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EXPLORATION OF ANTI-OVARIAN ANTIBODIES IN FEMALES UNDERGOING INTRAUTERINE INSEMINATION AND *IN VITRO* FERTILIZATION; A PRELIMINARY STUDY

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ABSTRACT

The current study has been carried to investigate the existence of anti-ovarian antibodies (AOA) in women undergoing intrauterine insemination (IUI) or *in vitro* fertilization (IVF). Serum samples from 21 women undergoing IUI, 24 women undergoing IVF and 25 fertile healthy controls were examined for the presence of anti-ovarian antibodies using ELISA method. AOA has been positive in 35.5% of infertile women, while none of the fertile controls were positive for AOA. The levels of these antibodies increased proportionally with the patient's age and the number of treatment attempts. Though the presence of AOA did not affect oocyte recovery rate, it resulted in decreased fertilization rate, cleavage rate, and pregnancy rate in infertile women. Moreover, these antibodies may also stuck pregnancy after IVF or IUI procedure as they attack the fertilized ova and thus ended up with a lower pregnancy rates. The study concluded that anti-ovarian antibodies might be a cause of premature ovarian failure in females who did IUI or IVF procedures. Also advanced age of women is not associated with lower pregnancy rates after IVF. that anti-ovarian antibodies may be a cause of infertility and existence of these antibodies could have adverse effects on the outcome of assisted reproductive techniques.

KEYWORDS: In vitro fertilization, AOA, intrauterine insemination, Sudan.

INTRODUCTION

Premature ovarian failure (POF) is a term usually used to describe women younger than 40 years of age who present with amenorrhea. Diagnosis of POF is on the basis of follicle stimulating hormone levels in the menopausal range associated with amenorrhea before the age of 40. Women diagnosed with the POF suffered from anovulation and hypoestrogenism and presented with primary or secondary amenorrhea, infertility, sex steroid deficiency, and elevated gonadotropins (Kalantaridou *et al*., 1998). POF is the causative factor in 10-28% of women presenting with amenorrhea and in 4-18% with secondary amenorrhea (Coulam *et al*., 1986; Anasti, 1998). Two mechanisms are probably involved in POF, namely follicle dysfunction and follicle depletion. The existing follicles in the ovary, in follicle dysfunction, do not function normally due to some pathological process such as for e.g. FSH-receptor mutation (Aittomäki, 1995). On the other hand, in women with follicle depletion there are no primordial follicles probably due to inadequate initial pool of primordial follicles or destruction of follicles due to toxins or autoimmune mechanisms (Nelson, 2009). Variety of possible causes reflects the heterogeneity of POF. None of the causes seem to predominate. In the majority of the cases, the cause of POF is unknown. The number of women with POF is increasing. Some of the causes can lead to complete absence of oocytes, and others can lead to inability of follicles to mature or to disordered folliculogenesis. The causes could be chromosomal, genetic, autoimmune, metabolic (galactosemia), infection (mumps), and iatrogenic (Goswami and Conway, 2005).

Among the several mechanisms that account for the pathogenesis of spontaneous POF, genetic and autoimmune mechanisms play a major role. Anti-ovarian antibodies (AOA) is another aggressive Th1 autoimmune disorders, the antibodies attack healthy ovarian tissue. This means that there is damage being done to the follicles and eggs in the ovaries, and is linked to a number of infertility problems like POF. Strong support for an autoimmune character of isolated POF would be the presence of antibodies to ovarian structures or AOA in the serum of these patients. Evidence favors the presence of an autoimmune disease of the ovary. In some cases AOA has been considered to be a suitable marker for identification of autoimmune POF (Luborsky *et al*., 1990; Fénichel *et al*., 1997; Pires *et al*., 2006) as well as immunological- related infertility in "*in vitro*" fertilization (IVF) patients (Gobert *et al*., 1992; Barbarino-Monnier *et al*., 2003; Pires *et al*., 2007).

Patients and samples

Seventy females who attended different fertility centers in Khartoum were selected in this study. 25 age-matched normal women with normal pregnancy were also included for control purposes. Women were subjected to clinical interview and serum samples were collected.

Determination of anti-ovarian Antibodies

Anti-ovarian IgMs and IgGs were investigated using a commercially purchased ELISA kit (Euroimmun, Luebeck, Germany) and the test was performed according to manufacturer instructions. Briefly, 50 µl of each patient's serum was added to each well and the plate was incubated at 37°C for 1 hour. The plate was washed 3X and 50 µl of the conjugate was added before incubation took place for 1 hour and then washed. 50 µl of Substrate was added and the plate was left for 30 minutes in dark. The reaction was stopped by adding 50 µl of stop solution and the optical density was read using ELISA reader at wave length 450 nm.

RESULTS

Out of the 70 females examined, 21 (46%) of them did intrauterine insemination and 53% did *in vitro* fertilization. The mean age of the studied women was 31.7 ± 6.6 years (age ranged between 16-45 years). Anti-ovarian antibodies were detected in 35.5% of infertile women, while none of the fertile controls was AOA positive. Women with higher levels of anti-ovarian antibodies aged between 40-45 years, Figure 1.

