Evaluation Hormonal State in Iraqi Infertility Females Under In-Vitro Fertilization Program

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Abstract

Background: Uniqueness of the current study at first time in Iraq specially, across the comparison the clinical parameters among Iraqi infertility females according the IVF program outcome: failures and success implantation. While, aspect were neglected in IVF field. Also, to difficulty of some parameters comparing with control.

Methods: This study done during (December 2019 to November 2020) on Iraqi infertile females with age range (19 – 45)ys. It included the two groups: success implantation and failure implantation females.

Result: The current study revealed to that , secondary infertility is a good marker in implantation success prediction. Central circumference increasing has been associated with implantation failure (P<0.05). When hormonal profile comparison among two groups. Failure implantation females have non-significant increasing in basic prolactin values. Also, E2, progesterone and LH values were higher significantly(P>0.001), among success implantation. The hCG values were highly significant in failure implantation (P>0.001). MII oocytes and G2 embryos were highly significant in success implantation. Our result support that no. of embryo cells have positive impact on IVF outcome. The S. TC and TG were significant increasing among failure implantation.

Key words: *hormonal state, Infertility female, IVF, toxicity*

Introduction

Implantation is phase at which the embryo adheres to the wall of the uterus(1). The implantation required the synchronism among the endometrium maturation and embryo(s) development(2). The clinical window of implantation (WOI) is the time frame; in which IVF embryo(s) must be transferred. The optimal period for WOI is known as the period (20-24) of natural cycles, or after 2 or 3 days of egg-retrieval in an IVF cycles (3). After IVF, 25-30 % of the embryo(s) transferred to the uterus are successful in implantation (4).

After birth, 1-2 million cells remain in the ovary; no new oocytes are formed. Follicle stimulating hormone (FSH) promotes the growth of multiple follicles at different stages of development (5). Luteinizing hormone (LH) triggers follicle bursts and the release of ova into the fallopian tube. The corpus luteum(CL) is created, when luteinized granulosa cells combine with luteinized theca and surrounding the stroma in the ovary(6). CL was a temporary endocrine organ that secreted the hormones progesterone and estradiol (E2). E2 causes uterine swelling, which may aid in pressing the blastocyst into the endometrium (7). After ovulation, progesterone is released during the luteal phase. The endometrium cannot be prepared for implantation, if progesterone levels are insufficient. While, Human chorionic gonadotropin (hCG) functions to maintain of gestational yellow body and controls the development of progesterone and estrogen (8).

The purposes of present study: Evaluate the hormonal and demographic state of Iraqi infertility women during IVF.

Materials and Methods

Study done over a ten months (December 2019 to November 2020), on 70 Iraqi infertile females during IVF, age range was 19 – 45 years. Samples collected from: the Kamal Al-Samurai hospital. Iraqi infertility females were assessed clinically by physician. IVF program was beginning 2-3 day of cycle. There are forma to determine the number and quality each of ova and embryo during IVF. The embryos were transferred, at two or three days after cleavage began.

Subject undergo to questioning for obtain some demographic and clinical information. Also, Subject divided into two groups according IVF outcome: Implantation failure group comprised 50 infertile Women. While, other group 20 subjects of implantation success. Serum lipid profiles were measured. Also, Serum hormonal profile tests done by VIDAS- auto analyzer as the following:

i. Basal hormonal profile(LH; FSH; TSH and prolactin) in the second day of menstrual cycle for normal ovarian reserve.

ii. E2 Follow- up of IVF to assessment of ovulation occurrence

iii. At embryo transfer (ET)(LH; Progesterone). Furthermore, β-HCG after 14day of ET to IVF outcomes reported.

