Evaluation of the Sperm Cervical Mucus Contact test (SCMC) for Assessment of Cervical Factor Infertility

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Abstract

Background:
Cervical mucus is a necessary component of human fertility. Cervical mucus is considered a main barrier for sperms for passing to the upper female reproductive tract, only less than 1% of the spermatozoa in vagina can penetrate the cervical mucus successfully.

Objective:
Use *in vitro* sperm cervical mucus contact test (SCMC) to evaluate the fertility of couples complaining from unexplained infertility.

Methods:
This study was conducted in the Infertility Unit/ Babylon Hospital of Gynecology and Children. Seminal fluid analysis was performed for all 45 studied specimens. SCMC test was performed by aspirating a cervical mucus at 12-14 day cycle of females. One drop of semen mixed with a drop of cervical mucus and covered with cover slide. The slide is then incubated at 37C° for 30 minutes.The sperm concentration and sperm motility due to grading activity were evaluated and compared with semen parameters.

Results:
The results showed a significant decrease ($p<0.001$) of sperm concentration and forward sperm motility (grade a & b), while a significant increase ($p<0.001$) in grade c and d sperm motility compared to their values in seminal fluid analysis.
The correlation (r) study showed significant negative correlation between forward sperm motility a+b with age of females, while a positive correlation between grade c of sperm motility and age of females, and non-significant correlation between all studied sperm parameters with infertility period.

Conclusion:
It was concluded that sperm SCMC test is necessary for detecting the causes of infertility for Normospermic men.

Key wards: Sperm cervical mucus contact test, cervical hostility, sperm quality.

الخلاصة

الخلفية العلمية:

يعد مخاط عنق الرحم مكون مهم لخصوبة البشر. يعتبر مخاط عنق الرحم الحاجز الرئيسي لعبور النطف الى الجزء الاعلى من القناة التناسلية الانثوية, فقط أقل من 1% من النطف في المهبل بإمكانها عبور مخاط عنق الرحم بنجاح.

الهدف:

اجراء فحص اتصال النطف بمخاط عنق الرحم لغرض تقييم خصوبة الازواج الذين يعانون من العقم غير المفسر.

طرائق العمل:

أجريت الدراسة في وحدة العقم/ مستشفى بابل للولادة والاطفال. تم اجراء فحص السائل المنوي لعينات المني المدروسة والبالغ عددها 45 عينة. تم اجراء فحص اتصال النطف بمخاط عنق الرحم عن طريق سحب مخاط عنق الرحم في اليوم 12−14 من دورة الطمث للزوجات. تم مزج قطرة واحدة من السائل المنوي مع قطرة من مخاط عنق الرحم على شريحة زجاجية وغطيت بغطاء الشريحة ووضعت الشريحة في

الحاضنة بدرجة 37م° لمدة نصف ساعة , ومن ثم تم اجراء الفحص المجهري لحساب تركيز النطف والنسبة المئوية لحركة النطف وحسب درجات نشاط النطف وقورنت مع قيمها في فحص السائل المنوي.

النتائج:

اظهرت النتائج نقص معنوي ($p<0.001$) في تركيز النطف والنطف ذات الحركة الامامية (a و b), في حين لوحظ زيادة معنوية ($p<0.001$) في حركة النطف درجة c و d عند مقارنتها بقيمها في فحص السائل المنوي. أما دراسة علاقة الارتباط فقد لوحظ علاقة سلبية معنوية بين حركة النطف بدرجة a+b وعمر الاناث, في حين وجدت علاقة ارتباط موجبة معنوية بين حركة النطف بدرجة c مع عمر الاناث, في حين لم تحصل أي معنوية بعلاقة الارتباط بين معايير النطف ومدة العقم.

الاستنتاج:

يستنتج من الدراسة بأن اختبار اتصال النطف بمخاط عنق الرحم ضروري لتشخيص حالات العقم للأشخاص الذين يعانون من العقم غير المفسر والذين لديهم معايير نطف طبيعية .

