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Taiwanese Journal of Obstetrics & Gynecology 52 (2013) 512–515

www.tjog-online.com

Original Article

Pelvic endometriosis with peritoneal fluid reduces pregnancy rates in women undergoing intrauterine insemination

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Accepted 15 November 2012

Abstract

Objective: This study investigated the occurrence of peritoneal fluid in women undergoing intrauterine insemination (IUI) and its correlation with the stage of pelvic endometriosis and its influence on pregnancy outcomes.
Materials and Methods: A retrospective case–control design was used to recruit 272 infertile women with pelvic endometriosis. The treatment protocol consisted of controlled ovarian hyperstimulation with downregulation and gonadotropin for IUI treatment following ultrasound and laparoscopic intervention. The amount and color of the peritoneal fluid were determined during laparoscopy.
Results: The mean amount of peritoneal fluid with pelvic endometriosis that was detected using transvaginal ultrasound was ~15.1 mL. Women whose cycles contained more peritoneal fluid had significantly lower pregnancy rates (17.2% and 31.3%, respectively). The total clinical pregnancy rate was not significantly different between the two groups with reddish and yellowish peritoneal fluid who had pelvic endometriosis.
Conclusion: Pelvic endometriosis and peritoneal fluid, detected through vaginal ultrasound, have negative effects on the pregnancy outcome of IUI treatment.

Keywords: infertility; intrauterine insemination; pelvic endometriosis; peritoneal fluid; pregnancy

Introduction

The effect of peritoneal fluid with endometriosis on pregnancy outcomes has been studied extensively in recent years. Most studies have reported that the peritoneal fluid of endometriosis exhibits embryo toxicity, and pregnancy rates are low in women with severe endometriosis [1–3]. This may be because fluid accumulates in the cul-de-sac, disturbing oocyte pickup and tubal sperm motility [4–6]. In addition, nitric oxide from peritoneal fluid, generated by endometriosis, can reduce the pregnancy rates in these patients [7]. An additional study, using peritoneal fluid recovered from a mouse with endometriosis, demonstrated its adverse effects on early embryonic development [8]. However, fluid accumulation within the cul-de-sac, which is detected using a transvaginal ultrasound prior to laparoscopy, has been sporadically reported in previous studies, and most of these cases were reported to entail pelvic endometriosis [9–11]. The frequency of occurrence for this complication in women with unexplained infertility and whether women with other indications of intrauterine insemination (IUI) have this problem remain unknown. This study reports on the original observation of peritoneal fluid in consecutive series of laparoscopic results related to the lower pregnancy rate of infertile patients undergoing IUI treatment.

Materials and methods

Patient selection and study design

This study was approved by the Institutional Review Board of Taipei Medical University Hospital. A total of 272 infertile

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1028-4559/$ - see front matter
http://dx.doi.org/10.1016/j.tjog.2013.10.010

women who received a laparoscopic operation for endometriosis and/or endometrioma were enrolled and retrospectively reviewed using our IUI database system from January 2005 to December 2011. The inclusion criteria comprised several factors: (1) prelaparoscopic transvaginal ultrasound revealed pelvic endometriosis and/or endometrioma; (2) laparoscopic intervention disclosed pelvic endometriosis with peritoneal fluid in the cul-de-sac; (3) chromopertubation indicated the patency of the bilateral tubes; and (4) a histologically confirmed diagnosis of endometriosis and/or endometrioma. Patients who had received exploratory laparotomy for endometriosis and/or endometrioma were excluded. Surgical treatment consisted of a thorough laparoscopic electrocauterization/ablation or the excision of the peritoneal and nonperitoneal endometriotic lesion, lysis of adhesion, and the appropriate enucleation of the endometrioma. The volume and color of the peritoneal fluid were recorded. Patients who had peritoneal fluid were classified into two groups. Group 1 consisted of 49 patients with peritoneal fluid that was detected using transvaginal ultrasound, and Group 2 consisted of 223 patients without peritoneal fluid, as determined by transvaginal ultrasound. Transvaginal ultrasound using an Ultramark 9 HDI (Advanced Technology Laboratories, Bothell, WA, USA) with a 5–9-MHz multi-frequency transvaginal probe was performed to detect the pelvic pathology and peritoneal fluid. Fluid accumulation within the cul-de-sac was defined as an echolucent ring configuration that was distended by a certain amount of fluid between the posterior uterine wall and the intestinal loop linings in a sagittal view.

