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Antispermatozoal Antibodies in Human Follicular Fluid

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ABSTRACT: **The possibility of antispermatozoal antibodies in women having significant effects in the higher regions of the female reproductive tract has been investigated. Follicular fluids (FF) and sera taken at the time of oocyte recovery from women undergoing in vitro fertilisation and embryo transfer (IVF-ET) were tested for the presence of antispermatozoal antibodies, and the concentrations of IgM, IgG, IgA, and complement C_3 were determined. The concentrations of immunoglobulins and C_3 in FF were consistent with transudation from serum inversely proportional to molecular weight. Titres of agglutinating and immobilising antibodies in FF were usually one or two dilution steps below those of serum except where immobilising activity was associated with IgM. IgG:IgA ratios were lower in FF from women with antispermatozoal antibodies, suggesting local production or enhanced transudation of IgA; however, a secretory component could not be detected in any of the follicular fluids in this study. Two women with antispermatozoal antibodies and infertility in excess of 5 years had successful IVF-ET and have delivered healthy infants. (Am J Reprod Immunol Microbiol. 1985; 7:113–117.)**

Key words: Antispermatozoal antibodies, follicular fluid, in vitro fertilisation, IgG and IgA ratios

INTRODUCTION

Immunity to spermatozoa, either isoimmunity or autoimmunity, is now accepted as being a cause, or a contributory factor, in human infertility. We have reported significant levels of antisperm antibodies in about 8% of the female partners of infertile couples examined.[1] Tests for antispermatozoal antibodies are generally performed on sera. Statistical analyses of results in surveys indicate that spermagglutinating activity at a titre greater than 32 in the gelatin agglutination test (GAT) will have a significant deleterious effect on fertility. Complement-dependent sperm immobilising antibodies are considered by many workers to correlate more closely with infertility than spermagglutinating antibodies, particularly in women. Since the testing procedure is less sensitive, much lower levels than a titre of 32 are commonly regarded as being significant.[2,3]

Cervical mucus from the human female contains appreciable concentrations of serum proteins, including immunoglobulins, and, in addition, the cervix has been shown to have local immune capacity both in concert with, and independent of the circulation.[5,6] Antispermatozoal antibodies have been detected in both the serum and the cervical mucus of some women and, in others, in the mucus alone.[7,8] The way in which such antibodies influence fertility is not well understood. However, Kremer has produced good evidence, confirmed by a number of workers, of a block to sperm progression through cervical mucus caused by antibodies against spermatozoa, particularly those of the IgA class.[9,10]

Immunosuppression, with consequent reduction in the levels of antispermatozoal antibodies, can be a successful procedure in restoring fertility to women with antispermatozoal antibodies.[11,12] In addition, other treatments, such as removing the immunogenic source by abstinence, using condoms, or avoiding the influence of antibodies in cervical mucus with intrauterine insemination, have had limited success.[10,13,14] Therefore, it seems that antispermatozoal antibodies may act higher in the tract than the cervix. Little is known of the immune capacity of the upper regions of the female tract. Immunoglobulins have been detected in the uterus and fallopian tubes, but at concentrations much lower than those in serum. Additionally, complement components have been detected but at levels that would appear to have little effect.[15]

There is an increasingly firm body of evidence from in vitro studies which indicates that some antispermatozoal antibodies are able to inhibit the attachment of spermatozoa to the zona pellucida.[16,17] Consequently, antispermatozoal antibodies in the higher parts of the female tract, at concentrations which are unable to produce agglutination or immobilisation, nevertheless may still have a significant effect on fertility.

This study presents the results of investigations of immunoglobulin and complement component C_3 levels, and of antispermatozoal antibody titres in the sera and follicular fluid collected from women undergoing in vitro fertilisation and embryo transfer (IVF-ET) at the University of Western Australia/Pivet Laboratory/Cambridge Hospital programme in Perth, Western Australia.

MATERIALS AND METHODS

Patients

Sera and follicular fluids were collected from patients at the time of oocyte recovery for IVF-ET. Sera were collected by venepuncture and follicular fluid was collected undiluted and only used in this investigation if confirmed microscopically to be free of blood contamination. The procedures for IVF-ET and follicular fluid collection have been reported elsewhere.[18,19] The follicular fluids in the control group were not chosen to match those from women with antispermatozoal antibodies in steroid hormone concentrations. In all patients, however, ovulation was triggered by 5000 units of human chorionic gonadotropin administered intramuscularly when the diameter of the leading follicle was 1.8 cm or greater and the average oestrogen production per large

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follicle was about 1500 pmol/L. All sera and follicular fluid were stored at −20°C and heated to 56°C to inactivate native complement before testing for antisperm antibodies.