الكلمات المفتاحية: اتصال النطف بمخاط عنق الرحم، عداء العنق، نوعية النطف.

Introduction

The causes of infertility are traditionally categorized according to several categories or factors. These are male, females involved: ovarian, cervical, uterine, tubal, peritoneal and unexplained (Fritz and Speroff,2011). Cervical mucus is a necessary component of human fertility and plays at least two important physiologic roles in fertility. First, cervical mucus is essential for sperm survival and transport (Dunson *et al.*,1999),second, cervical mucus has been described as a "biological valve" admitting sperm to the uterus at certain times of the cycle while inhibiting their entrance of other times (Hilgers and Prebil,1979).

Sims (1968), the first researcher determined the effect of cervical factors on infertility (Pandya *et al.*,2013). Cervical mucus was considered a main barrier for sperm for passing to upper female reproductive tract, only less than 1% of the spermatozoa in vagina can penetrate the cervical mucus successfully. The evaluation of the reaction between the spermatozoa and cervical mucus include post coital test, sperm cervical mucus contact test (SCMC) and sperm cervical mucus penetration test (Dhurvey *et al.*,2012).

Many couples with normal infertility tests which are performed separately, like seminal fluid analysis, clinic tests, and hormonal tests, remain not conceiving. So this study aimed to use in vitro test (sperm cervical mucus contact test) to evaluate the cervical mucus and its affecting on fertility in the couples complaining from unexplained infertility.

Materials and Methods

This study was conducted in the Infertility Unit/ Babylon Hospital of Gynecology and Children from December 2012 to February 2014. The study involved 45 couples complaining from unexplained infertility, men with normal seminal fluid analysis and normal females cases with clinic and hormonal checkup with age range 17-40 years old.

Seminal Fluid Analysis was performed for all studied males in accordance to WHO(1999). Semen specimens were collected in a wide disposable container after 3-5 days of abstinence.

Cervical mucus specimens collection:

Cervical mucus specimens were collected from the females in midcycle by opening the cervix using speculum. The vagina was cleaned from vaginal exocervical mucus by a

sterilized cotton. The cervical mucus specimens were aspirated by a syringe with a special tube and transferred directly to the lab to perform the test.

Sperm Cervical Mucus Contact test:

This test was performed by mixing a drop of cervical mucus with a drop of the husband`s semen on a slide and covered by a coverslip. The slide was left 30 minutes in an incubator at 37 C°. Then, the slide was read to evaluate the sperm concentration and sperm motility percent in each grade (a, b, c and d) (Majumdar, 2004).

Statistical Analysis:

The results were statistically analyzed by using t- test using SPSS V.19, and correlation coefficient for correlation relationship.

Results

Sperm cervical mucus contact test (SCMC) was performed for the couples with normal seminal fluid analysis with normal them wives . The results revealed significant decrease ($p<0.001$) of sperm concentration and sperm motility percent with grade a and b, while a significant increase ($p<0.001$) in grade c and d were noticed in SCMC compared to their values in seminal fluid analysis (Table 1).

The correlation coefficient between sperm concentration in SCMC and age of females showed non-significant negative ($p>0.05$) correlation: $r= -0.029$ (Figure 1). A significant negative decrease ($p<0.05$) correlation was shown between the grade a+b of the sperm motility and age of females: $r= -0.352$, while a positive significant increase ($p<0.05$) correlation in grade c of the sperm motility and age of females: $r= 0.289$, grade d of the sperm motility was insignificant increase ($p>0.05$) correlation: $r= 0.076$ with age of females (Figure 2).

The results of correlation coefficient between sperm parameters (sperm concentration and sperm motility percent) in SCMC and infertility duration, showed non-significant negative ($p>0.05$) correlation (Figures 3 and 4).

