Therefore, the study of hormonal state I is essential in the diagnosis of glands, non endocrine organs, or metabolic disorders [4].

Results: Significantly higher estradiol (E2) (p=0.024), anti-müllerian (AMH) (p=0.006), and lower prolactin (PRL) (p=0.002) in pregnant in comparing with failure group. Correlations studies indicated a significant correlation between AZP A and AMH in pregnant group, and there is a significant correlation between AZPA and E2 hormone in the failure group.

Conclusion: It can be concluded that serum AZPA level does not differ between women who had success or failure IVF. However, in women with failure pregnancy in IVF, AZPA is positively correlated with E2 and its negatively correlated with PRL. In pregnant group, AZPA is positively correlated with AMH.

Keywords: In vitro fertilization, Estradiol, Anti-zona pellucida antibodies

INTRODUCTION

Infertility is the incapability of a couple to achieve pregnancy over an average period of one year (in a woman under 35 y of age) or 6 mo (in a woman above 35 y of age) despite adequate, regular 3-4 times per week, unprotected sexual intercourse [1]. Infertility is a complex disorder with significant medical, psychosocial, and economic problems [2]. Hormonal imbalance is an important cause of anovulation. The disorder in the ovulation may lead to infertility. Hormonal irregularities that affect ovulation include hyperthyroidism, hypothyroidism, polycystic ovary syndrome and hyperprolactinemia [3]. Women with hormonal imbalance will not produce enough follicles to ensure the follicle stimulating hormone (FSH) and luteinizing hormone (LH) secretion, which are necessary for ovulation to occur.

Infertility is defined as the failure of a couple to achieve pregnancy after 1 year of unprotected intercourse. It is a complex disorder with significant medical, psychosocial, and economic problems [2].\n
Hormonal imbalance is an important cause of anovulation. The disorder in the ovulation may lead to infertility. Hormonal irregularities that affect ovulation include hyperthyroidism, hypothyroidism, polycystic ovary syndrome and hyperprolactinemia [3]. Women with hormonal imbalance will not produce enough follicles to ensure the follicle stimulating hormone (FSH) and luteinizing hormone (LH) secretion, which are necessary for ovulation to occur.

Therefore, the study of hormonal state I is essential in the diagnosis of infertility and treatment. IVF is a process by which an egg is fertilized by sperm outside the body: in vitro (in glass). The procedure involves monitoring and stimulating a woman's ovulatory process, removing an ovum or ova (egg or eggs) from the woman's ovaries and letting sperm fertilize them in a liquid in a laboratory. The fertilized egg (zygote) is cultured for 2–6 d in a growth medium and is then implanted in the same or another woman's uterus, with the intention of establishing a successful pregnancy [5].

The zona pellucida, found in mammalian eggs, is a glycoprotein membrane surrounding the plasma membrane of an oocyte. It is a vital constitutive part of the oocyte, external but of essential importance to it. The zona pellucida first appears in unilaminar primary oocytes and is secreted by both the oocyte and the follicular cells. It is surrounded by the cumulus oophorous. The cumulus is composed of cells that care for the egg when it is emitted from the ovary [6].

This structure binds spermatozoa and is required to initiate the acrosome reaction. In the mouse (the best characterized mammalian system), the zona glycoprotein, ZP3, is responsible for sperm binding, adhering to proteins on the sperm plasma membrane GaIT. ZP3 is then involved in the induction of the acrosome reaction, whereby a spermatozoon releases the contents of the acrosomal vesicle. The exact characterization of what occurs in other species has become more complicated as further zona proteins have been identified [7].

In humans, five days after the fertilization, the blastocyst performs zona hatching; the zona pellucida degenerates and decomposes, to be replaced by the underlying layer of trophoblastic cells. The zona pellucida is essential for oocyte death and fertilization. The zona pellucida's main function in reproduction is to bind sperm [8]. However, the correlation of the AZPA antibody with the result of IVF is not studied previously. The study of the factors affecting the rate of success of IVF cycles is a very important field of study. In the present study, the possible relationship between the AZPA, an antibody bound to a pellucida, and the outcome of IVF was studied in the present work. The second aim is to correlate the serum AZPA concentration with some associated hormones in women undergo IVF procedure.

