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Evaluation of Serum and Follicular Fluid Levels of Growth Hormone, IGFBP-3 and Estradiol in Assessing Oocytes Maturation and Embryo Development

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Abstract

Objective: The aim of this prospective, selective clinical trial was to measure the concentrations of E2, GH and IGFBP-3 in serum and follicular fluids collected from infertile women undergoing Control ovarian hyper stimulation (COH) using two different preparations long agonist and antagonist protocol and their effects on oocytes maturation and embryo development.

Method: A total of 40 infertile women undergoing COH were included in this study, 20 with long agonist protocol and the other 20 with antagonist protocol. Serum and FF samples were obtained from patients at the day of oocytes retrieval.

Result: The FF concentration of GH was significantly higher in patients treated with antagonist than in those treated with agonist while no significant differences were found in serum level of GH as well as FF levels of E2 and IGFBP-3. Regarding the oocytes and embryo development no significant difference observed between antagonist and agonist protocols. Both serum and follicular concentration of E2 had positive effects on the total number of retrieved oocytes, mature oocytes, immature oocytes, and embryo quality. Follicular GH level affects the total number of retrieved oocytes, MII, and MI oocytes numbers. Follicular level of IGFBP-3 had significant effects on the MI oocytes number.

Conclusion: Serum and follicular levels of both E2 and GH plays an essential role in IVF outcome and subsequently embryo development while IGFBP-3 has a little effect on IVF parameters.

Keywords: Growth Hormone; IGFBP-3; Estradiol; Oocytes Maturation; Embryo Development

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Introduction

ICSI is widely accepted as effective treatments for most causes of infertility [1]. Women undergoing COH prior to ICSI are treated by various protocols aimed at inducing multiple follicular growths which include agonists and antagonists protocols [2]. Nevertheless, several growth factors acting locally are involved in the process of follicle selection and maturation and embryo development [3]. E2 and GH are important regulators that participate in gonadal gametogenesis in several species including humans. The GH act either directly by its receptors on the ovarian tissues or indirectly by stimulating the production of insulin growth factor-I (IGF-I) and IGFBP-3, however, the role of IGFBP-3 in follicular fluid has not been clearly demonstrated [4].

Growth Hormone

GH is a multifunctional hormone with effects ranging far beyond those on linear growth. It is involved in the regulation of male and female infertility and has been used in the management of both male and female infertility [5]. It is produced also locally by the ovary not only by the pituitary but in more continuously manner and at lower levels [5,6]. It binds to GH receptors on the theca, granulose, and luteal cells, hence promoting gametogenesis and steroid genesis [7,8]. GH effects also vary throughout the ovarian cycle modulating Estradiol and progesterone release from human luteinized granulose cells [9].

Insulin like Growth Factor Binding Protein-3 (IGFBP-3)

It is a multifunctional protein, it is one of six different insulin like growth factor binding proteins, named IGFBP-1 to IGFBP-6 [10]. It is the predominant IGFBP and functions as the main IGF carrier protein in the blood which binds about 95% of the IGF-I in the circulation [11]. IGFBP-3 is synthesized mainly in the liver and regulated primarily by GH [12,13]. IGFBP-3 is expressed in various tissues in the body including ovaries and has also been shown to inhibit or

potentiate the actions of IGF in many peripheral tissues by restriction the bioavailability of free IGF and functions as a reservoir of IGF in plasma and regulate the transport of IGF between intra- and extra vascular spaces [14]. Furthermore, IGFBP-3 has been reported to have IGF-independent actions [10], it promotes the apoptosis in cellular and animal models by both IGF-1 independent and dependent pathways, and has growth-inhibitory effects in many systems [14,15].

Estradiol (E2)

It is the most active compound of estrogens [16]. Approximately all of the E2 comes from the ovary, it secreted mainly by the granulose cells of the developing ovarian follicles, the corpus lustrum, and the placenta [17]. The biosynthesis of E2 in granulose cells is depends on the aromatize enzyme activity that converts testosterone to E2, and it secreted into the FF [18].

Materials and Methods

This Prospective clinical trial involved patient who were undergoing COH during period of study between August 2016 and February 2017 in outpatient department of high institution for infertility diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, Baghdad-Iraq were recruited in this study. The present study involved 40 Iraqi infertile women aged (18-39 years) who were undergoing COH. Patients were classified according to the type of protocol, 20 patient with long agonist and 20 with antagonist protocols. The study was approved by the Local Medical Ethical Committee of high institution for infertility diagnosis and Assisted Reproductive Technologies Al-Nahrain University.

