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INFERTILITY IN FEMALES IN CONTEXT OF ANTISPERM ANTIBODIES IN PELVIC INFLAMMATORY DISEASE (PID) AND POLYCYSTIC OVARIAN SYNDROME (PCOS)

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ABSTRACT
The present study has been designed to evaluate the various causes of female infertility prevailing among Central Travancore women and to relate the incidence of antisperm antibodies (ASA) with altered hormonal profile in these infertile cases. A total of 500 women (21 – 35) who were subjected to infertility treatment at various infertility clinics in Kottayam, Pathanamthitta and Alappuzha districts and hundred normal healthy women (20 – 35) with proven fertility at the luteal phase were taken as subjects. The data were collected from hospital records as well as using an investigator administered questionnaire. The clinical studies revealed that polycystic ovary syndrome (PCOS) is the major cause of female infertility (33.6 %). High incidence of ASA was observed in various infertile cases. The ASAs were found in 13.8 % of the total infertile cases. Pelvic inflammatory disease (PID) constituted the maximum of cases (32%) followed by unidentified cases (25.71 %), polycystic ovary syndrome (PCOS) (22.6%) and other infertile cases (5.14%). The control group showed 5% incidence of ASAs. Markedly higher incidence of ASA in cases of PCOS as well as in PID, the increased insulin resistance with higher level of blood glucose, points to an enhanced production of ASA due to the insulin mediated endocrine disruption. This study points to an insulin dependent endocrine-immune disruption in cases of female infertility.

INTRODUCTION

Infertility is a commonly encountered situation occurring more or less equally in both sexes, the cause and prevalence rate of which may vary. Overall factors responsible for infertility comprise 30 - 40% in the male, 40 - 55% in the female, and 10% in both partners. In ~10% cases the causes of infertility remain unexplained. Immunological incompatibility may be the etiology of the infertility in some of the patients with unexplained infertility. Immunological infertility is assumed to be the cause of infertility in 9 - 20% of the concerned couples with unexplained infertility.

Female infertility can be affected by diseases or dysfunctions of reproductive tract, neuro endocrine system and immune system. The concept that an adverse immune response to certain tissues in the reproductive system causes female infertility is now more recognized than before (Kayama, 2005; Diekman *et al*., 2000). Consequently, the focuses are intensifying on the origin of endocrine and immunological imbalances and their subsequent consequences in the reproductive function (Peters and Coulam, 1992). Hormonal imbalance and immune dysfunction are thought to influence each other and bring about infertility. The anti sperm antibody (ASA) is one of the many immunological markers that gets consideration in evaluating immunological infertility (Kipersztok *et al*., 2003; Heidenreich *et al*., 1994). It has been shown that both male and female can make antibodies that react with human sperm and it should, therefore, be considered as a potential fertility parameter. Sperm immobilization, inhibition of cervical mucus penetration and interference with events that lead to sperm-oocyte binding are some of the mechanisms by which ASAs impede fertilization (Tasdemir *et al*., 1995; Francavilla *et al*., 1997**)**. On the other hand, review of numerous retrospective and prospective analyses of pregnancy rates for couples with circulating ASA leads one to question the prognostic value of ASA screening for diagnosis of infertility. Probably, infertility practioners across the world usually exclude ASA assay in screening infertility patient. This has created a scarcity of data on ASA in the current literature (Kayama, 2005). Without sufficient information on ASA, the true relevance of ASA in female infertility remains undetermined. Therefore, in this study, we have investigated the incidence of ASAs in various causes of female infertility prevailing among Central Travancore women and have been made an attempt to relate altered hormonal profile with immunological dysfunction in PCOS and PID cases.

MATERIALS AND METHODS

This was a prospective study done from 2004 - 2010 in specialist infertility clinics in Kottayam, Pathanamthitta and Alappuzha districts. The specialist infertility clinics selected for our study were Abraham's Infertility Research and Gynaec Centre Athakkatt Chambers, Changanacherry, Kottayam

District, Kerala, India, and Lifeline Hospital, Adoor, Pathanamthitta District, Kerala, India. A total of 500 women at reproductive age (21 - 35) who admitted with complaints and symptoms suggesting infertility problems were evaluated. Hundred healthy volunteer females (20 – 35) with proven fertility at the luteal phase were taken as control. The data were collected from hospital record as well as using an investigator administered questionnaire.

