of the uterus as well as the thickness and pattern of the endometrium
3. Serial folliculometry.
4. Hormonal profile, early follicular phase (day 3) serum E2, FSH.

All patients will undergo the same ovarian stimulation protocol (the long luteal phase protocol).

2.2.1. The ovarian stimulation

The long down-regulation protocol (Suprefact®, Hoechst) will be used in all patients as a daily subcutaneous dose of 0.5 mg started on cycle day 21. Once the serum oestradiol concentration is suppressed to $\leqslant$50 pg/ml, the dose will be reduced to 0.2 mg and continued until the day of hCG administration. Ovarian stimulation with recombinant FSH (Gonal F®, Serono) 150 IU as well as urinary human menopausal gonadotrophin (u-HMG) (Merional®, IBSA) 75 IU will begin following pituitary down-regulation. The standard initial dose will be 225 IU.

The ovarian response will be monitored by serial serum estradiol concentrations and trans-vaginal ultrasound beginning on day 5 of stimulation until the day of hCG administration. Based on these results, the FSH/HMG dose and subsequent monitoring will be individualized. Ovarian stimulation will be continued until at least three follicles reached a mean diameter of $\geqslant$20 mm, at which time hCG (Choriomon®, IBSA) 10,000 IU s.c. or i.m. will be administered 36 h before oocyte recovery. Following oocyte retrieval, the patients will receive luteal phase support in the form of natural progesterone vaginally in a dose of 400 mg/day (Prontogest Supp®, Marcyrl) to continue preparing the endometrium. After oocyte retrieval, the cumulus and corona radiate will be removed mechanically under a stereomicroscope, after exposure to 80 IU/ml hyalouronidase solution for 30 s. ICSI will be done to mature oocytes, this will be followed by transfer of embryos in the appropriate time.

2.2.2. Assessment of fertilization and cleavage

Oocytes will be examined for fertilization 16–18 h after ICSI and cleavage of the oocytes will be assessed on day 2 (48 h) and day 3 (72 h) before transfer into the uterus.

B-hCG will be measured for diagnosis of pregnancy 14 days after embryo transfer and then will be measured serially to monitor the rise in its titer. Implantation will be noted later by appearance of the gestational sac in the uterus using transvaginal ultrasonography (TVS).

2.2.3. Blood sampling

In all patients blood will be sampled on the day of hCG administration. The isolated sera will be frozen and stored at −20 °C for later hormonal analysis.

2.2.4. Follicular fluid sampling

Oocyte retrieval will be taken under vaginal ultrasound guidance. Only aspirates from follicles larger than 17 mm, uncontaminated with blood will be included in this study. Follicular fluid sample will be centrifuged for 10 min, and the supernatant will be stored at −20 °C until hormone measurements will be carried out.

Assay of estradiol, progesterone and androstenedione in the follicular fluid as well as estradiol and progesterone in the serum:

All samples will be analyzed using commercially available kits.

The primary outcome measures will be

a. The number of mature oocytes per cycle of induction,
b. the grading of embryos obtained,
c. implantation rate (number of sacs per number of embryo transfer),
d. serum estradiol and progesterone levels on day of hCG administration and
e. follicular fluid level of estradiol, progesterone and androstenedione on the day of oocyte retrieval.

The secondary outcome measures will be
Pregnancy rate which will be diagnosed by:

1. Serum B-hCG assay 14 days after embryo transfer.
2. Clinical pregnancy will be confirmed by observing fetal cardiac pulsations 2 weeks after positive pregnancy test by TVS.

In these cases, the administration of progesterone will be continued up to week 12 of gestation.

3. Data analysis

Statistical analysis was done using SPSS program "version 17" (Statistical Package of social sciences, Chicago, USA). Quantitative data were expressed using range, mean and standard deviation while Qualitative data were expressed in frequency and percent. Quantitative data were analyzed using student's *t*-test to compare between two categories while *F*-test (ANOVA) was used to compare more than two categories, Least significant difference (LSD) is basically a *t*-test, used only when the *F* value is significant to detect the presence of significance between each of the two groups. Pearson correlation coefficient was used to analyze correlation between different parameters. Significance was considered at $p \leqslant 0.05$.

