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The Immunity Parameters in Sera of Infertile Women During Intracytoplasmic Sperm Injection (ICSI) Protocol

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Abstract: The recent study focused on infertile women by whom they subjected in antagonist ICSI protocol without progressions. Three groups were under study: 25 ICSI women by ages 18-42 years: 20 infertile women(positive control): 20 healthy women(negative control), both of two controls were within fertile period. ICSI protocol practiced with a regimen of Intramuscular (IM) or Subcutaneous (SC) hormones injections (FSH,75 IU/day and LH,75 IU/day) at cycle day 2. While Cetrorelix hormone (0.25 mg/day) was subcutaneously injected at cycle day 7-10. Also single (IM) or (SC) injection of HCG (10000 IU) hormone was administered to ICSI women until we obtain mature follicles. Clinical investigations were done for the studied groups at cycle day 2, 7 and 10.The cycle day 2 of menstruation revealed good selection for infertile women then subjected with ICSI protocol according to significant decrease in sera Estradiol (E2) hormone level in which related to suitable level of FSH (6.49 0.45 m IU/mL) with a significant increase in LH level (6.18±0.94 mIU/mL) in $p<0.05$ and no significant differences in prolactin hormone level. The above equal amount of sera FSH and LH lead to predict for endometriosis or polycystic ovary syndrome which verified by ultra sound examination and by resultant of serum IgG and IgA increment as anti-FSH and anti-LH. ICSI protocol women sera revealed to significant decrease in serum sugar (110.78±3.19 mg/dL) due to administer Glucophage, significant elevation in SGOT (21.53±2.44 U/L), $p<0.05$ because of treatment and no significantly differences in patients sera for alkaline-phosphatase and creatinine while there was a significant decreased in C-reactive protein, this due to mild ovarian stimulation, no inflammatory response and the patients reached to follicular phase. ICSI protocol women sera revealed no significant differences in their immune-globulins precursors concentrations due to treatment as in sera protein electrophoresis results whereas sera revealed significant decreasing in C-reactive protein concentration 42.20±17.45, $p<0.05$.The patients were in low immune responding and ought to administer immune-globulins to improve their pregnancy success percentage more than 20%.

Key words: Immunity, infertile , ICSI protocol, women, globulins, pregnancy

INTRODUCTION

Along the past years, the researchers were focused on the interference procecus to have much oocytes from infertile woman. Ovarian Hyper-Stimulation Syndrome (OHSS) is an iatrogenic complication of Controlled Ovarian Hyper-stimulation (COH) used in Assisted Reproduction Treatment (ART) (Aljawoan *et al*., 2012; Romito *et al*., 2017). Furthermore, some of patients response to (CHO) will be increased resulting in ovary deformity, so as ovarian cystic enlargement with biochemical alterations which leading to an increment in vascular permeability thereby, shift of fluid from the intra-vascular compartment to the third space (Busso *et al*., 2018). Seemingly, the Ovarian Hyper Stimulation Syndrome (OHSS) response can cause uncomfortable for the patients with some signs, abdominal swelling, abdominal pain, vomiting, diarrhea, nausea and drowsiness. The critical cases can also lead to admission in the hospital due to some risks onto patient such as dysuria, venous thrombosis and embolization (Choux *et al*., 2017). In this study, we focused on pathological effects of mild ovarian stimulation in ICSI protocol without any complications by using antagonist protocol, meanwhile to assess patient immune response affection.

MATERIALS AND METHODS

The research was undertaken in Biotechnology Research Center, Department of Medical and Molecular Biotechnology, Al-Nahrain University. The clinical

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Fig. 1: a) The reaction of serum specific antibody (IgG) for groups under study then to determine the concentration of (IgG) and b) The reaction of serum specific antibody (IgA) for groups under study then to determine the concentration of (IgA)

specimens were collected from patients of Al-Imamayn Al-Kadhomiyayn Medical City, Um Al-Baneen Fertility and IVF Center.