Table 1 : Sperm parameters in sperm cervical mucus contact test and seminal fluid analysis

Sperm Parameters	Seminal Fluid Analysis (Mean± S.E)	Sperm Cervical Mucus Contact test (Mean± S.E.)
Sperm concentration (million/ ml)	48.73 ±2.85	39.37 * ±2.85
Grade a sperm motility percent	21.00 ±0.01	5.00 * ±0.01
Grade b sperm motility percent	31.00 ±0.01	16.00 * ±0.02
Grade c sperm motility percent	25.00 ±0.01	39.00 * ±0.03
Grade d sperm motility percent	23.00 ±0.01	40.00 * ±0.03

Semen Samples No. :45

*P<0.001 significant differences compared to seminal fluid analysis (control).

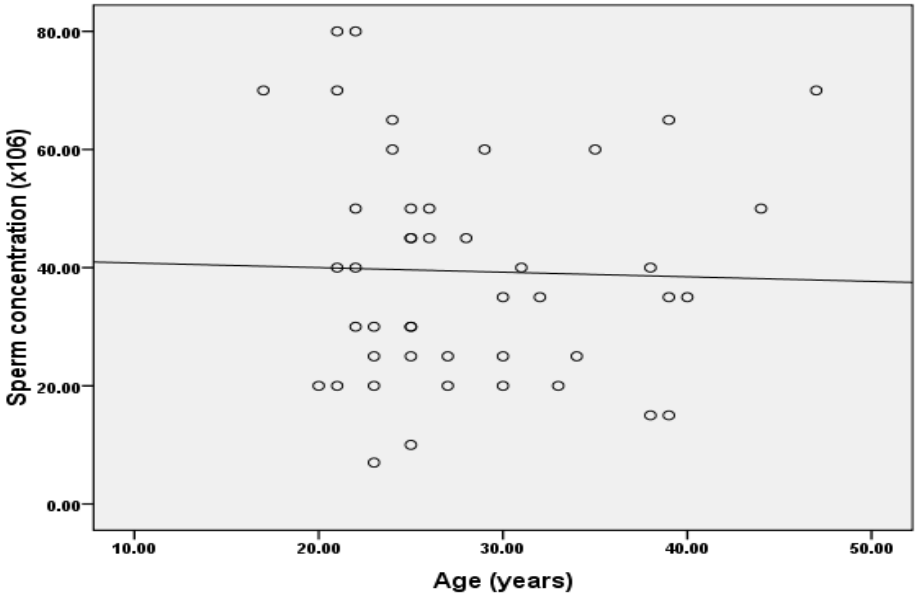

Figure 1 : Correlation between sperm concentration in SCMC and age of females(p>0.05) non-significant differences