4. Results

The two groups were compared with respect to all baseline characteristics. Data in (Table 1) revealed that, there was a statistical significance difference between two groups regarding the basal estradiol level with a mean of 49.48 ± 7.48 and 84.98 ± 8.85 (P = .0001) in unexplained and PCOS groups, respectively. There was a significant difference between the two groups regarding serum estradiol level on day of hCG with a mean 4990.07 ± 2775.534 and 7495.33 ± 3726.982 (P = .001) in unexplained and PCOS groups respectively showing a higher level of serum estradiol in the PCOS group while serum progesterone was showing no statistical difference in both groups. The follicular fluid estradiol, progesterone and androstenedione levels on the day of retrieval were comparable between the two groups showing no statistical significant difference. Regarding the number of oocytes retrieved, the two groups showed statistical significant difference (P value = .0001) with a mean of 13.58 ± 7.5 in the unexplained group and 23.84 ± 10.68 in the PCOS group with a higher number of oocytes in the PCOS group. There was also a statistical significant difference between the two groups as regards M2 oocytes where it was higher in the PCOS group with a

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Table 1 Differences between the two groups.

	Unexplained	PCO	*t*	*P*
Basal estrogen	49.48 ± 7.48	84.98 ± 8.85	20.547	.0001
Serum estradiol	4990.07 ± 2775.534	7495.33 ± 3726.982	3.617	.001
Serum progesterone	1.25 ± .914	1.17 ± 0.618	.494	.622
Follicular fluid estradiol	101927.96 ± 29988.923	93489.47 ± 29682.645	1.342	.183
Follicular fluid progesterone	6917.08 ± 3225.230	6196.36 ± 5155.919	.795	.429
Follicular fluid androstenedione	4.48 ± 2.053	4.69 ± 2.124	.49	.62
Oocyte number	13.58 ± 7.5	23.84 ± 10.68	5.259	.0001
M2	10.6 ± 6.95	19.56 ± 11.42	4.491	.0001
M1	1.09 ± 2.81	1.07 ± 2.359	.041	.968
Germinal vesicle	.62 ± 1.051	.53 ± 1.984	.266	.791
ClassA embryo	6.82 ± 4.792	14.89 ± 8.873	5.366	.0001
Class A	1.56 ± 1.925	3.16 ± 3.723	2.561	.013
Class B	.42 ± .812	.18 ± .442	1.775	.080
Embryo transfer	4.11 ± 2.003	4.80 ± 1.198	1.98	.52

mean of 19.56 ± 11.42 when compared with that of the second group (10.6 ± 6.95), *P* value = .0001. There were no statistical significant difference between the two groups as regards M1, germinal vesicles and fractured oocytes. There was a statistical significant difference between the two groups in favor of the PCOS patients as regards embryo quality grade A and 4A, with a mean of 6.82 ± 4.792, 1.56 ± 1.925 (*P* value = .0001) and 14.89 ± 8.873, 3.16 ± 3.723 (*P* value = .013) in order in unexplained and PCOS groups respectively. There was no statistically significant difference between the two groups regarding the number of embryos transferred.

The coasting rate was compared in both groups and showed a statistical significant difference showing a higher coasting rate for the PCOS group (8.88% of cases in unexplained group & 40% of cases in PCOS group), *P* = .001. There was no statistical significant difference in cancelation rate regarding both groups. There was no statistical significant difference between both groups as regards the outcome of pregnancy. Out of 45 cases in the unexplained group 26 (57.77%) cases were pregnant and 19 cases (42.22%) were not pregnant, however in the PCOS group out of 45 cases 3 cases (6.66%) were chemical pregnancy cases, 19 cases (28.88%) were not pregnant and 29 cases (64.44%) were pregnant (Table 2).