The study involved on three groups of patients as follows: 1-25 infertile women with ages ranged from 18-42 years thereby, the ovarian stimulation by antagonist protocol on going to ICSI were practiced for them by a regimen of hormones ampoules (Wang *et al*., 2017) as for administering them intramuscularly or subcutaneously by continuous injections. Daily doses were began by FSH (75 IU/day) alone or in combination with LH (75 IU/day) that were injected by increasing or decreasing the doses according to the patient response which started within day 2 of menstrual cycle then follow up the patient every subcutaneously injected by cetrorelix (0.25 mg/day) at 2-3 days to assess her responding, thereby the patient cycle day 7-10 according to ultra soundexamination and E2 hormone level, till we obtained mature follicles then to administer injection of HCG 10000 IU intramuscularly for induction of ovulation. Some patients may need doses of Glucophage started with 500 m g/day. Then after 34 h of HCG injection, the picked up oocytes were fertilized by standard procedure of Intra-Cytoplasmic Sperm Injection (ICSI). The clinical investigations were done at cycle day 2, 7 and 10. The 20 infertile women were without ICSI they subjected as a positive control. The 20 healthy women subjected as a negative control.

Clinical tests were applied on all women according to Radesic and Tremellen (2011) as follows: The blood was collected then sera were examined for all investigations at cycle day 2, 7 and 10 to determine hormones levels within 1 h. like LH, FSH, prolactin and Estradiol (E2) by using ELISA technique and according to each hormone quantify

Fig. 2: The reaction of serum specific antibody (IgM) for groups under study then to determine the concentration of (IgM)

instruction. Also sera were examined for determination of (glucose after 2 h of meal, creatinine, alkalinphosphatase and glutamic oxaloacetic transaminase concentrations) by using bio system kits procedure .

Sera were examined to determine concentrations of Immune-Globulins (IgG, IgA and IgM) by using radial immune-diffusion plates (LTA Sri via. Milano-Kits) as shown in Fig. 1a, b and 2 also to determine the acute phase protein (C-reactive protein) by using AGAPPE diagnostics Switzerland GmbH).

Sera were examined by protein electrophoresis technique by using Helena Bio Science Europe kit, according to their instructions to determine