Results

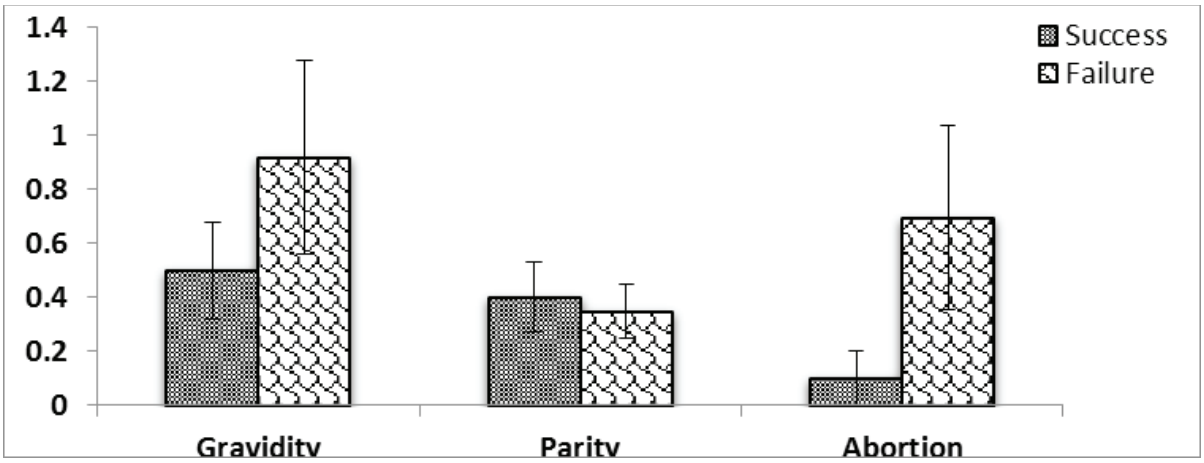
Current study descripted the demographic and clinical data of Iraqi infertility females. 58.57% have primary, 41.43% secondary infertility type (P>0.05). Also, the study recorded the highly significant increasing (P<0.001) of abnormal ovulation through 36 cases (51.43%), 19 cases (27.14%) have unexplained infertility. While, Uterine factor were seen in 10 cases (14.29%). only 3 cases (4.29%) and 2(2.86%) cases with endometriosis and tubal factor, at respectively. While, patients distributed according outcome of implantation: Success implantation forms 28.57%, failure implantation 71.43% (P<0.001). Infertility duration distributed into four categories: 1-5 ys (37.14%), 6-10 ys (41.43%), 11-15 ys (15.71%) and >15 ys (5.71%) (P<0.01). Ages distributed between 20-25 ys (30%), 26-30 ys (34.29%) and >30 ys (35.71%) (P<0.05). While, BMI distributed significantly(P>0.05) into three categories : 18-24 Kg (21.43%), 25-30 Kg (54.29%) and >30 Kg (24.295%). Waist/hip ratio (WHR) (Kg) recorded lower significant (P<0.05) for group <0.85 (25.71%) than for group >0.85(74.29%).

The success of implantation strongly (P<0.05) in secondary infertility (60%) than primary Iraqi infertility females (40%) as following in table(1):

Table (1) Outcome of Iraqi Infertility Females Implantation According of Type of Infertility via IVF

Parameters		Success (No./%)	Failure (No./%)	Chi-square	P- Value
Type of infertility	Primary	8(40%)	33(66%)	3.980	P<0.05 *
	Secondary	12(60%)	17(34%)		

In obstetric history: Gravidity has non-significant increasing of failure implantation group than success. The later can be disturbuted to non- significant increasing to abortion among failure implantation group, as shown in Figure(1).



Figure(1) Comparison of Obstetric History between Success and Failure implantation Groups

Non-significant differences of demographic parameters(Age, BMI, Waist/Hip Ratio, and Duration of Infertility) of Iraqi infertility females among two groups during IVF (P>0.05). While, This study recorded significant increasing(P<0.05) in central circumference among failure implantation group, as shown in table 2:

Table (2) Demographic Characteristics of Study Samples among Success and Failure Implantation Groups.

Parameters	Success (Mean± S.E)	Failure (Mean± S.E)	P- Value
Age (Years)	29.1±1.27	29.65±0.85	P>0.05
BMI	28.731±0.752	29.244±0.908	P>0.05
Waist/ Hip ratio	0.80±0.01	0.81±0.01	P>0.05
Central circumference (Cm)	99±1.67	104.31±1.45	P<0.05*
Duration of Infertility(ys)	7.4±1.075	8.163±0.569	P>0.05

The current study recorded non - significant increasing (P>0.05) in LH and FSH basic in success implantation. While, non-significant decreasing of prolactin basic among the same group. E2 levels for success implantation had highest value (857.11±88.96) at (P>0.001).