Basic Steps in the ICSI

Control Ovarian Hyper Stimulation (COH)

Two type of COH protocols involved in this study: The long GnRH agonist protocol which included down-regulation of pituitary with SC injection of GnRH-agonist (0.1 mg/d) that was begun in day 21 of the preceding menstrual cycle, then gonadotropin administered SC on the second or third day of menstruation. GnRH antagonist protocol included administration of Gonadotropin on the second or third day of menstruation then down-regulation of pituitary with SC injection of GnRH antagonist when follicular size reached 14 mm in diameter. The Gonadotropin was given in different dosages according to the patient's age and previous response of ovulation induction. In both protocol when at least three follicles had reached 18mm in diameters, HCG injection (10,000 IU) was used for triggering [19]. Oocytes pickup (OPU) usually performed after 34-36 hours of hCG injection [20].

Oocytes Preparation and Maturity Assessment

In the ICSI laboratory, the retrieved oocytes were immediately classified according to their maturity by the embryologist. After denudation of oocytes a rapid morphological evaluation is performed, including evaluation for any abnormality, and grading according to nuclear maturity as MII, MI, or GV. Oocytes nuclear maturity, as assessed by light microscopy, is assumed to be at the MII stage when the PBI is visible in the PVS, whereas the presence of an intracytoplasmic nucleus called the 'germinal vesicle' (GV). The oocytes that have neither a visible GV nor PBI these oocytes are generally classified as MI oocytes.

ICSI and embryo culture

ICSI was performed 3-6 h after oocytes recovery. Only oocytes at MII stage that did not show obvious signs of degeneration were used for ICSI. The injected oocytes were cultured at 37°C in IVF medium. The fertilization was assessed 16-20 h after ICSI. The normally fertilized oocytes were considered only for final embryo transfer. These were cultured for an additional 24-30 h in fresh CO2-equilibrated IVF medium.

Embryo Grading

Embryos were graded morphologically on days 2 and 3 (48 and 72 hrs. after oocytes pickup) as follows:

Grade 1, symmetrical with equal- sized blastomeric and<10% extracellular fragmentation.

Grade 2, with unequal blastomeric and<10% extracellular fragmentation.

Grade 3, unequal blastomeric with 10%-50% extracellular fragmentation.

Grade4, >50% blastomeric fragmentation with uneven blastomeric [21].

Samples Collection

At the day of oocytes retrieval 5 ml of blood was taken from each patient and put it in dry plain tube, then allowed to coagulate for 30 min at room temperature then centrifuged at 3000 rpm for 10 min, the supernatant serum aspirated and put in small tubes to be stored at -20°C for subsequent analysis for measurement of GH, IGFBP-3 and E2. Follicular fluid was collected after oocytes pickup, put in plain tube then centrifuged at 3000 rpm for 10 min; the supernatants were taken to other clean tubes and stored at -20°C for subsequent assay for GH, IGFBP-3 and E2. Samples with massive blood contamination were excluded from further analysis.

Hormonal Assay

Serum and FF concentrations of IGFBP-3 was determined by using commercial enzyme immunoassay kits (Human IGFBP-3 ELISA KIT) for serum and other biological fluid in the body, purchased from kono Biotech Co., Ltd and the ELISA kit number is KN0975Hu which measured by ELISA reader and washer (BioTek ELx80, US). Serum and follicular fluid concentrations of E2 and GH were measured by using fully automated diagnostic Chemiluminescence Immunoassay (CLIA) equipment (snibe Maglumi 800, UK).

Results

Agonist and antagonist group comparison

Table 1 exemplify that no significant difference was declared between agonist and antagonist group in the age, BMI, and laboratory IVF parameters including the total number of retrieved oocytes, MI, MII, GV, abnormal oocytes, fertilization rate and grade I embryo transferred. There were significant different in follicular level of GH between agonist and antagonist group (P=0.017) while no significant difference in the serum GH and all other serum and follicular level of E2 and IGFBP-3.

Correlation of E2, GH, and IGFBP-3 with the Laboratory IVF Parameters

As seen in the Table 2, there were positive significant association of both serum and follicular levels of E2 with the total number of oocytes and the number of mature oocytes (p<0.001), the number of grade

Table 1: Comparison of demographic, laboratory IVF parameters and hormonal concentrations between Agonist and Antagonist by unpaired t test.