The results of ASAs, follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), thyroid stimulating hormone (TSH), thyroxine (T_4), tri - iodo thyroxine (T_3), progesterone, and total testosterone of several blood tests were collected. The hormonal levels were estimated using RIA technique and ASAs were assayed using immunobead assay. The data were subjected to statistical analysis using Student's 't' test.

RESULTS

Table 1 represents the various causes of infertility among 500 patients. The hospital records revealed that in six years (2005-2010), PCOS constituted the highest percentage of all infertile cases (33.6%). The other causes were fibroid uterus (9.8%), primary amenorrhea (4.8%), endometriosis (2.2%), pelvic inflammation (5%), para ovarian cysts (5%), multiple factors (2.4 %), unidentified cases (7%) and male factors (30.2%).

Table 2 represents incidence of ASAs in various infertile cases. Highest incidence of ASAs was observed in PID followed by unidentified cases and PCOS. Total 500 infertile females were studied for presence of ASAs. 25 patients were suffering from PID, 168 were of PCOS and 9 were with unexplained infertility. Amongst the 25 PID cases, 32% were positive for ASA and in PCOS cases the incidence of ASA was 22.61%. In unexplained cases, 25.71% were positive for ASA. In the remaining 272 cases, 5.14% were positive for ASA. The ASAs constituted 13.8% of the total cases where as the control group showed 5% incidence of ASA.

Table 1: The causes of infertility among 500 patients

Causes	No. of cases	% cases
PCOS	168	33.6%
Fibroid uterus	49	9.8%
Primary amenorrhea	24	4.8%
Endometriosis	11	2.2%
PID	25	5%
Para ovarian cysts	25	5%
Multiple factors	12	2.4%
Unidentified cases	35	7%
Male factors	151	30.2%

Table 2: The incidence of ASAs in various infertile groups

Causes	Incidence of ASAs	% incidence
PID	8 / 25	32%
PCOS	38 / 168	22. 61 %
Unidentified cases	9 / 35	25.71%
Other infertile cases	14 / 272	5.14%
Total infertile cases	69 / 500	13.8 %
Control	5 /100	5%

Normal value (0 – 20Iu/mL); Infertile cases (3 – 45Iu/mL)

Table 3: The hormonal profile in infertile and control groups

Hormone	Normal range	Control	Infertile
FSH(mIu/mL)	1.6 – 8.7	4.97 ± 0.83	6.25 ± 2.88
LH(mIu/mL)	0 -6.0	5.00 ± 0.79	4.77 ± 2.84
Insulin(μIU/mL)	2 - 8.5	10.27 ± 2.42	*39.61 ± 5.2
Prolactin(ng/mL)	3.0 -20.0	21.95 ± 2.41	*99.09 ± 8.34
Estradiol(pg/mL)	50 -155	102.5 ± 20.6	*228.61 ± 268.53
Progesterone (ng/mL)	1.6 – 21.0	13.00 ± 2.10	*18.61 ±8.78
TSH(uIu/mL)	0.3 -5.0	1.88 ±0.49	*5.48 ± 2.3
T3(ng%)	80 -180	107.5±17.16	*140.27 ± 20.08
T4(ug%)	4.5 -11.5	9.27±1.68	*18.23 ± 9.63

Values are mean ±S.E; *Significant ($p < 0.05$)

Table 3 represents the hormonal profile in infertile and control groups. It was observed that out of 500 cases, only20 patients showed normal LH: FSH ratio of 1:1. About 82 patients have LH: FSH ratio greater than 1: 1 and the remaining have ratio less than 1: 1. Prolactin, estrogen and progesterone and insulin levels were significantly ($p < 0.05$) increased in infertile groups when compared to control. TSH, T_3 and T_4 levels were also found to be significantly ($p < 0.05$) higher in infertile groups when compared to control.