Screening of couples for antispermatozoal antibodies was one of the initial investigations undertaken during this series prior to inclusion on the IVF-ET programme. Patients N1–N13 are women in the IVF-ET programme who were shown not to have antispermatozoal antibodies. They were chosen at random from a large number of women falling into this category. The serum antispermatozoal antibody status was rechecked at the time of oocyte recovery.

Patients P1–P4 are women in the IVF-ET programme with serum antispermatozoal antibodies which were considered to be influencing their fertility.

Patient P1 (P1a and P1b) had primary infertility for 2 years at the time of initial testing in October 1981, and she has been monitored since that time. Her levels of antispermatozoal antibodies in the serum have fluctuated between titres of 40 and 160 in the GAT and between 4 and 64 in the sperm immobilisation test (SIT). She has poor quality mucus and required clomiphene citrate to induce satisfactory ovulation. Intrauterine AIH, with washed spermatozoa for six treatment cycles,[20] was unsuccessful, and 6 months of using condoms during intercourse (occlusion therapy) failed to produce a significant lowering of sperm antibody levels. Fluids from two attempts at IVF-ET were available from this patient.

Patient P2 had primary infertility of 20 months when first tested in June 1981. Her antispermatozoal antibody levels have fluctuated between titres of 40 and 80 in the GAT and between 8 and 16 in the SIT. Occlusion therapy was unsuccessful in this patient, as was intrauterine AIH using washed spermatozoa for four treatment cycles, and also 3 months of a daily immunosuppressive regime of 3 mg dexamethasone acetate.

Patient P3 had primary infertility of 12 months when first tested in November 1979. Her GAT titres have fluctuated between 40 and 80, and her SIT titres between 16 and 32. Ten weeks of the immunosuppressive regime with dexamethasone acetate caused disappearance of the immobilising antibodies but did not affect the levels of the agglutinating antibodies, and the patient failed to conceive.

Patient P4 also had primary infertility for 12 months when first tested in April 1980. At that time, her GAT titre was 10 and her SIT titre was 4. At the time of IVF-ET, the titres were 160 in the GAT and 32 in the SIT. During the intervening period, occlusion therapy led to a reduction in spermimmobilising antibodies, but the patient failed to conceive.

Tests for Antispermatozoal Antibodies

The gelatin agglutination test and the sperm immobilisation test were carried out essentially as described by Rose et al[21] with the following minor modifications. 1. In the GAT, dilutions were performed in Tyrode's[22] solution containing 2% human serum albumin (HSA) and using a dilution series of 10, 20, 40, 80, 160, 320, 640, and 1,280. 2. The SIT was performed using fresh human serum as a complement source and Tyrode's solution containing 2% HSA as the diluting medium. A negative control was included on every occasion of testing for sperm-immobilising antibodies, as was a control using the serum under test (undiluted) with the addition of inactivated complement.

Assays for Immunoglobulin and Complement Component C_3 Concentration

Determinations of the concentrations of immunoglobulins G, A, and M in sera and follicular fluid were carried out by the Hunter Immunology Unit, Mater Misericordiae Hospital, Newcastle, New South Wales, using nephelometric procedures.[23]

Determinations of complement component C_3 in sera and follicular fluid, and additional and confirmatory determinations of immunoglobulin concentrations were made using NOR-Partigen immunodiffusion plates (Behring) using the Behring Ig C_3 standard serum (human) as control.

Absorption of Active Sera With Anti-Immunoglobulin Sera

In order to determine which class of immunoglobulin was responsible for the antispermatozoal antibody activity, the active sera were absorbed with rabbit antisera to human IgG, IgA, and IgM (Behring). The commercial antisera, and a normal rabbit (control) serum, were dialysed to remove spermotoxic preservative (sodium azide), and were absorbed four times with human red cells to remove any antispecies activity. They were then tested and found to be inactive in both the GAT and SIT. Equal amounts of aliquots of the active human sera and each of the prepared commercial antisera (and the normal rabbit (control) serum) were mixed, and incubated at 37°C for 1 hour and at 4°C overnight. The mixtures were then centrifuged to remove immune complexes and tested for antispermatozoal activity in the GAT and SIT, with the original sera being tested again at the same time.