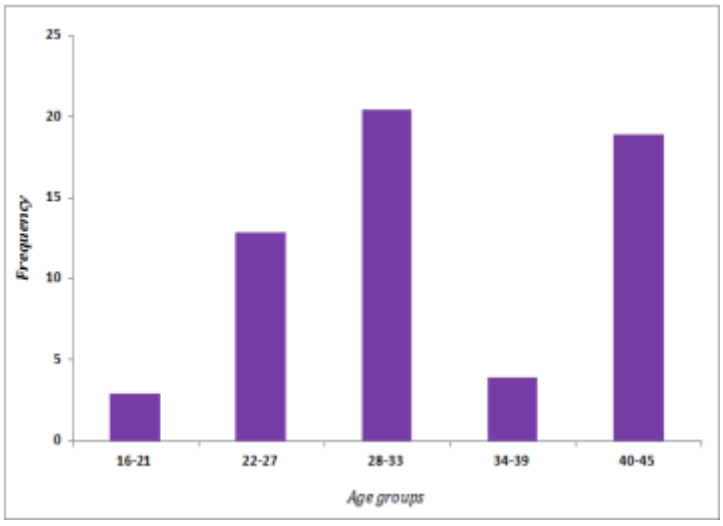

Figure 1: Anti-ovarian antibodies in studied women according to their ages.

Instead of similar parameters, the present study showed that IVF is a procedure that remains preferable and commonly performed in fertilization centers in Khartoum compared to IUI, Figure 2.

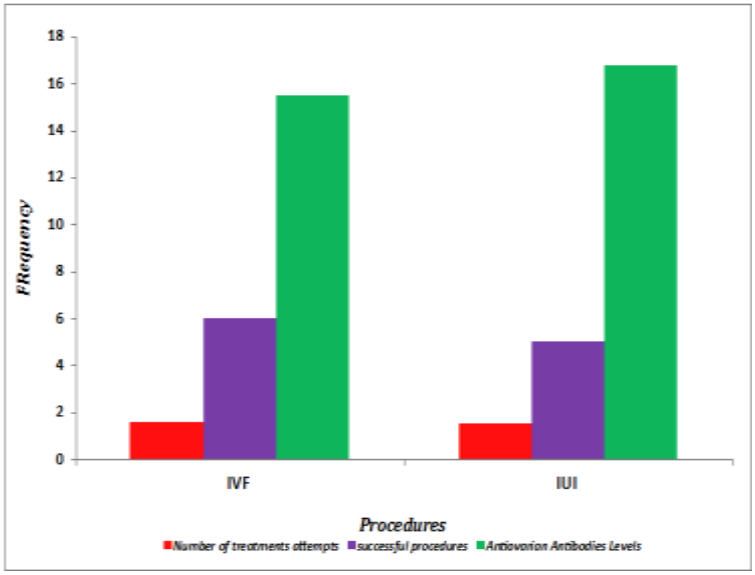

Figure 2: Comparison of anti-ovarian antibodies according to procedures.

DISCUSSION

Normal fertility is highly dependent on female age. It is also well established that the outcome of couples treated by *in vitro* fertilization (IVF) and embryo transfer is significantly influenced by advancing maternal age. It is therefore a logical assumption that the outcomes after IVF and embryo transfer with ICSI would be similarly affected by maternal age. It has been demonstrated previously that maternal age influences pregnancy outcome after ICSI (Oehninger *et al.*, 1995; Devroey *et al*., 1996). In Sudan, the prevalence of females infertility is 11.5%, 2.7% with primary infertility and 8.8% are those who perform IVF. The first IVF babies were twin females, delivered on 15th of April 2001, with birth weights of 2.56 and 2.5 kg. Since then 300 babies were delivered. Sudan Assisted Reproductive Center is the second one in Sudan since 2000 and the first outcome babies (a twin of one male and one female and a singleton male) were delivered on March 2004 with weights of 2.1, 2.5, 2.5kg respectively (Gaily *et al*., 2010).

The results of the present study revealed that the majority of those who reported to fertility centers were women at mid ages although some at other ages were also seen. These results are conforming to other findings that reported *in vitro* fertilization (IVF) in both young and older women at various successful rates (Baart *et al*., 2005). In Sudan no age-dependent

marriage i.e. some tribes and/or families believe in early marriage, that why women with younger ages were found to undergo IVF. Such cases are also common in many parts of the world and particularly in Africa (Sighn *et al*., 1996; Goldman, 1986). It has been known that anti-ovarian antibodies are detected commonly by fluorescent method, which lacks enough specificity and sensitivity. This study has carried out ELISA to attain proper results with higher sensitivity and specificity (Novosad *et al*., 2003). Autoimmune premature ovarian failure (POF) has been considered to be a mechanism possibly responsible for primary idiopathic failures. Several studies revealed the association of anti-ovarian antibodies (AOA) with POF. The incidence of AOA in patients with POF ranges widely (20-67%), according to the type of antibody, methods used for selection of patients and measurement of auto antibodies (Fenichel *et al*., 1997; Wheatcroft *et al*., 1997). In the current work using an ELISA, a 35.5% of circulating AOA has been detected in sera of a group of 45 patients with primary POF compared to negative readings for all the control samples. A number of investigators showed an association between infertility and ovarian antibodies i.e. ovarian antibodies can be a cause of premature ovarian failure (Moncayo *et al*., 1989; Meyer *et al*., 1990; Gava *et al*., 1994; Nip *et al*., 1995; Ebbiary *et al*., 1994). Furthermore, these antibodies may also stuck pregnancy after IVF or IUI procedure as they attack the fertilized ova and thus ended up with a lower pregnancy rates (Gava *et al*., 1996a).

CONCLUSION

In conclusion, we have shown that anti-ovarian antibodies might be a cause of premature ovarian failure in females who did IUI or IVF procedures. These antibodies can be triggered after these procedures and their level increases proportionally with patient age and number of treatments attempt.

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