In the unexplained group there was a positive correlation between serum estradiol and the number of oocytes retrieved (*P* = .0001), number of M2 oocytes (*P* = .0001), and grade A embryos (*P* = .001). There was no significant correlation between serum progesterone, follicular fluid estradiol, progesterone and androstenedione to the number, oocytes maturity, embryo grading & number of transferred embryos. There was also no correlation between serum & follicular fluid hormones to the outcome of pregnancy (Tables 3 and 4).

In the PCOS group there was a positive correlation between serum estradiol (*P* = .001), serum progesterone and class 4A embryos (*P* = .002). There was no correlation between follicular fluid estradiol, progesterone and androstenedione to the number, maturity of oocytes, embryo grading and number of embryos transferred. There was also no correlation between serum and follicular fluid hormones to the oocyte number, oocyte maturity, embryo grading, number of embryos transferred and the outcome of pregnancy in the PCOS group (Tables 5 and 6).

5. Discussion

In the current study we used the differential expression of steroids in the serum and the follicular fluid of the patients undergoing ICSI as a predictor for the number, quality, maturity and the outcome of ICSI in both the unexplained as well as the PCO patients.

Volpe et al. (21) compared the follicular fluid content of estrogen and androstenedione in a group of patients with polycystic ovary disease (PCO) and normally-ovulating infertile women in an IVF/ET program. PCO patients showed significantly higher follicular fluid androstenedione and similar follicular fluid estradiol levels compared to controls. These data indicated a normal intrinsic potential aromatase activity in ovaries from PCO patients stimulated with gonadotropins and suggested that PCOs do not derive from inherent ovarian aromatase deficiency, increased follicular fluid androstenedione content following gonadotrophin stimulation may result from theca cell hyperactivity and androgen accumulation in the follicular antrum of rescued hyperandrogenic follicles as well as from inhibitory factors that may inhibit aromatase

Table 2 Differences between the two groups in coasting, cancelation rates and pregnancy outcome.

	Unexplained		PCO		X^2	*r* or *p*
	Number	Percentage (%)	Number	Percentage (%)		
Coasting	4/45	8.88	18/45	40	11.791	*r* = .001
Cancelation	1/45	2.22	1/45	2.22	.001	*r* = 1.000
Chemical pregnancy	0/45	0	3/45	6.66	4.28	*p* = .117
Not pregnant	19/45	42.22	13/45	28.88		
Pregnant	26/45	57.77	29/45	64.44		

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Table 3 Correlation between serum & follicular fluid hormones to the ICSI outcome in the unexplained group.

	Serum estradiol	Serum progesterone	Follicular fluid estradiol	Follicular fluid progesterone	Follicular fluid androstenedione
Oocyte	R .568	.041	.05	.048	.144
Number	P.0001	.789	.743	.755	.344
M2	.597	.115	.178	.096	.147
	.0001	.458	.249	.536	.342
M1	.057	.190	.273	.025	.194
	.714	.216	.073	.874	.206
Germinal	.006	.089	.083	.041	.129
vesicle	.968	.567	.591	.794	.403
Class A	.543	.091	.143	.065	.128
	.001	.555	.354	.673	.408
Class A4	.128	.098	.017	.173	.003
	.408	.528	.913	.260	.986
Class B	.251	.039	.138	.213	.010
	.1	.801	.372	.165	.947
Embryo	.435	.012	.037	.190	.162
transfer	.003	.939	.812	.217	.295

Table 4 Outcome of pregnancy in the unexplained group in relation to serum & follicular fluid hormones.

	No.	Pregnant	No.	Non pregnant	*t*	*p*
Serum estradiol	26	5447.384 ± 2724.226	19	4306.89 ± 2796.407	1.372	.180
Serum progesterone		1.1427 ± .4946		1.3953 ± 1.29009	.914	.426
FF. estradiol		99810.8054 ± 104825.431		33155.9857 ± 25616.4418	.550	.570
FF. progesterone		6571.1373 ± 7390.475		3059.82261 ± 3466.19521	.839	.416
FF. androstenedione		4.5538 ± 2.13733		4.3684 ± 1.98440	.296	.766

Table 5 Correlation between serum & follicular fluid hormones to the ICSI outcome in the PCOS group.