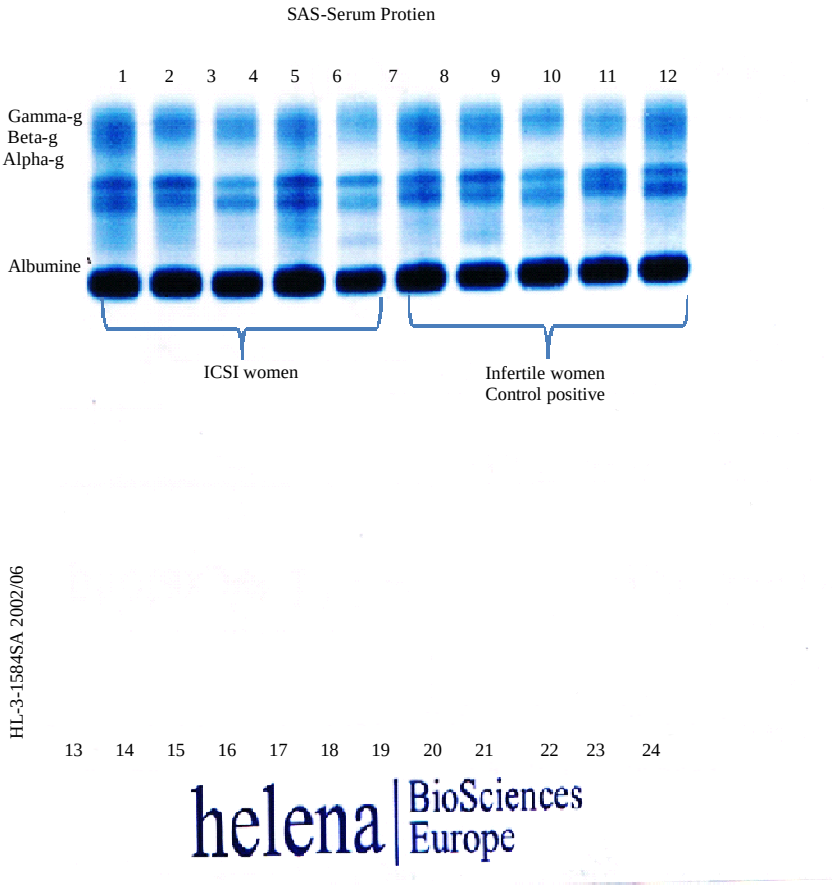

Fig. 3: Serum protien electrophoreses examination for ICSI women and infertile women which demonstrate bands of protien fractions

concentrations of total protein, albumin, α-1-globulin, α-2-globulin, β-globulin and γ-globulin as shown in Fig. 3.

Statistical analysis: The Statistical Analysis System (SAS) program was used to effect of difference factors in study parameters. Least Significant Difference (LSD) test was used to significant compare between means in this study.

RESULTS AND DISCUSSION

The current study revealed to an obvious significant differences by decreasing in FSH mean of infertile women (positive control) 6.13± 0.73 mIU/mL in comparison with both of ICSI FSH mean 6.49±0.45 mIU/mL and healthy women (negative control) FSH mean 8.99±1.75 m IU/mL, $p<0.05$. Also E2 hormone mean value of ICSI women 28.41±3.43 mIU/mL was significantly decreased in comparison with both of infertile women (positive control) and healthy women (negative control) by 44.34±6.38 pg/mL and 100.12±31.22 pg/mL, respectively $p<0.05$ as shown in Table 1. While there were significant differences by the elevation in mean value of LH in ICSI women 6.18±0.94 m IU/mL in comparison with LH means of infertile women (positive control) 3.36±0.52 m IU/mL and healthy women (negative control) LH mean 3.21±0.84 mIU/mL, respectively $p<0.05$. Hence, there were no significant differences in prolactin hormone levels between groups under study as shown in Table 1.

Chemical parameters results were revealed to significant increase in S.GOT mean of ICSI women 21.53±2.44 mU/mL in comparison to infertile women (positive control) mean 18.96±2.54 mU/mL and healthy women (negative control) mean 13.53±1.81 mU/mL, $p<0.05$ as shown in Table 2. In contrast to CRP mean was significantly decreased, so as the highest mean of CRP were in healthy women (negative control) 48.18±18.84 mg/L and infertile women (positive control) 54.58±14.51 mg/L $p<0.05$ but the lowest mean was in ICSI protocol women 19.12±6.12 mg/L, $p<0.05$ as shown in Table 2.

Also, there was a significant decreased in serum sugar mean of ICSI women 110.78±3.19 mg/dL in comparison with infertile women (positive control) mean

Table 1: Compare between difference groups in hormones level

	Mean±SE			
The group	FSH (mIU/mL)	E2 (pg./mL)	LH (mIU/mL)	Prolactin (ng/mL)
ICSI (n = 25)	6.49±±2.15[a]	28.41±3.43[b]	6.18±0.94[a]	0.45 B19.69
Positive control (n = 20)	6.13±0.73[b]	44.34±6.38[b]	3.36±0.52[b]	19.40±1.72[a]
Negative control (n = 20)	8.99±1.75[a]	100.12±31.22[a]	3.21±0.84[b]	16.95±2.02[a]
LSD value	2.179*	40.41*	2.318*	5.795 NS

Table 2: Compare between difference groups in chemical parameters

	Mean±SE				
The group	S.C (mg/dL)	S.GOT (mU/mL)	S.Alk.Pho. (U/L)	CRP (mg/L)	S. Sugar (mg/dL)
ICSI (n = 25)	1.568±0.04[a]	21.53±2.44[a]	424.09±35.46[a]	19.12±6.12[b]	110.78±3.19[a, b]
Positive control (n = 20)	1.541±0.04[a]	18.96±2.54[a, b]	426.71±42.89[a]	54.58±14.51[a]	100.71±8.78[B]
Negative control (n = 20)	1.481±0.04[a]	13.53±1.81[B]	508.73±77.11[a]	48.18±18.84[a]	124.52±10.92[a]
LSD value	0.119 NS	7.023*	140.74 NS	17.25*	21.88*