At ET: There was non-significant increasing in LH among success implantation. Also, non-significant increasing in progesterone hormone among success implantation. But, the current study recorded highly significant increasing (P>0.001) in hCG values that were (41.60) in failure implantation than(12.05) in success, as shown table (3):

Table(3) Hormonal Profile in Success and Failure Implantation Groups via IVF

Hormonal profile		Outcome of implantation		P- Value
		Success (Mean± S.E)	Failure (Mean± S.E)	
Hormonal basic (CD2)	LH	6.36±1.37	4.65±0.68	P>0.05
	FSH	5.75±0.98	5.76±0.57	P>0.05
	Prolactin	18.59±3.21	22.73±2.29	P>0.05
	TSH	3.61±1.06	2.13±0.16	P>0.05
Follow up IVF	E2	857.11±88.96	563.44±43.40	P<0.001*
At embryo transfer	LH	4.58±1.50	2.11±0.63	P>0.05
	HCG	12.05±3.63	41.60±4.22	P<0.001*
	Progesterone	41.91±5.25	40.56±3.38	P>0.05

There are highly significant in total no. ova in success(12.40± 0.97) than (7.58±0.62) failure implantation group of Iraqi infertility women during IVF (P>0.001). Also, highly significant (P>0.001) recorded in MII oocytes in success (7.95±0.88) than failure group (4.10±0.55). Total embryo(s) have highly significant increasing (P>0.001), among success implantation group. The mean were (4.65±0.48) in success, While it was (3.26±0.27) in failure implantation. Non-significant increasing of Grade 1(G1) embryos (P>0.05). While, highly significant for Grade 2 (G2) embryos means, that were 1.25±0.39 in success and 0.26±0.08 of failure implantation. Mean embryo cells no. has significant increasing (P<0.01) in success implantation (6.80) than failure implantation group (5.44), as shown in table (4).

Table(4) The Ova and Embryos Quality in Success and Failure Implantation Groups via IVF

Parameters		Outcome of implantation		P- Value
		Success(Mean±S.E)	Failure (Mean± S.E)	
Ova Quality	Total Ova	12.40±0.97	7.58±0.62	P<0.001*
	GV	0.90±0.35	0.76±0.16	P>0.05
	MI	1.50±0.44	1.04±0.21	P>0.05
	MII	7.95±0.88	4.10±0.55	P<0.001*
	Rapture	1.95±0.478	1.44±0.254	P>0.05

Cont... **Table(4) The Ova and Embryos Quality in Success and Failure Implantation Groups via IVF**

Embryo Quality	**Total Embryo**	**4.65±0.48**	**3.26±0.27**	**P<0.01***
	G1	2.40±0.37	1.58±0.28	P>0.05
	G2	1.25±0.39	0.26±0.08	P<0.01*
	G3	0±0	0.12±0.05	P>0.05
	Embryo Cells No.	6.80±0.47	5.44±0.32	P<0.01*

MI Oocyte(pre-ovulatory); MII Oocyte(mature); GV Oocyte(immature)

According the Table (5): The total cholesterol (TC) levels have highly significant increasing in failure than success implantation (P>0.001). The Means were 133.94±5.64 in failure, 111.9±5.77 in success group. Also, there was increasing significant for S. triglyceride and S. HDL between failure and success groups (P>0.05, P>0.01). Means were (95.4±6.33), (113.57±5.62) for S. triglyceride(TG) and (51.12±1.95), (42.75±2.78) for S.HDL at receptively. While, non-significant deference was reported in S.LDL among failure and success implantation. VLDL had highly significant (P>0.01), in implantation failure than success group.

Table(5) Lipid Profile in Success and Failure Implantation Groups via IVF

Lipid profile	Outcome of Implantation		P- Value
	Success (Mean± S.E)	Failure (Mean± S.E)	
S.TC	111.9±5.77	133.94±5.64	P<0.001*
S.TG	95.4±6.33	113.57±5.62	P<0.05*
S.HDL Chol.	42.75±2.78	51.12±1.95	P<0.05*
S.LDL Chol.	54.19±5.5	60.9±4.17	P>0.05
VLDL	19.11±1.27	22.67±1.14	P<0.01*
AIP	0.28±0.05	0.39±0.04	P>0.05

Discussion

Infertility type has significantly effect on outcome of implantation among Iraqi infertile females. Current result agree with Hambartsoumian,1998 study(9). Also, the present result, can be contributed according the Moragianni, 2012 suggest, to the counseling and psychological support are required to enhance the IVF success [10]. In present study: Gravidity means seem non-significant higher among failure than success implantation. This is back to non-significant increasing of abortion in failure than success group . Although the current study does not accept with significant lowering for gravidity and parity among infertility females as

compared to the control [11].

The present study found no effect of age on the implantation success, which agree with Abdalla *et al.* study[12]. Also, Other study reported no significant relation for BMI and age means between primary infertility and control[13]. Furthermore, the age means for infertile females and the control group included in the study were identical [14]. There was highly significant increasing of success implantation rate in BMI<30 than >30(15). Central obesity lead to decrease the chance of implantation success according the current study . Central circumference is a tool to assess lipid excess and ART prediction of infertility women [16].