Table 4 represents the hormonal profile in PCOS and control groups. Out of the total infertile cases, 168 patients were reported to have PCOS. In PCOS, serum FSH and LH levels were found to be significantly ($p < 0.05$) increased when compared to control. The LH: FSH ratio in PCOS cases is 3: 2 and in control the ratio is 1: 1. The estrogen level was significantly ($p < 0.05$) increased and progesterone level was significantly ($p < 0.05$) decreased in PCOS when compared to control. In PCOS, there was a significant ($p < 0.05$) increase observed in the levels of insulin, prolactin and testosterone in comparison to the control. The levels of TSH and T_3 did not alter significantly in PCOS and control groups. However, T_4 level was found to be to be significantly ($p < 0.05$) increased in PCOS when compared to control. An increase in blood glucose level was observed in PID cases ($p < 0.05$) and PCOS when compared to control.

DISCUSSION

The present study was undertaken to gain insight into the prevalence of various causes of female infertility in Central Travancore women. Our study also explored the possible association between the incidence of ASAs and the hormonal imbalances and thereby an immune endocrine disruption in these cases.

PCOS is the most common cause of female infertility with 33.6% incidence. PCOS is a highly prevalent hormonal and metabolic disorder among reproductive aged women worldwide. It occurs in approximately 5% to 10% of the female population and may be the leading cause of infertility in reproductive age (Knochenhauer *et al*., 1998; Azziz *et al.,* 2004). PCOS is characterized by insulin resistance, obesity, chronic anovulation, hyperandrogenism, polycystic ovaries and decreased fertility (Ehrmann *et al*., 1995; Frank, 1995; Azziz *et al.,* 2004). In the long term, PCOS is associated with

Table 4: The hormonal profile and blood glucose level in PCOS and control groups

Hormone	Control	PCOS	PID
FSH(mIU/mL)	4.97 ± 0.83	*12.30 ± 9.5	8.25 ± 2.88
LH(mIU/mL)	5.00 ± 0.79	*18.4 ± 7.4	8.77 ± 2.84
Estradiol(pg/mL)	102.5 ± 20.6	*185 ± 23.13	*83.61 ± 5.2
Progesterone(ng/mL)	13.00 ± 2.10	*7.11 ± 4.2	*19.09 ± 8.34
Prolactin(ng/mL)	21.95 ± 2.41	*26.67 ± 8.2	*28.61 ± 268.53
Insulin(μIU/mL)	14.27 ± 2.92	*35.61 ± 5.3	*24.61 ±8.78
Testosterone(ng/dL)	46.70 ±12.21	*77.18 ± 14.45	48.2 ± 2.3
TSH(μlu/mL	1.88 ±0.49	1.72 ±0.34	1. 40± 0.32
T3(ng%)	107.5±17.16	99.62 ± 11.67	*118.23 ± 9.63
T4(ug%)	9.27±1.68	*18.90 ± 2.6	16.25 ± 2.88
Blood Glucose (mg/100mL)	97.8 ± 3.64	143. 35 ± 6.9	*154.45 ±12. 83

Values are mean ±S.E; *Significant ($p < 0.05$)

an increased risk of Type 2 diabetes, cardiovascular disease, dyslipidemia and endometrial cancer (Ehrmann *et al*., 1995). Therefore, the syndrome is recognized as having a major impact throughout life on the gynecological and metabolic health of women. Recently, the number of women with PCOS has increased worldwide (ACOG, 2006). Researchers attributed that this increased incidence of PCOS was apparently due to change in the life style factors. The socio economic studies carried out in this patients reveal that they belong to high income families and have a high intake of dietary sugar (AACE, 2005).

Uterine fibroids of the present study are very common non cancerous growths that develop in the muscular wall of the uterus. They are clinically obvious in 20 – 25% of women of reproductive age (Goodwin *et al*., 2008**)**. For women with endometriosis, the monthly fecundity diminishes by 12 to 36%. Current studies demonstrate that pregnancies are not improved by treating minimal endometriosis (Harrison and Barry-Kinsella, 2000).