Parameter	Agonist N=20 Mean±SD	Antagonist N=20 Mean±SD	P value
Age (yr)	29.5±5.44	31.25±3.58	0.237
BMI (kg/m2)	28.85±3.88	28.28±4.65	0.675
Total oocyte No.	7.2±3.91	8.0±5.15	0.583
MII	5.3±3.28	4.65±3.48	0.547
MI	0.55±0.69	1.05±1.1	0.093
GV	0.1±0.31	0.5±1.4	0.218
Abnormal	0.5±0.76	1.2±1.54	0.077
Fertilization rate	78.85±24.42	77.19±26.86	0.839
NO. of G1 ET	2.85±1.98	2.15±1.95	0.268
S.IGFBP-3 (µg/l)	27.71±12.76	25.08±8.64	0.446
F.IGFBP-3 (µg/l)	25.76±8.58	24.83±7.29	0.713
S. E2 (pg/ml)	1304.07±878.93	1454.2±993.89	0.616
F. E2 (pg/ml)	1609.14±867.46	1857.12±963.53	0.398
S.GH(ng/ml)	1.89±1.4	1.41±1.05	0.227
F.GH(ng/ml)	0.58±0.52	1.04±0.63	0.017

Table 2: Correlation of S.E2 and F. E2 with other parameters in all cases.

Parameters	S.E2		F. E2	
	r	P	r	P
total oocyte No.	0.77	<0.001	0.638	<0.001
MII	0.721	<0.001	0.577	<0.001
MI	0.374	0.017	0.334	0.035
GV	0.154	0.343	0.063	0.699
Abnormal	0.261	0.103	0.284	0.075
Fertilization rate	-0.058	0.724	-0.164	0.313
No. of G1 ET	0.523	0.001	0.399	0.011

1 embryo transferred (P=0.001, p=0.011, respectively). In addition, significant positive correlation was observed between the number of MI oocytes with the serum as well as follicular level of E2 (p=0.017, p=0.035, respectively). In Table 3, The F.GH demonstrate a positive significant effects on the total number of oocytes and the number of both MII and MI oocytes with no significant effects on the number of GV and grade 1 embryo as well as the fertilization rate while the S.GH had a positive significant effects on the fertilization rate however no significant effects on the other IVF parameters.

Table 3: Correlation of S.GH and F.GH with other parameters in all cases.

Parameters	S.E2		F. E2	
	r	P	r	P
total oocyte No.	0.059	0.716	0.402	0.01
MII	0.193	0.232	0.379	0.016
MI	0.045	0.785	0.322	0.043
GV	-0.073	0.653	0.108	0.508
Abnormal	-0.087	0.592	0.186	0.25
Fertilization rate	0.414	0.008	-0.04	0.802
No. of G1 ET	0.282	0.078	0.143	0.379

Table 4, show no significant association between the serum level of IGFBP-3 and the laboratory IVF parameters, whereas a significant correlation was observed between the follicular level of IGFBP-3 and MI oocytes number in all cases (*p=0.036*), however, no significant association of follicular level with other IVF parameters.

Table 4: Correlation of S.IGFBP-3 and F. IGFBP-3 with other parameters in all cases.

Parameters	S.E2		F. E2	
	r	P	r	P
total oocyte No.	0.227	0.159	0.213	0.186
MII	0.233	0.147	0.22	0.172
MI	0.171	0.291	0.332	0.036
GV	0.067	0.683	0.019	0.905
Abnormal	0.023	0.886	-0.04	0.813
Fertilization rate	-0.253	0.115	-0.23	0.157
No. of G1 ET	-0.102	0.531	-0.13	0.443