Table 3: Compare between difference groups in immunity parameters

	Mean±SE		
The group	IgG (mg/dL)	IgM (mg/dL)	IgA (mg/dL)
ICSI (n = 25)	1676.73±138.95[a]	198.45±13.87[a]	276.56±21.13[a, b]
Positive control (n = 20)	965.02±102.23[b]	186.75±9.88[a]	222.78±18.3[b]
Negative control (n = 20)	1413.67±139.23[a]	202.42±20.32[a]	319.15±15.38[a]
LSD value	369.21*	40.784 NS	56.438*

Table 4: Compare between patients and control groups in serum protein fractions parameters

	Mean±SE						
The group	T. Protein(g/dL)	Albumin (g/dL)	Alpha-1-g(g/dL)	Alpha-2-g (g/dL)	Beta-g (g/dL)	Gama-g (g/dL)	CRP (mg/L)
ICSI (n = 5)	6.91±0.13[a]	3.67±0.12[a]	0.346±0.05[a]	0.924±0.05[a]	0.700±0.04[a]	1.262±0.07[a]	42.20±17.45[b]
Positive control (n = 5)	6.65±0.31[a]	3.50±0.18[a]	0.354±0.03[a]	0.904±0.05[a]	0.626±0.04[a]	1.272±0.12[a]	83.60±32.39[a]
LSD value	0.789 NS	0.508 NS	0.142 NS	0.168 NS	0.141 NS	0.330 NS	31.44*

*($p<0.05$); [a, b]Means having with the different letters in same column differed significantly

Table 5: Compare between patients and control groups in immunity parameters

	Mean±SE		
The group	IgG (mg/dL)	IgM (mg/dL)	IgA (mg/dL)
ICSI (n = 5)	1327.02±353.55[a]	165.64±42.12[a]	254.74±47.76[a]
Positive control (n = 5)	1329.34±176.16[a]	164.92±16.78[a]	307.62±58.91[a]
LSD value	910.90 NS	104.56 NS	174.90 NS

NS: Non-Significant; [a, b]Means having with the different letters in same column differed significantly

100.71±8.78 mg/dL and healthy women (negative control) mean 124.52±10.92 mg/dL, $p<0.05$ as shown in Table 2.

The results revealed to significant difference by increasing IgG concentration mean in ICSI women sera 1676.73±138.95 mg/dL in comparison with infertile women (positive control) mean 965.02± 102.23 mg/dL but not with healthy women (negative control) IgG mean 1413.67±139.23 mg/dL ($p<0.05$). While, there was a significant decreased in sera IgA concentration mean of infertile women (positive control) 222.78±18.3 mg/dL in comparison with healthy women (negative control) mean 319.15±15.38 mg/dL, ($p<0.05$) and slight decrease in ICSI women sera IgA. Hence, there were no significant differences in sera IgM concentrations means between groups under study as shown in Table 3.

Serum protein electrophoresis results were revealed to no significant differences by sera concentrations means for Total Protein (T.P), albumin, α-1-globulin, α-2-globuli, β-globuli, γ-globulin and serum immune-globulins (IgG, Ig, IgA) between ICSI women and infertile women (positive control) as shown in Table 4 and 5 ($p<0.05$). While the acute phase protein (CRP) concentration mean was differed significantly by decreasing in ICSI women sera 42.20±17.45 mg/L in comparison with infertile women (positive control) 83.60±32.39 mg/L, ($p<0.05$) as shown in Table 4.