PID was more than twice as common in women with a history of sexually transmitted diseases (26 per cent) than among women who had never reported an STD (10 per cent). The relationship between PID and infertility is relatively well accepted. PID causes infertility by the scarring process that occurs during the healing of sexually transmitted infections in our study, about 20 -30% of the patients with unidentified cases remain infertile even after 9 years of attempting to conceive. Dysregulation in immune system reactions with enhanced production of autoantibodies and ASAs is putative etiologic candidate for this group of patients (Haller *et al*., 2006**)**

Ovarian cysts are common and, in the vast majority of cases, they are benign (non-cancerous) in patients younger than 35. Primary amenorrhea is the failure of menses to occur by age 16 years, in the presence of normal growth and secondary sexual characteristics. Multiple factors for infertility include hormonal deficiencies, problems in the reproductive organs and some illness. Some of the male factor problems are identifiable and reversible, such as ductal obstruction and hypogonadotrophic hypogonadism. Other conditions are identifiable, but not reversible such as bilateral testicular atrophy secondary to viral orchitis

In the present study, increased incidence of ASAs was observed in PID, unidentified cases and PCOS. Another study (Varuni *et al*., 2011) also reported increased incidence of ASAs in PID (1 /5, 20% incidence). Markedly higher association of ASA was observed in females with PID. However, the precise mechanism of generation of ASAs is yet to be discovered. The female reproductive tract is unique in that it needs to protect itself against invading pathogenic organisms while not mounting a significant destructive immune response against allogenic sperm cells and the developing concep-tus. The female reproductive tract is not an immunoprivi-leged site but that the immune system functions locally a protective role against infectious agents and in the establishment and maintenance of pregnancy but adversely on fertility by the generation of ASAs (Naz and Menge, 1994**)**. The mechanical or chemical disruption of the female genital tract mucosal layer or sperm penetration into the mucosal membrane of genital systems may permit ASA formation (Novak and Berek, 2007**)**. PID patients in our study have diabetes mellitus type II and urinary tract infections. In these patients, long term IR developed into diabetes mellitus type II and cause inflammation in the female genital tract and may elicit a response from the immune system by producing ASAs. No other hormonal imbalances related to IR was not found in PID cases.

In our investigations, no obvious cause of infertility was found for 7% of cases with 25.71% incidence of ASAs. It has been found that dysfunction of the immune system and associated production of ASAs play an important role unexplained and various other infertile cases. Earlier studies also showed the highest prevalence of ASAs in women with symptoms suggesting of PCOS like hyper prolactinemia, hypertension, hypercholesterolemia and diabetes mellitus - type II (Varuni *et al*., 2011). PCOS patients showed diabetes mellitus - type II and hyper prolactinemia with increased prevalence of ASAs in this study.

The increased prevalence of ASAs in PCOS and other infertile cases in our study can be attributed to the hormonal imbalances observed in these cases. In our study, inappropriate gonadotropin secretion (altered LH: FSH ratio) cause abnormal steroid hormone (estrogen, progesterone andtestosterone) synthesis in PCOS and other infertile cases. The altered hormonal levels stimulate the formation of ASAs through cytokine action and humoral immunity.

In the present study, increased level of insulin, LH, estrogen and testosterone with low progesterone were observed in PCOS as well in PID. The altered hormonal profile in these cases was due to IR stimulated in these patients. An estimated

50% to 70% of women with PCOS are insulin resistant and experience weight gain, difficulty in losing weight, hypoglycemic episodes and intense cravings for carbohydrates (Michel more *et al*., 2001**)**. The exact mechanisms of IR in PCOS is unclear. The pituitary and ovaries of a patient who is insulin resistant may be stimulated by higher levels of insulin (Nestler, 1999). This results in increased leptin levels and a preferential increase in LH, but not FSH levels. The net effect of these changes is to stimulate the partial development of follicles that secrete supernormal levels of estrogen and testosterone, but which rarely ovulate. The menstrual cycle is dominated by increased estrogen and androgen production without progesterone. These changes are exacerbated by insulin-induced reduction in sex hormone binding globulin (SHBG), from liver which amplifies ovarian testosterone production (Wu *et al*., 2000). In PCOS, it is observed that insulin induced alterations in the hormonal profile in the brain and the ovaries can interfere with immune system, which stimulate the formation of ASAs through cytokine action and humoral immunity. No correlation was found between thyroid hormones and ASAs in PCOS and other infertile cases.