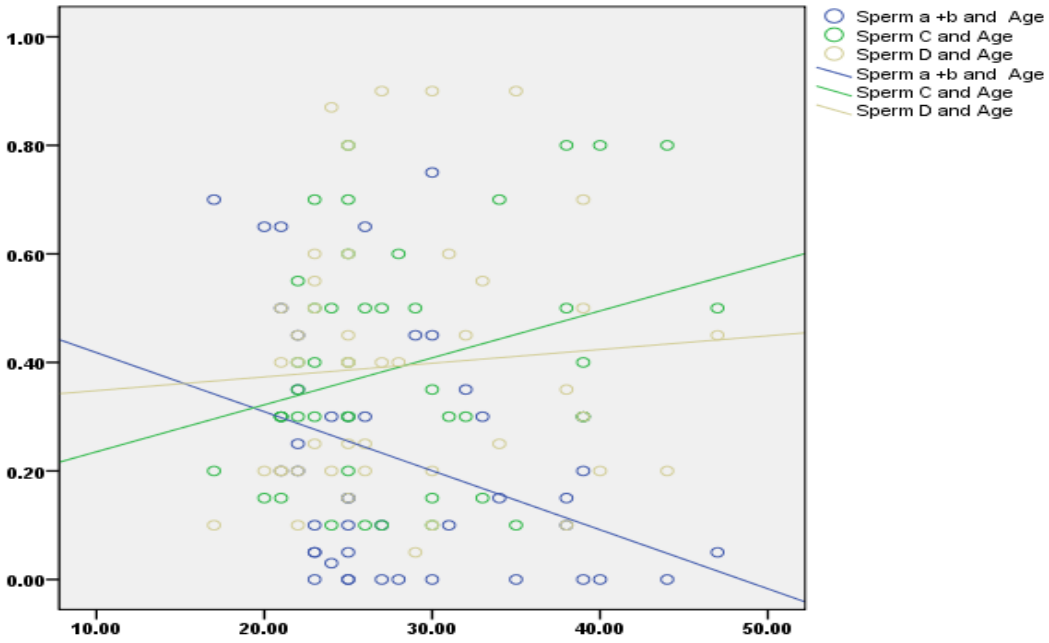

Figure 2 : Correlation between sperm motility in SCMC and age of female (p<0.05) significant differences in grade a+b and c of sperm motility in SCMC with age of females

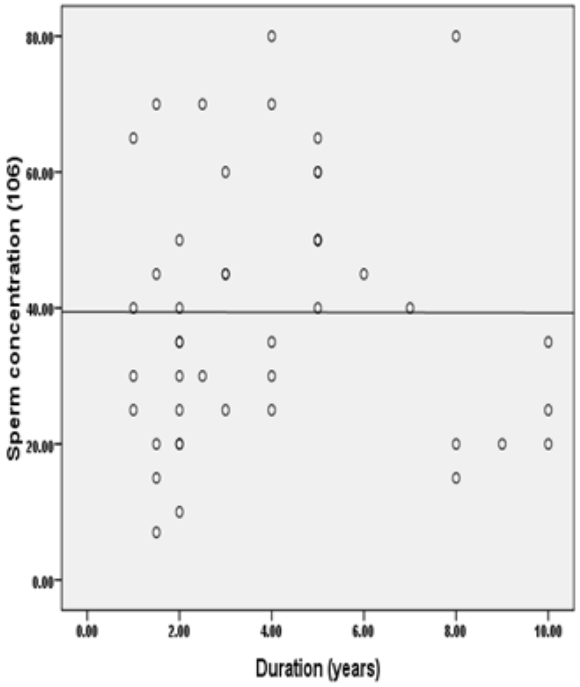

Figure 3 : Correlation between sperm concentration in SCMC and infertility duration (p>0.05) non-significant differences

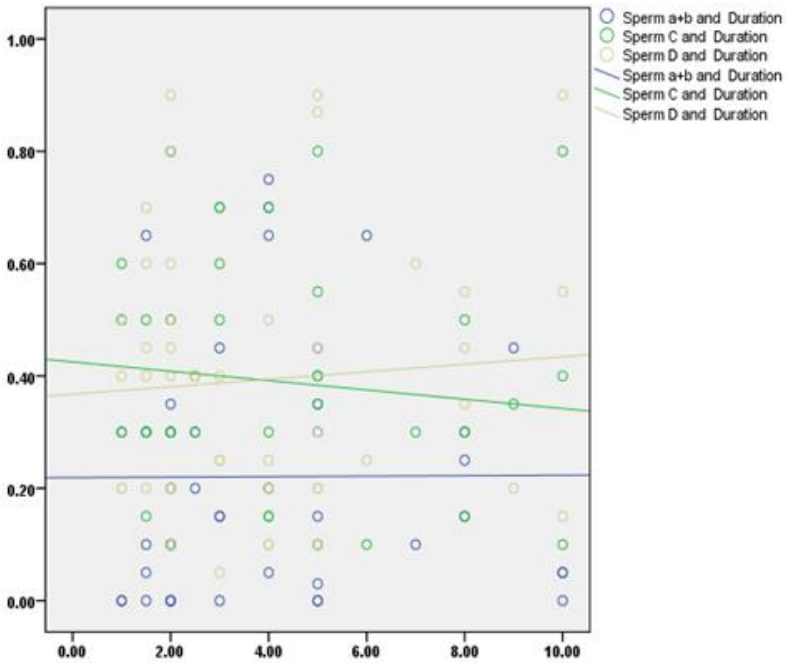

Figure4: Correlation between sperm motility in SCMC and infertility duration. (p>0.05) non-significant differences