Detection of Secretory Component in Follicular Fluid

In order to ascertain whether secretory piece was present in follicular fluid, radial immunodiffusion was used. Follicular fluids were placed in exterior wells in 1% agarose (Pharmacia) around a central well containing antihuman secretory piece prepared from human colostum (Behring). The array of wells was that obtained using a commercial punch template available from the Gelman Company. A control well containing human colostrum was included on every plate.

RESULTS

Immunoglobulin Levels in Randomly Selected Follicular Fluids

The concentrations of the immunoglobulins G, A, and M in follicular fluid from nine patients without antispermatozoal antibodies are presented in Table I. The IgG concentrations varied between 5.5 and 9.6 g/L. The IgA concentrations varied between 0.33 and 1.6 g/L. The IgM concentrations should be viewed with caution since they are at or near the minimum detection level of the system used (g/L) but, nevertheless, the level of the concentrations is clear.

Immunoglobulin and Complement C_3 Levels in Serum and Follicular Fluid

Immunoglobulin and C_3 concentrations in follicular fluids and sera taken at the same time are presented in Table II. Information is presented from four patients who did not have circulating antibodies to spermatozoa at the time of oocyte recovery and from the four patients with antibodies. Comparisons between the concentrations of these proteins in serum and in follicular fluid are given in Table III. The IgG content of follicular fluid varied between 54 and 95% of serum (average 76.6%); IgA varied between 38 and 90% (average 57.7%); IgM varied between 5 and 25% (average 11%); and complement component C_3 varied between 57 and 85% (average 70%). Although the numbers of samples are too small to conduct a meaningful statistical analysis, there are no clear differences between the levels in these patients with and those without antispermatozoal antibodies. The percentages of the serum immunoglobulin concentrations found in follicular fluid are inversely related to the molecular weight of the immunoglobulins and are consistent with transudation of these serum proteins into follicular fluid. This is also reflected by ratios of the different immunoglobulins in serum and in follicular fluid.

TABLE I. Immunoglobulin and Antispermatozoal Antibody Levels in Follicular Fluid From Patients Without Detectable Antispermatozoal Antibodies in Their Sera

Patient	IgG (g/L)	IgA (g/L)	IgM (g/L)	Sperm antibodies (SIT or GAT)
N1	9.5	1.2	0.5	Neg
N2	7.4	1.2	0.03	Neg
N3	7.5	0.7	0.3	Neg
N4	6.2	0.8	0.04	Neg
N5	8.5	1.0	0.07	Neg
N6	9.6	1.6	0.08	Neg
N7	5.5	0.33	0.12	Neg
N8	9.7	1.2	0.11	Neg
N9	7.5	1.0	0.03	Neg

The ratio of IgG to IgA in follicular fluid was found to be significantly lower ($P < 0.05$) in the women with antispermatozoal antibodies than in those without them. This is due to the mean IgG concentration's being lower, and the mean IgA concentration's being higher, in the women with the antibodies than in the women without them. However, in view of the small numbers involved, further data need to be collected before this relationship could be accepted as reflecting a real effect.

TABLE II. Immunoglobulin and Complement Component C_3 Levels in Sera and Follicular Fluids From (a) Patients Without Antispermatozoal Antibodies in the Serum and (b) Patients With Antispermatozoal Antibodies in the Serum

	Serum				Follicular Fluid			
Patient	IgG (g/L)	IgA (g/L)	IgM (g/L)	C_3 (g/L)	IgG (g/L)	IgA (g/L)	IgM (g/L)	C_3 (g/L)
a								
N10	10.8	2.5	4	0.6	9.5	1.2	0.4	0.4
N11	8	1.8	1.6	1.1	6.2	0.8	0.04	0.7
N12	10.6	1.1	0.8	0.8	8.5	1.0	0.07	0.6
N13	10.2	3.2	2.2	0.9	9.7	1.2	0.11	0.6
b								
P1a	10.6	2.6	1.2	0.7	6.0	1.2	0.1	0.4
P2	7.9	2.3	2.2	0.7	4.3	1.5	1.12	0.6
P3	8	2.7	2.5	0.6	7.0	1.0	0.24	0.4
P4	11.5	2.6	1.2	0.9	9.9	1.6	0.3	0.7
P1b	12	2.5	1.2	0.9	7.7	1.4	0.3	0.7

Levels of Antispermatozoal Antibodies in Follicular Fluid

High titres of both spermagglutinating and spermimmobilising antibodies were found in the follicular fluid of patients with circulating antispermatozoal antibodies. The results are presented in Table IV. Commonly, the titre in the follicular fluid was one or two dilution steps below the titre for the same activity in the serum. This general result appears to be consistent with expectations based on relative immunoglobulin concentrations. However, patient P3 appears to be an exception to this finding. Examination of antibody titres of the follicular fluid of this patient showed low titres in the GAT and negative findings in the SIT, in contrast to the high titres in both of these tests of the serum.