MATERIALS AND METHODS

Materials

Sixty women underwent IVF patients participated in the study. Written consents were obtained for each patient and controls. Their age range was 27.35±7.6 1 y. IVF cycles were conducted in the “Center of Fertility” in Al-Sadr Teaching Medical City in Najaf Governorate-Iraq during the period from June-August 2014. IVF process involves monitoring and stimulating a woman's ovulatory process, removing an ovum or ova from the woman's ovaries and letting sperm fertilize them in a liquid in a laboratory. The fertilized egg (zygote) is cultured for 2–6 d in a growth medium and is then
implanted in the same woman’s uterus, with the intention of establishing a successful pregnancy. All the patients signed an informed consent form prior to the start of the study. The study was approved by the institutional review board at Kufa University, Iraq.

The study excluded the patients with any obvious major systemic diseases including diabetes mellitus, hereditary diseases, or other endocrine disorders. Women were divided into two groups (pregnant and failure) according to the results of the IVF after few weeks of IVF process. Blood samples were collected from women before the operation. Women group that had conceived are expressed as “pregnant group” while women who hadn’t are expressed as “failure group.” All groups had treatment regimen depend on parameters: total gonadotrophin dosage, recombinant FSH 75–200 IU was started on day 2 of the cycle was continued until the day of final oocyte maturation trigger. Oocyte number and maturity are recorded after the embryologists spread each cumulus-oocyte complex immediately after oocyte retrieval.

Methods

Serum hormones: Luteinizing hormone (LH), Follicle stimulating hormone (FSH), (E2), Progesterone (PRG), (PRL) were measured using ready for use ELISA kits supplied by Monobind®, AMH was measured using ready for use ELISA kits supplied by Ansh Labs, USA. AZPA ELISA kit was supplied from Qayee Bio-Technology Co., USA.

Statistical analysis

The distribution types of the variable results were examined by using the Kolmogorov-Smirnov test. Analysis results divided the variables into two types, namely, normally distributed variables and nonparametric variables, according to the statistical distribution. For the normally distributed variables, the results were expressed as a mean±standard deviation. Pooled t-test was used for the comparison between the patients and control groups. Pearson’s correlation coefficients (r) were used to estimate the correlation between parameters. For the nonparametric variables, the results were expressed as medians in addition to (mean±standard deviation). The Mann-Whitney U test was used for the comparison between the patients and control groups. Spearman’s correlation coefficients (ρ, rho) were used to estimate the correlation between parameters. All statistical analysis was measured by using the SPSS Statistics Version 21 (2013) by IBM-USA.

RESULTS

1-Comparison between pregnant and failure groups

Fig. 1 showed the serum AZPA level in pregnant and failure groups. Serum AZPA showed the insignificant difference between groups.

![Fig. 1: Serum AZPA level in pregnant and failure groups](image)

The results of hormones in pregnant and failure groups are presented in table (1). The results showed a significant higher E2 (p=0.024) AMH (p=0.006), and lower PRL (p=0.002) in pregnant in comparing with failure group.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pregnant</th>
<th>Failure</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH (mIU/ml)</td>
<td>12.48±3.01</td>
<td>12.47±4.68</td>
<td>0.991</td>
</tr>
<tr>
<td>AZPA (ng/ml)</td>
<td>109.3±20.37</td>
<td>101.7±19.68</td>
<td>0.149</td>
</tr>
<tr>
<td>FSH (mIU/ml)</td>
<td>6.28±2.71</td>
<td>6.58±4.72</td>
<td>0.34</td>
</tr>
<tr>
<td>E2 (pg/ml)</td>
<td>567.8±319.11</td>
<td>461.1±245.47</td>
<td>0.024*</td>
</tr>
<tr>
<td>PRG (ng/ml)</td>
<td>10.96±6.56</td>
<td>20.3±69.62</td>
<td>0.470</td>
</tr>
<tr>
<td>PRL (ng/ml)</td>
<td>8.94±4.39</td>
<td>15.7±4.89</td>
<td>0.002*</td>
</tr>
<tr>
<td>AMH (ng/ml)</td>
<td>2.53±1.38</td>
<td>1.46±0.69</td>
<td>0.006*</td>
</tr>
</tbody>
</table>

*Significant difference (p<0.05) between pregnant and failure groups. Results are expressed as mean±SD, n=60 LH, luteinizing hormone; AZPA, anti-zona pellucida antibodies; FSH, follicle-stimulating hormone; E2, estradiol; PRG, progesterone; PRL, prolactin; AMH, anti-müllerian hormone.