In our study, the result of decreasing in E2 hormone was in cycle day 2 and it was good indicator to practice ICSI procedure. This result who made our decision onto infertile women (positive control) to involve them in ICSI procedure in agreement with Blockeel (2012). While the

elevation in E2 hormone increase in healthy women (100.12±31.22 pg/mL) may be due to functional ovarian cyst.

Also the studied ICSI women revealed to suitable level of FSH (6.49±0.45 mIU/mL) at cycle day 2 in agreement with Perkins (2017). LH levels in ICSI women were revealed to slight increase by 6.18±0.94 mIU/mL at cycle day 2 in relation to their FSH level 6.49±0.45 of menstruation which verified by Perkins, (2017) who published that base line levels of LH<7 mIU/mL at the above mentioned day. Hence, the equal means values for both of FSH and LH (6.49±0.45 and 6.18±0.94) in ICSI women lead to predict that some patients suffered from polycystic ovary syndrome which was verified by ultra sound examination and in agreement with Sterling. Furthermore by the evidence of equal serum means of prolactin hormone concentration of both ICSI women and infertile women (positive control) 19.69± 2.15 ng/mL and 19.40±1.72 ng/mL respectively, in agreement with (Hashim *et al*., 2010).

ICSI women revealed to decrease of serum sugar, this result mostly, may due to administer of glucophage as treatment for ovarian cyst, so, those events were by resultant effect of insulin resistance which may form ovarian cyst in agreement with Cakiroglu *et al.* (2017) and Marshal and Dunaif (2012). While ICSI women were revealed for mild elevation in SGOT (21.53±2.44 mU/mL) which was due to the effect of medication-associated hepatic cell injury in agreement with Giboney (2005) and Oh *et al*. (2017).

In this study, ICSI women revealed to significant decrease in their sera C-Reactive Protein (CRP) this may due to the patient reached to the follicular phase that lead to the expected day of ovulation in agreement with (Gaskins *et al*., 2012; John, 2017) as well as the using suitable ovarian stimulation. While infertile women (positive control) had a highest value of CRP because of their ovarian cyst in agreement with Timur *et al.* (2015) or due to any inflammatory response of hidden infection such as in healthy women (negative control) in agreement with Almagor *et al*. (2004) and in some cases of obesity in agreement with Abdalmageed *et al*. (2016) and Cakiroglu *et al*. (2016) and the study suggested that some patients were with chronic subclinical inflammation in agreement with Vodolazkaia *et al.* (2011). While serum alkaline-phosphatase level and serum creatinine amount were not revealed any significant differences between the groups under study, so, there were no effects on renal and hepatic functions due to treatments onto ICSI women in agreement with Romito *et al*. (2017).

The under studied ICSI women revealed to low immune response despite slight increase in sera IgG concentration which may come from either chronic infection or Polycystic Ovary (PCO), vice versa to slight decrease in sera IgA concentration in endometriosis; this IgG titer may behaved as anti-FSH that leaded to low level of FSH in agreement with Haller-Kikkatalo *et al*. (2011), Opoien *et al*. (2012) and Wahd *et al*. (2014) .

CONCLUSION

Seemingly, ICSI women immune response were not significantly affected in this procedure of ovarian stimulation by the evidence of no differences in means of sera immunoglobulin precursors concentration and proteins as to serum protein electrophoresis investigation in agreement with Gaskins *et al*. (2012), also by the evidence of slight decreasing in sera CRP concentration as a pro-inflammatory response in ICSI women in agreement with John (2017). The present study revealed low percentage of pregnancy success (20%) this is may be due to predisposing factors so as patients age and body mass index, professional doctors in oocyte pick up and embryo transfer, E2 and progesterone hormone levels on Human Chorionic Gonadotropin (HCG) day in agreement with Wang *et al*. (2017). Whereas the increasing of pregnancy success ratio in patients under study may lead to boost their immunoglobulin levels by an intravenous immunoglobulin (IVIg) doses in agreement with Han and Lee (2018), Krivonos *et al*. (2017), Sung *et al*. (2017) and Virro *et al*. (2012).

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