Through comparable hormonal profile of current study, among success and failure implantation during IVF. Current basic hormonal were similar to Tawfeek *et al*. for each of: prolactin; FSH and LH [14]. Thyroid dysfunction associated to fertility decreasing [17]. Other research suggests that infertility women with an abnormal menstrual cycle should have checked TSH [18]. The current study agree with important of E2 for induction of implantation to improved IVF efficiency [19].

At ET: non-significant decreasing of LH between failure implantation women of current study. This can be supported by the report that refer to that, Luteal phase deficiency (LPD) is widespread among failed implantation females[20]. The change in progesterone levels in LPD, leads to a rise implantation failure rate [21]. For high significance of hCG among failure implantation group of this study; disagree with non-significant differences of implantation rates in higher or lower the hCG cycles [22]. The current study matched with Strom *et al.* 2012 decreased of hCG levels through ET at cleavage stage in success implantation[23]. Also, Other report refer to: high doses of hCG administration is associated significantly with decreased of implantation, (24). The current study showed non-significant increasing progesterone level, that comparable with previous studies; through the progesterone kept the fetus from the mother's immune, during secretory phase of cycle [25].

This study agree with Khalaf Allah *et al*., 2020 through the oocyte no. is essential to be evaluated the IVF outcome[26]. Also, The no. of eggs obtained has no impact on the probability of implantation [22]. With increasing MII oocyte and G2 embryos among success group in current study. Other reports refer to non-association with in MII no. [27].

Present results suggest the lipid may be used as indicator of fertility. Other designs revealed to the TG, TC, LDL levels were highest, but HDL were lowers in un-explain infertility females than fertiles [28;29].

Conclusion

Although, afraid the couples of IVF outcome. But, There are many factors that effect in the implantation success rate during IVF. Specially, central obesity and hormonal profile. So, the control of these factors across the BMI , LH, Progesterone, E2, hCG balance, Oocyte pick up number and quality will lead to significant increasing in success implantation during IVF.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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References

1. Griffith OW, Chavan AR, Protopapas S, Maziarz J, Romero R, Wagner GP. Embryo implantation evolved from an ancestral inflammatory attachment reaction. Proc Natl Acad Sci U S A. 2017;114(32): 6566–6575.
2. Valdes CT, Schutt A, Simon C. Implantation failure of endometrial origin: it is not pathology, but our failure to synchronize the developing embryo with a receptive endometrium. Fertil Steril. 2017; 108(1):15–28
3. Katzorke N, Vilella F, Ruiz M, Krüssel JS, Simón C. Diagnosis of Endometrial-Factor Infertility: Current Approaches and New Avenues for Research. Geburtshilfe Frauenheilkd. 2016;76(6):699–703.