Earlier, it was shown that exogenous administration of estradiol in the rats significantly increased the IgA and IgG type of antibodies in the uterus, suggesting estradiol-dependent development of specific antibodies in the reproductive tract (Wira and Stern, 1991). Steroids, such as estrogen and progesterone, are known to regulate lymphocyte proliferation through cytokine action (Grekova *et al*., 2002) and participate in humoral immune responses by modulating antibody synthesis (Canellada *et al*., 2002). Higher estrogen and progesterone levels may be responsible for higher levels of antibodies in the reproductive tract, which might ultimately hamper the motility of spermatozoa.

In our study markedly higher incidence of ASA was observed in females with PID, unidentified cases and PCOS. In PCOS, the hormonal imbalances in brain and ovaries induced by the insulin resistance stimulated increased production of ASAs. In PID, increased risk of diabetes mellitus - type II elicit immune responses by producing ASAs. Immune-regulation is a complex process, which involves both the central nervous system and endocrine system. The reason for this immune endocrine dysfunction is not known. The altered hormone synthesis leads to either stimulation or inhibition of certain protein molecules such as cytokines which stimulate the production of antibodies against spermatozoa (Wira and Stern, 1991). Further studies are required to understand the underlying mechanism. Increasingly, immune and endocrine systems are seen as an intertwined web of signals and responses, mediated by often identical messengers of information. Nowhere is this more apparent than in the fields of neuroendocrinology and neuroimmunology.

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REFERENCES

AACE: American Association of Clinical Endocrinologists. 2005. Position statement on metabolic and cardio vascular consequences of polycystic ovary syndrome. *Endocrine Practice*. **11(2):** 126-134

ACOG: American College of Obstetricians and Gynecologists. (2002, reaffirmed 2006). Management of infertility caused by ovulatory dysfunction. ACOG Practice Bulletin No.34. *Obstet. Gynecol* .**99 (2):** 347 – 358

Azziz, R., Woods, K. S., Reyna, R., Key, T. J., Knochenhauer, E. S. and Yildiz, B. O. 2004. The prevalence and features of the polycystic ovary syndrome in an unselected population. *J. Clin. Endocrinol Metab*. **89:** 2745-2749

Canellada, A., Blois, S., Gentile, T. and Margni Idehu, R. A. 2002. In vitro modulation of protective antibody responses by Estrogen, Progesterone and Interleukin-6. *Am. J. Reprod. Immunol*. **48:** 334-343.

Diekman, A. B., Norton, E. J., Westbrook, V. A., Klotz, K. L. and Herr, J. C. 2000. Anti-sperm antibodies from infertile patients and their cognate sperm antigens: A Review. *Amer. J. Reprod. Immunol*. **43:** 134-143.

Ehrmann, D. A., Barnes, R. B. and Rosenfield, R. L.1995. Polycystic ovary syndrome as a form of functional ovarian hyperandrogenism due to dysregulation of androgen secretion *Endocr. Rev*. **16:** 322-353.

Francavilla, F., Romano, R., Santucci, R., Marrone, V., Properzi, G. and Ruvolo, G. 1997. Interference of antisperm antibodies with the induction of the acrosome reaction by zona pellucida (ZP) and its relationship with the inhibition of ZP binding. *Fertil. Steril*. **67:** 1128–1133.

Frank, S. 1995. Polycystic ovary syndrome. *New Engl J. Med*. **333:** 853-861.

Goodwin, S. C., Spices, J. B., Worthington-Kirsch, R., Peterson, E., Pron, G., Li, S. and Myers, E. R. 2008. Uterine artery embolization for treatment of leiomyomata : Long term outcomes from the Fibroid Registry. *Obset. Gynecol*. **111(1):** 22-33.