Discussion

Cervical mucus has been suggested to influence spermatozoa functions in many ways (1) removal of spermatozoa from the hostile vaginal environment; (2) exclusion of seminal plasma and its components; (3) selective exclusion of certain sperm cells with morphological and possibly functional abnormalities; and (4) retention of spermatozoa for later migration to the upper tract (Eggert-Kruse *et al.*,1995). Furthermore, cervical mucus may also play a role in initiating or even completing the process of capacitation (Overstreet and Vande Voort 1989). Sperm transport from the vagina to the oviducts is greatly dependent on the properties of human cervical mucus including mucus quality, thickness and hydration (Ola *et al.*,2003).

The results of SCMC test showed significant decrease of sperm concentration compared to control, this result may be due to presence of microbial organisms like *Staphylococcus aureus* in cervical mucus, Emokae and coworkers (2009) reported that the presence of this bacteria should not be ignored as it can lead to decrease in the number of spermatozoa, the suppression of their motility, change in their morphology and fertilizing capacity. Also the complements bound with antibodies in cervical mucus caused spermatozoa analysis, then reduction of sperm number in cervical mucus (Chiu and Chamley 2004).

The results revealed a significant decrease in grade a & b of sperm motility in SCMC test, this results may refer to cervical enmity because the transport of spermatozoa from the vagina to oviducts depend on the cervical mucus properties. The nonimmunological cervical mucus hostility when low pH of the mucus (<6) or lack or absence of adequate amount of cervical mucus in preovulatory phase caused inadequate cervical mucus (Kirby *et al.*,1991). One study reported that separated *S. aureus* from the cervix of infertile women caused reduction in sperm motility in *in vitro* studies (Kaur *et al.*,2009).

Grade c & d sperm motility significantly increased in SCMC test. This result may refer to antisperm antibodies in cervical mucus (Mortimer,1994) IgA in cervical mucus caused shaky head motion of the spermatozoa (Pretorius et al.,1987). The bedeviling of sperm motility after binding with antibodies results from relevancy of spermatozoa with glycoprotein fibers in cervical mucus which cause a shaky head motion of spermatozoa (Zraly et al.,2003). The bounding of antibodies to the surface sperm antigens which are predominantly functional molecules such as receptors and enzymes can induce enhanced sperm agglutination, modulate acrosome reaction (Bohring et al.,2001), inhibits metabolic processes of sperms and thus decrease their motility and ability to penetrate into oocyte (Witkin and David 1998). Less quantity and poor quality of cervical mucus may be due to inadequate oestrogen level or less utilization of oestrogen through receptors is the main factor of cervical factor infertility (Pandya *et al.*,2013).

The correlation (r) between the sperm concentration and age of the females in SCMC test was non-significant differences, while there was a negative significant correlation between grade a & b sperm motility and age of the females. These results may be due to elevation of antisperm antibodies in cervical mucus with aging. The larger presence of antisperm antibodies is in the age group 31-40 years old (Sreenivas *et al.*,2011). The results showed non-significant correlation between sperm concentration and sperm motility in SCMC test and duration of infertility, these results may be due to not effect of infertility duration on cervical factors, referring to the study done by the author has

shown that there was no statistically significant difference between the incidence of ASA and duration of marriage as well as duration of infertility (Tennakoon 2013).

It was concluded that cervical factors affected sperm quality, and SCMC test operant for detecting cervical factors.

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