Class of Immunoglobulins Associated With Antispermatozoal Antibodies in Patient's Sera

Absorption of active sera with anti-IgG, anti-IgA, and anti-IgM showed reductions in both spermagglutinating and spermimmobilising activity only with anti-IgG in sera from patients P1 (a and b), P2, and P4. This indicates that the immunoglobulin class involved in these activities in these sera was primarily IgG.

The exception to the above result was patient P3. The results of absorption with the three antisera are presented in Table V. Absorption with anti-IgG and anti-IgM markedly reduced spermimmobilising activity, and all three antisera reduced the spermagglutinating activity. In this patient, therefore, it appears that the antispermatozoal antibody activity is associated with

TABLE III. Comparisons Between the Immunoglobulin and Complement Component C_3 Concentrations in Serum and in Follicular Fluid

	Follicular Fluid Concentration as a % of Serum Concentration				Ratios of Immunoglobulin Concentrations			
					Serum		Follicular Fluid	
Patient No.	IgG	IgA	IgM	C_3	IgG:IgA	IgG:IgM	IgG:IgA	IgG:IgM
N10	88	48	10	67	4.3:1	18:1	7.9:1	23.8:1
N11	78	44	2.3	63	4.4:1	7.2:1	7.8:1	155:1
N12	80	90	8.8	75	9.6:1	13:1	8.5:1	121:1
N13	95	38	5	67	3.2:1	12:1	8.1:1	88:1
P1a	57	46	8.3	57	4.0:1	15:1	5:1	60:1
P1b	64	56	25	78	4.8:1	13.3:1	5.5:1	25.7:1
P2	54	65	5.4	85	3.4:1	11.3:1	2.9:1	35.8:1
P3	88	70	9.6	67	3.0:1	13.3:1	3.7:1	29.2:1
P4	86	62	25	78	4.4:1	12.7:1	6.2:1	33:1

TABLE IV. Titres of Antibodies to Spermatozoa in Serum and Follicular Fluid From Patients With Antibodies Previously Detected in the Serum

Patient	Fluid	Test Procedure GAT	SIT
P1a	Serum	40	16
	follicular fluid 1[a]	10	4
	2	20	4
	3	40	2
	4	20	2
	5	40	8
P1b	Serum	80	16
	follicular fluid 1	40	8
	2	20	4
	3	20	4
	4	20	4
	5	20	4
P2	Serum	40	26
	follicular fluid 1	20	8
	2	20	8
P3	Serum	160	16
	follicular fluid 1	20	−ve
	2	10	−ve
	3	10	−ve
	4	10	−ve
	5	10	−ve
	6	10	−ve
P4	Serum	320	32
	follicular fluid	160	16

[a]Follicles from which fluid was collected from each patient at oocyte recovery.

immunoglobulins of all three classes. In particular, the immobilising activity was associated mainly with IgM. This appears to be the reason for the lack of spermimmobilising activity in the follicular fluid of this patient.

Presence of Secretory Component in Human Follicular Fluid

Using radial immunodiffusion, no secretory component could be detected in any of the patients' follicular fluid. Lines were obtained between the antiserum and the human colostrum control.

DISCUSSION

Fertilisation in humans takes place in a milieu to which follicular fluid is likely to be major contributor. The rupturing follicle floods the area around the fimbria with the fluid, and, no doubt, some enters the oviduct along with the ovum. In addition, the ovum has been nurtured in this fluid during its development, and significant amounts undoubtedly remain interspersed within the cumulus mass surrounding the oocyte.