Grekova, S. P., Vodyanik, M. A. and Chernyshov, V. P. 2002. The effect of progesterone and estrogen on proinflammatory cytokine co-stimulatory proliferative activity. *Am. J. Reprod. Immunol*. **48:** 147-147.

Haller, K., Sarapik, A., Talja, I., Salumets, A. and Uibo, R. 2006. Controlled ovarian hyperstimulation changes the prevalence of serum autoantibodies in in vitro fertilization patients,. *Amer. J. Reprod. Immunol*. **56:** 364-370.

Harrison, R. F. and Barry-Kinsella, C. 2000. Efficacy of medroxyprogesterone treatment in infertile women with endometriosis: a prospective, randomized, placebo-controlled study. *Fertil. Steril*. **74:** 24 -28.

Heidenreich, A., Bonfig, R., Wilbert, D. M. and Engelmann, U. H. 1994. Risk factors for anti-sperm antibodies in infertile men. *Amer. J. Reprod. Immunol*. **31:** 69-76.

Kayama, K. 2005. Immunologic tests: anti-sperm antibody a review. *Japanese J. Clin. Med.* **63(7):** 581-584.

Kipersztok, S., Kim, B. D., Drury, K. C., Williams, R. S. and Rhoton-Vlasak, A. 2003. Validity of a rapid assay for anti-sperm antibodies in semen. *Fertil. Steril* . **79(3):** 522-528.

Knochenhauer, E., Key, T., Kahsar-Miller, M., Waggoner, W., Boots, L. and Azziz, R. 1998. Prevalence of the polycystic ovary syndrome in unselected black and white women of the Southeastern United States; a prospective study. *J. Clinical Endo. Crinol. Metab*. **83:** 3078-3082.

Michel more, K. L., Balen, A. H. and Dunger, D. B. 2001. Polycystic ovaries and eating disorders: Are they related? *Hum. Reprod*. **16(4):** 765 – 769.

Naz, R. K. and Menge, A. C. 1994. Antisperm antibodies: origin, regulation, and sperm reactivity in human infertility. *Fertil. Steril*. **61:** 1001–1013.

Nestler, J. E. 1999. Insulin resistance effects on sex hormones and ovulation in the polycystic ovary syndrome. In Reaven, GM, Laws A, (Eds). Insulin Resistance: The Metabolic syndrome. Human Press; Totowa, N. J. pp. 347- 365.

Novak, E. and Berek, J. S. 2007. Berek and Novak's gynecology; Infertility 14th Ed. Philadelphia Williams and Wilkins. 1221-1222.

Peters, A. J. and Coulam, C. B. 1992. Sperm Antibodies: Review. *Amer. J. Reprod. Immunol.* **27:** 156-162.

Tasdemir, I., Tasdemir, M., Fukuda, J., Kodama, H., Matsui, T. and Tanaka, T. 1995. Effect of sperm-immobilizing antibodies on the spontaneous and calcium-ionophore (A23187)-induced acrosome reaction. *Int. J. Fertil*. **40:** 192–195.

Varuni, T., Surangi, G. Y. and Deepal, S. W. 2011. Possible risk factors for the formation of antisperm antibodies in a subfertile population. *Sri Lanka J. Obstet. Gynaecol*. **33:** 12-19

Wira, C. R. and Stern, J. 1991. Endocrine regulation of the mucosal immune system in the female reproductive tract: Control of IgA, IgG and secretory component during the reproductive cycle, on implantation and throughout pregnancy. In: Pasqualini JR, Scholler R(Eds), Hormones and Foetal Patho-physiology . Pp 343-367. Marcel Decker Inc. New York, USA.

Wu, X., Sallinen, K., Zhou, S., Su, Y., Pollanen, P. and Erkkola, R. 2000. Androgen excess contributes to altered growth hormone/insulin-like growth factor-1 axis in nonobese women with polycystic ovary syndrome. *Fertil. Steril*. **73:** 730-734.