2-Correlation between AZPA and hormones in pregnant group

The results in table 2 showed a significant correlation between AZPA and AMH hormone in pregnant group.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>LH</th>
<th>FSH</th>
<th>E2</th>
<th>PRG</th>
<th>PRL</th>
<th>AMH</th>
<th>BMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>AZPA</td>
<td>r</td>
<td>0.15</td>
<td>-0.16</td>
<td>0.95</td>
<td>0.74</td>
<td>0.52*</td>
<td>-0.01</td>
</tr>
<tr>
<td>p</td>
<td>0.41</td>
<td>0.54</td>
<td>0.39</td>
<td>0.06</td>
<td>0.46</td>
<td>0.05</td>
<td>0.95</td>
</tr>
</tbody>
</table>

*Significant correlation (p<0.05) between parameters in pregnant group, LH, luteinizing hormone; AZPA, anti-zona pellucida antibodies; FSH, follicle-stimulating hormone; E2, estradiol; PRG, progesterone; PRL, prolactin; AMH, anti-müllerian hormone; BMI, body mass index.

3-Correlation among parameters in failure group

The results in table 3 showed a significant correlation between AZPA and E2 hormone in the failure group.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>LH</th>
<th>FSH</th>
<th>E2</th>
<th>PRG</th>
<th>PRL</th>
<th>AMH</th>
<th>BMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>AZPA</td>
<td>r</td>
<td>0.05</td>
<td>0.50*</td>
<td>0.18</td>
<td>-0.40*</td>
<td>0.03</td>
<td>0.23</td>
</tr>
<tr>
<td>p</td>
<td>0.76</td>
<td>0.67</td>
<td>&lt;0.01</td>
<td>0.34</td>
<td>0.03</td>
<td>0.88</td>
<td>0.22</td>
</tr>
</tbody>
</table>

*Significant correlation (p<0.05) between parameters in failure group, LH, luteinizing hormone; AZPA, anti-zona pellucida antibodies; FSH, follicle-stimulating hormone; E2, estradiol; PRG, progesterone; PRL, prolactin; AMH, anti-müllerian hormone; BMI, body mass index.
DISCUSSION

The results in Table 1 are in disagreement with results of a study that showed that PRL within gonadotroph cells are controlled by dopamine, the main hypothalamic inhibitory regulator of PRL release in vivo, this specific actions of PRL within the gonadotroph and the cell signalling interactions that ultimately underlie hyperprolactinemia-induced infertility [9]; hyperprolactinemia can occur in physiological and pathological conditions [10] such as psychotic stress, severe mental illness or other causes, and the reproductive dysfunction affecting about one-third of infertile women [11].

Hyperprolactinemia is often associated with amenorrhea, anovulation, reduced libido, and orgasmic dysfunction in women, up to 20% of secondary amenorrhea in women is attributed to elevated PRL. PRL elevation lead to hypogonadism this in turn impaired gonad steroid secretion, which alters positive feedback effects at the hypothalamic and pituitary levels, lead to lack of gonadotropin cyclicity (FSH and LH levels decline) and to infertility [12]. Some argue that AFC (antral follicle count) may better reflect the specific damage to the operated ovary since this biomarker controls for the laterality of the injury. AMH, on the other hand, reflects the ovarian reserve of both gonads and can be influenced by compensation of the healthy ovary for the reduced reserve of the affected ovary [13].

Celik et al. (2012) have reported an increase in AFC 6 mo postoperatively despite a decrease in AMH [14]. The use of AMH and AFC as ovarian reserve markers before and after surgical treatment of endometriomas continues to be debated. In a recent meta-analysis study, Muzii et al. (2014) have challenged the conclusion that AMH declines after removal of an endometrioma [15].