The protein concentration of follicular fluid is similar to that of serum, and experiments have shown a rapid equilibration between the serum proteins and follicular fluid.[24] Previous work has demonstrated transudation of serum proteins into follicular fluid in amounts inversely proportional to molecular weight.[24–26] The results presented here are in agreement with that concept. The immunoglobulin concentrations found here are higher than those in some previous reports but are within the ranges of reported determinations.[24,25]

The developing follicle is not regarded as a secretory organ. However, the apparent increased amounts of IgA in the fluid of patients with circulating antispermatozoal antibodies are of interest. Despite the fact that secretory component which is commonly associated with locally produced IgA was not detected in the fluids tested in this study, the concept of local antibody production in the ovarian follicle is worthy of investigation in larger numbers of patients and by using more sensitive testing procedures. In women, secretory component has been demonstrated within the epithelial cells lining the cervix, uterus, and fallopian tubes, and direct evidence has been advanced for the local synthesis of antibodies in these organs.[2,15,26] The ovary and developing follicle have not been included as part of this system, but it seems that they should be further investigated. Although there is firm evidence for the local synthesis of immunoglobulin (particularly IgA) in the female tract, the origin of the plasma cells involved is not well understood. Most plasma cells in other mucosal sites, such as the intestinal and respiratory systems, are derived from precursors in the lymphoid tissues draining these areas. The cells are stimulated by absorbed antigen, released into the circulation and targeting in on the tissue of origin, as well as on other related tissues in that system.[27,28] It is possible that such events occur in the female genital tract and that the ovary is also involved. Women with circulating antibodies to spermatozoa presumably will have been stimulated through the vaginal/cervix area leading to both a systemic and/or a local response.

The levels of antispermatozoal antibodies in follicular fluid are closely related to the levels in serum. In the fluids tested in this study, titres were one or two dilution steps lower. It is clear, therefore, that treatments for effects of antispermatozoal antibodies that involve overcoming a block of sperm progression in cervical mucus are of little benefit while there are detectable levels of antispermatozoal antibodies in the serum, which will be reflected in the follicular fluid. Of course, immunosuppression is expected to reduce levels of antibodies in all locations, but there is some reluctance to use such treatment in cases of infertility, due to the possibilities of major or minor sequelae and the uncertainty of teratogenic effects.[12,29]

Where follicular fluid cannot be obtained for testing, it may be useful to ascertain the immunoglobulin class(es) of the antispermatozoal antibodies in the serum. As is illustrated with patient P3, a considerable proportion of her serum antibodies was of the IgM class which was not being transferred into the follicular fluid. Such information could be of importance when considering possible treatment, especially if IVF-ET is not a possibility.

Two of the patients reported on here (P1 and P2) have had successful in vitro fertilisations and embryo transfers by replacing maternal serum with donor serum in

TABLE V. Titres of Antispermatozoal Antibodies in Patient P3 After Absorption with Various Antisera

Sperm Antibody Test	Serum Titre (Untreated)	Titre After Absorption With: Normal Rabbit Serum	Anti-IgG	Anti-IgA	Anti-IgM
Sperm immobilisation (SIT)	8	8	2	8	−ve
Gelatin agglutination (GAT)	80	80	16	32	16

the culture system, and they have delivered healthy infants.[19] Where IVF-ET is an available form of treatment for various reproductive defects, it is suggested as a suitable treatment for women with antispermatozoal antibodies which are considered to be influencing their fertility.