	Serum estradiol	Serum progesterone	Follicular fluid estradiol	Follicular fluid progesterone	Follicular fluid androstenedione
Oocyte	R.129	.160	.260	.032	.105
Number	P.149	.294	.085	.835	.493
M2	.112	.133	.161	.024	.110
	.466	.383	.291	.875	.473
M1	.059	.043	.136	.058	.020
	.702	.778	.374	.707	.896
Germinal	.107	.127	.150	.030	.095
vesicle	.483	.405	.324	.843	.534
Class A	.175	.124	.106	.042	.185
	.251	.417	.490	.785	.225
Class A4	.460	.445	.154	.021	.212
	.001	.002	.313	.891	.162
Class B	.005	.009	.069	.194	.062
	.974	.953	.652	.202	.688
Embryo	.004	.171	.050	.009	.117
transfer	.977	.262	.744	.954	.444

activation, partially counteracting the effect of gonadotrophins. These results disagree with the current study in regard to follicular fluid androstenedione as there was no significance difference between both the groups. This may be referred to the use of different stimulation protocols, however it agrees with our results in regard to follicular fluid estradiol levels.

Teissier et al. (22) studied the relationship between steroid concentrations and the follicle size, oocyte quality and fecundability from normal and polycystic ovaries in women undergoing IVF. Fifty-nine patients (31 normal, 28 PCOS) underwent conventional IVF with r.FSH induction. Follicular diameter was classified as small (8–13 mm) or large (> 14 mm) and sex steroid content was analyzed for each group. Oocyte maturity was studied according to nuclear maturation on the day after fertilization. In both ovulation groups, estradiol and progesterone concentrations were significantly higher in large follicles with meiotically competent oocytes compared with those containing meiotically incompetent oocytes, with no difference in between corresponding sub-groups of follicles with meiotically competent oocytes. This study agrees with our study in relation to estradiol level and the number of oocytes ($P = .0001$), maturity of oocytes M2 ($P = .0001$) but progesterone did not show a

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Table 6 Outcome in the PCO group in relation to serum and follicular fluid hormones.

	Pregnant $x \pm$ SD	Non pregnant $x \pm$ SD	Chemical pregnancy $x \pm$ SD	*F*	*P*	LSD
Serum estrogen	5.1379 ± 2.43082	5.4615 ± 2.63361	5.333 ± 4.0414	.072	.931	P **I** – p **II** = .710 P **I** – p **III** = .902 P **II** – p **III** = .939
Serum progesterone	1.1524 ± .51118	1.2992 ± .84511	.7533 ± .28501	.977	.385	P **I** – p **II** = .481 P **I** – p **III** = .293 P **II** – p **III** = .175
FF. estradiol	96943.3945 ± 33174.88703	90598.6708 ± 19216.50554	72628.4033 ± 29657.46727	.999	.377	P **I** – p **II** = .525 P **I** – p **III** = .184 P **II** – p **III** = .350
FF. progesterone	6167.9897 ± 6032.47989	5660.8985 ± 3038.64253	8790.9700 ± 2827.03524	.439	.648	P **I** – p **II** = .773 P **I** – p **III** = .412 P **II** – p **III** = .355
FF. androstenedione	4.8138 ± 2.01808	4.8462 ± 2.32473	2.8333 ± 2.05508	1.245	.298	P **I** – p **II** = .964 P **I** – p **III** = .130 P **II** – p **III** = .144

P **I** = clinical pregnancy. P **II** = non pregnant. P **III** = chemical pregnancy.

relation to the maturity of oocytes in our study in reverse of Teissier et al., this may be due to the different number of patients between the two studies and the use of different stimulation protocol, as in Teissier's study the patients underwent the conventional IVF with r.FSH.