FSH is not significantly changed in the present study between pregnant and failure groups with IVF-ICSI. FSH is frequently used in assisted reproductive technology (ART). The most commonly used protocol in ART consists of controlled ovarian hyperstimulation (COH) with daily injections of recombinant human FSH (rhFSH) to induce multiple follicle growths in the ovaries. To prevent premature oocytes from ovulation, LH surge, and premature ovulation gonadotropin-releasing hormone agonist or antagonist is injected daily. The pituitary down-regulation (endogenous pituitary suppression) that is achieved with analogs creates an environment where LH is deficient or very low and which may be detrimental to the development of normal healthy follicles. It has been shown that growing follicles become increasingly sensitive to and ultimately dependent on, the presence of LH for their development [15].

Ovulation is controlled by pregnancy hormones such as the pituitary gonadotropins, FSH, LH, PRG, and E2, and gives rise to the surge in LH which precedes ovulation [16].

To prevent prematurely, LH surge and premature ovulation agonist or antagonist are injected daily. The pituitary down-regulation (endogenous pituitary suppression) that is achieved with analogs creates an environment where LH is deficient or very low and which may be detrimental to the development of normal healthy follicles. It has been shown that growing follicles become increasingly sensitive to and ultimately dependent on, the presence of LH for their development [15].

Once PRG concentration has breached that compatible with a successful outcome, then the solution might be vitrification of all embryos and embryo transfer in the natural cycle [17]. This approach is supported by Melo et al. (2006) [18], who concluded that PRG rise does not appear to have a negative impact on ongoing pregnancy rate in oocyte donation program. This confirms the negative impact of PRG rise on the endometrium rather than the oocyte/embryo quality. Furthermore, Polotsky et al. (2009) [19] and Shapiro et al. (2010) [20], demonstrated that in cycles with elevated preovulatory PRG, the probabilities of implantation and ongoing pregnancy are increased if all 2-pronamolar oocytes are cryopreserved and subsequently thawed and cultured to the blastocyst stage before transfer. PRG should be measured in each cycle using appropriate assay methods and defined threshold values. Furthermore, the design of prospective randomized studies comparing embryo cryopreservation and transfer in a subsequent cycle in one arm and fresh transfer in the other arm, when PRG concentration is over 1.5 ng/mL seems to be necessary, in order to draw solid conclusions regarding the effect of PRG elevation on pregnancy outcomes.

There are many factors that may influence endometrial-embryo synchrony in fresh embryo transfer cycles. Endometrial factors such as duration of PRG before embryo transfer, the length of estrogen administration [21] and lack of ovarian stimulation with gonadotrophins as in fresh IVF-embryo transfer [23] may play a role. It is clear that supraphysiologic levels of E2 are inevitably attained during COH owing to the development of multiple ovarian follicles, each contributing significantly to E2 production which can reach levels up to 10 times or more those found during spontaneous cycles. The effect of such supraphysiologic E2 levels on the outcome of IVF-ICSI have remained controversial [23-25]. The purpose of the current study was to evaluate the association between E2 levels on the day of human chorionic gonadotrophin administration and pregnancy achievement in IVF cycles where gonadotrophin down-regulation was used.

The most recognized role for AMH measurement is prior to IVF because AMH levels can be predictive of ovarian response, namely poor and hyper-response [26]. However, it is currently not known if serum AMH levels also reflect oocyte quality and the chance of successful live birth pregnancy. Recent studies have shown that AMH may predict ovarian reserve and the subsequent success of IVF [27, 28]. However, other reports did not find any value for predicting pregnancy outcomes [29, 30].

The relatively high E2 level observed in these test subjects may be an indication of annulation consequent of defective follicular development, failure of the formation of Graafian Follicle in the ovary, or due to deterioration of corpus luteum and hence drop in Estrogen levels. Estrogens are normally produced by dominant follicle in the ovary [1].

CONCLUSION

It can be concluded that serum AZPA level does not differ between women who had success or failure IVF. However, in women with failure pregnancy in IVF, AZPA is positively correlated with E2 and it’s negatively correlated with PRL. In pregnant group, AZPA is positively correlated with AMH.

CONFLICT OF INTERESTS

Declared none

REFERENCES