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REFERENCES

1. Kay DJ, Boettcher B. Incidences of antispermatozoal antibodies in sera from infertile couples from Newcastle, Adelaide and Perth: A statistical comparison of results. Shulman S, Dondero F, Nicotra M, eds. Immunological Factors in Human Reproduction. London: Academic Press, 1982:29–38.
2. Jones WR. Assessing immunity to sperm. Clin Reprod Fertil. 1982; 1:3–12.
3. Rumke PL. Autoantigenicity of spermatozoa. In: Cohen J, Hendry WF (eds) Spermatozoa, Antibodies and Infertility. Oxford, England: Blackwell Scientific Publications, 1978:67–79.
4. Menge AC, Medley NE, Mangione BS, Dietrich JW. The incidence and influence of antisperm antibodies in infertile human couples on sperm cervical mucus interactions and subsequent fertility. Fertil Steril. 1982; 38:439–446.
5. Cinader B, de Weck A (eds.) Immunological Response of the Female Reproductive Tract. Copenhagen: Scriptor, 1976.
6. Rebello R, Green FHY, Fox H. A study of the secretory immune system of the female reproductive tract. Br. J Obstet Gynaecol. 1975; 82:812–816.
7. Moghissi KS, Sacco AG, Borin K. Cervical mucus, antibodies and the post coital test. Am J Obstet Gynecol. 1980; 136:941–946.
8. Coghi IM, Dondero F, Cerasaro M, Gentile E, Bartozini T, Massaccesi A, Nicotra M. Post coital test in infertile women with antisperm antibodies. In: Shulman S, Dondero F, Nicotra M, eds. Serono Symposium No. 45. Immunological Factors in Human Reproduction. London: Academic Press, 1982:59–65.
9. Kremer J, Jager S. The sperm-cervical mucus contact test: A preliminary report. Fertil Steril. 1976; 27:335–340.
10. Kremer J, Jager S, Kuiken J. The clinical significance of antibodies to spermatozoa. In: Boettcher B, ed. Immunological Influence on Human Infertility. Sydney: Academic Press, 1977:47–66.
11. Schulman S. Sperm isoimmunity: Sperm antibodies in women. In: Shulman S, Dondero F, Nicotra M eds. Serono Symposium No. 45. Immunological Factors in Human Reproduction. London: Academic Press, 1982:5–18.
12. Alexander NJ, Sampson JH, Flugham DL. Pregnancy rates in patients treated for antisperm antibodies with prednisone. Int J Fertil. 1983; 28:63–67.
13. Ansbacher R, Kwok K-Y, Behrman SJ. Clinical significance of sperm antibodies in infertile couples. Fertil Steril. 1973; 24:305–308.
14. Isojima S. Relationship between antibodies to spermatozoa and sterility in females. In: Edwards RG, ed. Immunology and Reproduction. London: IPPF, 1969; 267–279.
15. Schumacher GFB. Humoral immune factors in the female reproductive tract and their changes during the cycle. In: Schumacher GFB, Dhindsa DS, eds. Immunological Aspects of Infertility and Infertility Regulation. New York: Elsevier, North Holland, 1980; 93–98.
16. Bronson RA, Cooper GW, Rosenfeld DL. Sperm-specific isoantibodies and auto-antibodies inhibit the binding of human sperm to the human zona pellucida. Fertil Steril. 1982; 38:724–729.
17. Haas GG, Sokoloski JE, Wolf DP. The interfering effect of human IgG antisperm antibodies on human sperm penetration of zona-free hamster eggs. Am J Reprod Immunol. 1980; 1:40–43.
18. Yovich JL, Stanger JD, Tuvik AF, Yovich JM. In-vitro fertilisation in Western Australia: A viable service program. Med J Aust. 1984; 104:645–649.
19. Yovich JL, Stanger JD, Kay DJ, Boettcher B. In-vitro fertilisation of oocytes from women with serum antisperm antibodies. Lancet. 1984; ii:369–370.
20. Boettcher B, Kay DJ, Fitchett SB. Successful treatment of male infertility caused by antispermatozoal antibodies. Med J Aust. 1982; 2:471–474.
21. Rose HR, Hjort J, Rumke Ph, Harper AJK, Vyazov O. Techniques for detection of iso- and auto-antibodies to human spermatozoa. Clin Exp Immunol. 1976; 23:175–199.
22. Bavister BD. Environmental factors important for in-vitro fertilisation in the hampster. J Reprod Fertil. 1969; 18:544–545.
23. Buffone GJ, Lewis SA, Jolliff CR, Markowitz H, Uirella G, Fudenberg HH, Muxioz J, Burdash N, Buchner P. Manual immunochemical nephelometric assays for serum immunoglobulins Igh, IgA and IgM. Clin Chem. 1979; 25:1009–1013.
24. Edwards RG. Follicular fluid. J Reprod Fertil. 1974; 37:189–219.
25. Velazquez A, Reyes A, Chargoy J, Rosado A. Amino acids and protein concentrations of human follicular fluid. Fertil Steril. 1977; 28:96–100.
26. Shalgi R, Kraicer P, Rimon A, Dixito M, Soferman N. Proteins of human follicular fluid: The blood follicle barrier. Fertil Steril. 1973; 24:429–434.
27. Ping WW. Sperm antibody activity in human fallopian tube fluid. Fertil Steril. 1979; 32:682–684.
28. McDermott MR, Clark DA, Bienstock J. Evidence for a common mucosal immunologic system. II. Influence of the estrous cycle on B immunoblast migration into genital and intestinal tissues. J. Immunol. 1980; 124:2536–2539.
29. Winter AJ. Microbial immunity in the reproductive tract. J Am Vet Med Assoc. 1982; 181:1069–1073.
30. Hargreave TB, Elton RA. Treatment with intermittent high dose methyl prednisolone or intermittent betamethasone for antisperm antibodies: Preliminary communication. Fertil Steril. 1982; 38:586–590.