Another study was done by Xia and Younglai (23) to investigate the relationship between oocyte morphology and follicular fluid steroid concentrations in patients being treated with intracytoplasmic sperm injection. A total of 82 IVF cycles were evaluated in patients aged (24–40) years and were treated with long luteal protocol. Oocytes at metaphase II were graded into four groups according to the status of the first polar body and the size of the perivitelline space. The proportion of oocytes at the germinal vesicle and germinal vesicle breakdown stages, and the proportion of degenerated oocytes and oocytes with a large polar body were compared with different concentrations of estradiol and progesterone in the follicular fluid. The results showed that the developmental potential of human oocytes, as assessed by the status of the first polar body and the size of the perivitelline space, is not associated with the absolute concentrations of estradiol and progesterone in the follicular fluid, and this agrees with the results of our study.

A prospective randomized study done by Sadeghipour et al. (24) compared the steroid contents of follicular fluid of PCOS to that of normogonadotrophic patients. The results showed no statistical significance in the level of follicular fluid estradiol between the two groups with a significance ($p = 0.56$) and this agrees with results of our study.

In a prospective randomized study conducted by Westergard (7), the concentration of gonadotrophins and steroids in pre-ovulatory follicular fluid and serum were related to the type of stimulation protocol as well as the outcome of assisted reproduction in 280 women subjected to the long protocol gonadotrophin-releasing hormone agonist pituitary down-regulation and ovarian stimulation with either HMG or recombinant FSH. The results revealed that the serum concentration of estradiol, progesterone and androstenedione were similar in conception and non-conception cycles, also follicular fluid progesterone and androstenedione were similar in conception and non-conception cycles while the estradiol level in the follicular fluid was higher but only with borderline significance ($p = 0.056$). This finding indicated that it is possible to establish a direct relation between concentrations of hormones in the follicular fluid from follicles that correspond to the transferred embryos in women who achieved pregnancy or failed to do so. These results agree with the results of our study.

Asimakopoulos et al. (25) studied the levels of steroid hormones and cytokines in individual follicles and studied their association with the fertilization outcome after intracytoplasmic sperm injection in a prospective study that included 43 women, they received ovarian stimulation with multi dose GnRH antagonist protocol, ICSI was performed then the concentrations of estradiol, progesterone were measured by immunoassay methods in the follicles from which the mature oocytes were derived. The results showed that the intrafollicular concentrations of the above factors were not significantly associated with the fertilization outcome and were not correlated with embryo quality, leading to the conclusion that the intrafollicular concentrations of the steroids cannot predict the fertilization outcome after ICSI and this agrees with the results of our study.

Alberto Revelli and Luisa Delle (26) studyied the follicular fluid contents and their effect on the oocyte quality. This study revealed that intrafollicular estrogenic environment is associated with good follicular growth and has anti-atresia effects. In addition, estradiol enhances the cytoplasmic maturation of oocytes via a direct non-genomic action at the plasma membrane level, elevated estradiol in follicular fluid indicates a more advanced stage of oocyte maturation and has been repeatedly found to be associated with a higher chance of achieving pregnancy.

There is also conflicting evidence regarding the meaning of progesterone levels in follicular fluid. Several authors found that a high follicular fluid progesterone concentration was predictive of oocyte maturation, subsequent implantation and pregnancy. On the other side, oocytes from follicles having high follicular fluid progesterone were frequently found in association with post mature oocytes that fertilized abnormally and gave rise to multi pronuclear embryos. It seems that while an optimal exposure to progesterone has positive effects over oocyte characteristics, an excessive exposure leads to a rapid worsening of the cell quality; a clear knowledge of the thresh-

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old at which progesterone begins to damage the oocyte is presently lacking. In the current study there was no significant relation between follicular fluid estradiol, progesterone and the oocyte maturation or the pregnancy outcome.

6. Conclusion

It was concluded from the current study that the use of serum steroids as a marker to predict oocytes number, maturity and to detect embryo grading may be of good help, however, serum and follicular fluid steroids do not have a direct correlation with ICSI outcome.

7. Conflict of interest

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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