4. Achache H, Revel A. Endometrial receptivity markers, the journey to successful embryo implantation. Hum Reprod Update. 2006;12(6):731–746.
5. Alisha M. Truman, Jonathan L. Tilly and DCW. Ovarian regeneration: The potential for stem cell contribution in the postnatal ovary to sustained endocrine function. Mol Cell Endocrinol. 2018;15(445):74–84.
6. Reed BG,Carr BR. The normal menstrual cycle and the control of ovulation. 2017; http://south dartmouth www. endotex. org/.
7. Cunningham FG, Leveno KJ, Bloom SL, Dashe JS, Hoffman BL, Casey BM, Spong CY. Williams Obstetrics, 25edition. 2018; MCGraw Hill, New york:1344pp.
8. Radwanska E, Frankenberg J, Allen EI. Plasma progesterone levels in normal and abnormal early human pregnancy. Fertil Steril 1978;30(4):398–402.
9. Hambartsoumian E. Leukemia inhibitory factor (LIF) production by human decidua and its relationship with pregnancy hormones. Gynecol Endocrinol. 1998;12(1):17–22.
10. Moragianni VA, Jones SML, Ryley DA. The effect of body mass index on the outcomes of first assisted reproductive technology cycles. Fertil Steril. 2012;98(1):102–108.
11. Margioula-Siarkou C, Prapas Y, Petousis S, Milias S, Ravanos K, Kalogiannidis I, et al. LIF and LIF-R expression in the endometrium of fertile and infertile women: A prospective observational case-control study. Mol Med Rep. 2016;13(6):4721–4728.
12. Abdalla HI, Wren ME, Thomas A, Korea L. Age of the uterus does not affect pregnancy or implantation rates; A study of egg donation in women of different ages sharing oocytes from the same donor. Hum Reprod. 1997;12(4):827–829.
13. Rasheed O, Ali N, Abdulrasul E. Serum vitamin D and anti- mullerian hormone levels in Iraqi infertile women at Baghdad city. Int J Basic Appl Sci. 2015;4(4):375-380.Sadler TW, Leland J, Tosny, K, Chescheir N, Hytham L.Medical imperiology, 12th edition. Wolters Kluwer, philadelphia, 2012:400PP.
14. Tawfeek, MA, Eid, MA, Hasan, AZ, Mostafa, M., El-Serogy H, Assessment of leukemia inhibitory factor and glycoprotein 130 expression in endometrium and uterine flushing: a possible diagnostic tool for impaired fertility. ABMC Women's Health.2012; 12(1):1-6.
15. Kohl TO, Ascoli CA. Immunometric double-antibody sandwich enzyme-linked immunosorbent assay. Cold Spring Harb Protoc. 2017;2017(6):458–462.
16. Orvieto R, Meltcer S, Nahum R, Rabinson J, Anteby EY, Ashkenazi J. The influence of body mass index on in vitro fertilization outcome. Int J Gynecol Obstet. 2009;104(1):53–55.
17. Tasnim N, Begum R. Study on Thyroid Hormone Status in Case of Female Infertility. Anwer Khan Mod Med Coll J. 2017;8(2):117–120.
18. Magtooph MG. Study of Thyroid and Reproductive hormones levels in fertile and infertile women. Univ Thi-Qar J Sci. 2019;5(2):31–36.
19. Yang ZM, Chen DB, Le SP, Harper MJK. Differential hormonal regulation of leukemia inhibitory factor (LIF) in rabbit and mouse uterus. Mol Reprod Dev. 1996;43(4):470–476.
20. Mesen, T B, Young SP. Progesterone and the Luteal Phase: A Requisite to Reproduction. GCNA. 2015 42(1): 135–151
21. Haines BP, Voyle RB, Pelton TA, Forrest R, Rathjen PD. Complex conserved organization of the mammalian leukemia inhibitory factor gene: regulated expression of intracellular and extracellular cytokines. J Immunol. 1999;162(8):4637–4646.
22. Mizrachi Y, Horowitz E, Farhi J, Levran D, Raziel A, Weissman A. Human chorionic gonadotropin serum levels following ovulation triggering and ivf cycle outcome. J Assist Reprod Genet. 2018;35(5):891–897.
23. Strom CM, Bonilla-Guererro R, Zhang K, Doody KJ, Tourgeman D, Alvero R, et al. The sensitivity

and specificity of hyperglycosylated hCG (hhCG) levels to reliably diagnose clinical IVF pregnancies at 6 days following embryo transfer. J Assist Reprod Genet. 2012;29(7):609–614.

24. Venetis CA, Kolibianakis EM, Bosdou JK, Tarlatzis BC. Progesterone elevation and probability of pregnancy after IVF: A systematic review and meta-analysis of over 60 000 cycles. Hum Reprod Update. 2013;19(5):433–457.

25. Wahid B, Bashir H, Bilal M, Wahid K, Sumrin A. Developing a deeper insight into reproductive biomarkers. Clin Exp Reprod Med. 2017;44(4):159–170.

26. Khalaf Allah A, Elmekkawi S, Megahed A, Megahed A. Obesity Index That Better Predict Ovarian Response: Body Mass Index, Waist Circumference, Waist Hip Ratio, or Waist Height Ratio in Women Undergoing ICSI : A Pilot Study. Evid Based Women's Heal J. 2020;10(1):54–64.

27. Wintner EM, Hershko-Klement A, Tzadikevitch K, Ghetler Y, Gonen O, Wintner O, et al. Does the transfer of a poor quality embryo together with a good quality embryo affect the In Vitro Fertilization (IVF) outcome? J. Ovarian Res.2017;10(1):1–5.

28. Szkup M, Jurczak A, Karakiewicz B, Kotwas A, Kopeć J, Grochans E. Influence of cigarette smoking on hormone and lipid metabolism in women in late reproductive stage. Clin Interv Aging. 2018;13:109–115.

29. Hansen KK, Knopp RH, Soules MR. Lipoprotein-cholesterol levels in infertile women with luteal phase deficiency. Fertil Steril .1991;55(5):916–921