Discussion

Comparison between Agonist and Antagonist

Comparing GnRH agonist and GnRH antagonist cycles reported that there was no different in the total number of retrieved oocytes, mature and immature oocytes, abnormal oocytes, fertilization rate and grade I of embryo transferred table 1 and this demonstrated in other studies [22,23], where as some other studies results found that the agonist protocol revealed significant increase in the total number of oocytes retrieved, number of MII and total number of embryos obtained, there has been significant increase in long protocol over antagonist protocol. This was explained by the more oocytes recruitment observed in long protocol with higher number of good quality oocytes which results in more good quality embryos; also, the more degree of pituitary suppression in the long protocol [24,25]. Controversially, another study concluded that the antagonist protocol produced significantly higher oocytes numbers in poor responders who were previously subjected to GnRH long protocol treatment [26]. However, no studies found compare the follicular growth hormone level in agonist and antagonist protocols, the present study shows significant higher follicular GH level when antagonists were used. This may explained in the study that the most patient with antagonist protocol were polycystic which most of them characterized by high level of testosterone hormone that's by a number of studies may increase the GH secretion and relieve the negative feedback (by IGF-I) on the GH secretion [27], another explanation that the action of antagonists can decrease of growth factors that may be was suggested as a putative cause of increased GH secretion [28]. Conversely, no difference in serum GH, serum and follicular level of E2 and IGFBP-3 in between GnRH agonist and GnRH antagonist as in Table 1, this is in agreement with other studies regarding to E2 level [28,29], and in the same manner with IGFBP-3 studies [1,30]. Other investigator illustrates a higher E2 level on day of HCG in agonist protocol group [31], the other exhibit higher E2 in antagonist and elucidated that due to the mode of administration, were LH levels remain unsuppressed during the early follicular phase and enhance E2 production [32].

Correlation of E2, GH, and IGFBP-3 with the Laboratory IVF

With the increased understanding of the physiology of ovarian folliculogenesis and steroid genesis it is now understood that the ovarian microenvironment is very important for normal physiology [33]. Although there are many local factors affecting the ovarian physiology, the importance of IGFBP-3 has been better understood in more recent studies. The IGFBP-3 exerts dual functions in modulating IGF action in vivo. IGFBP-3 inhibits IGF-I action by sequestering and preventing IGF-I receptor binding. The increase IGFBP-3 levels in FF may preserve the bioavailability and bioactivity of IGF-I in stimulating luteal formation, late oocytes maturation, and embryo development [14,34], these may give explanation of our studies that found a significant correlation between follicular level of IGFBP-3 and the MI oocytes, as seen in Table 4. On the other hand no correlation found between follicular IGFBP-3 with total oocytes number, mature oocytes, fertilization rate and embryo quality similar to some studies that rule out any relationship of IGFBP-3 and IVF parameters [33,35]. Some research shows statistically significant relationship between

follicular IGFBP-3 levels and Oocytes maturation in a positive manner [36], or Oocytes maturation and the fertilization rate [34]. Regarding the GH, and parallel to another studies [37], the present study shows a significant effect of follicular GH on the total number of oocytes retrieved, mature oocytes (MII), MI while the serum GH effect on the fertilization rate, as seen in Table 3. In vitro studies demonstrated an important role of GH in prenatal follicle growth and differentiation through their binding with the GH receptors, which are located both in the oocytes and follicular somatic tissues. Furthermore, GH stimulates the development of small antral follicles to Gonadotropin dependent stages, as well as maturation of oocytes [8]. Of significant interest is the possibility that GH might improve oocytes quality when administered in vitro. Early GH exposure in vitro (prior to both IVM and IVF) also increases the percentage of oocytes resuming meiosis, suggesting that GH might establish optimal conditions for nuclear maturation, perhaps by promoting follicular development [9]. The ability of GH to promote nuclear maturation of denuded oocytes from humans suggests that GH acts directly at the oocytes. Some studies demonstrated no difference in oocytes number, maturation, and fertilization and IVF outcome in the routine use of adjuvant growth hormone in IVF protocols [38]. Indeed, some studies observed that the addition of GH to the IVM maturation medium enhanced the eventual number of cleaved embryos and blast cysts [39], while other studies observed similar numbers but enhanced quality [40,41]. Both serum and follicular level of E2 were strongly associated with total number of retrieved oocytes, MII, MI and number of good embryo quality, Table 2. This support the recent researches that demonstrated a correlation of E2 with IVF outcome, these studies showed a significant positive association of serum and follicular E2 levels with number of obtained oocytes, oocytes nuclear maturation and embryo grading [42,43]. This relationship may explained that during the follicular phase in day 5-8 of the menstrual cycle, the activity of aromatize enzyme starts in granulose cells of follicles bigger than 6-8 mm and begins producing Estradiol, but the dominant follicle produce more Estradiol than other follicles in the cohort, and being higher in oocytes matured nucleus normally fertilized and developed into good quality embryo [42]. Also, can be study other parameter and at the level gene to more explain this case [44-46].

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