ART protocols

Following laparoscopic operation, patients received the gonadotropin-releasing hormone agonist leuprorelin (Leuplin Depot, 3.75 mg; Takeda Chemical Industries, Osaka, Japan) at 1.88 mg/month for 2 consecutive months. Four weeks following the administration of the second dose of leuprorelin, each patient received exogenous intramuscular gonadotropin of recombinant FSH (Serono, Geneva, Switzerland). When the leading follicle reached 18 mm in diameter, 250 μg recombinant human chorionic gonadotropin (hCG) was administered subcutaneously. IUI was performed ~36 hours later. The luteal phase was supported with micronized progesterone, 600 mg/day transvaginally for 2 weeks, and an additional 1500 IU hCG was administered on Day 6 following IUI.

Pregnancy was achieved by increasing the serum β-hCG levels to >5 mIU/mL on Day 16 following IUI, and transvaginal ultrasound was used to reveal heart activity until Week 6 of gestation.

Parameters of comparison

The parameters of comparison included maternal age, paternal age, peak serum estradiol, progesterone level, and sperm motility. The pregnancy rates were compared between the two groups. The stage of pelvic endometriosis and the amount and color of the peritoneal fluid were assessed.

Statistical analysis

Continuous data were summarized as means ± standard deviation. The Mann–Whitney rank-sum test was used for mean comparisons, and Fisher's exact test was used to compare the proportions. The significance of the groups was evaluated using the Student t test. Statistical analyses were performed using SPSS version 18.0 (SPSS Inc., Chicago, IL, USA). All p values were two-sided, and $p < 0.05$ was considered statistically significant.

Results

Table 1 shows the color of the peritoneal fluid in relation to the stage of pelvic endometriosis. Reddish peritoneal fluid accounted for 88.9% of the fluid, whereas yellowish fluid accounted for 11.03%. Reddish and yellowish peritoneal fluid accounted for 89.6% and 10.3% in Stage I, 90.4% and 9.5% in Stage II, 88.8% and 11.1% in Stage III, and 80% and 20% in Stage IV, respectively. No differences in the color of the peritoneal fluid were observed in correlation with the stage of pelvic endometriosis ($p = 0.612$).

Table 2 shows a comparison of the clinical characteristics of the two groups after IUI treatment. Preoperative peritoneal fluid accumulation detected by transvaginal ultrasound had an incidence of 18.0% (49/272). Substantially more peritoneal fluid was detected using vaginal ultrasounds (15.14 ± 0.63 vs. 8.54 ± 0.39 mL, $p < 0.001$), and pregnancy rates were lower among women with cycles in which peritoneal fluid was detected through vaginal ultrasound (16.3% vs. 31.3%, $p = 0.035$). There were no significant differences among patient age, duration of infertility, gonadotropin dose, motility, amount of washed sperm, estradiol on hCG day, progesterone on hCG day, endometrial thickness on the day of IUI, mean number of follicles, or multiple pregnancies. No ovarian hyperstimulation syndrome occurred in this study.

Table 3 shows the correlation of peritoneal fluid color with pregnancy outcome. The pregnancy rate with reddish fluid was 30.4% in Stage I, and 36.8% with yellowish fluid. The pregnancy rate with reddish fluid was 23.6% in Stage II, and 25% with yellowish fluid. The pregnancy rate with reddish fluid was 16.6% in Stage III, and 33.3% with yellowish fluid. The pregnancy rate with reddish fluid was 37.5% in Stage IV, and 0% with yellowish fluid. For the yellowish fluid, pregnancy rates were higher in Stages I–III than in Stage IV, although the P value was not significant ($p = 0.491$). The total clinical pregnancy rate was not significantly different between the

Table 1
Analysis of the stages of pelvic endometriosis and correlation with the color of peritoneal fluid determined by laparoscopy.

	Reddish color (n = 242)	Yellowish color (n = 30)	p
Stage I	164 (89.62%)	19 (10.38%)	0.003
Stage II	38 (90.48%)	4 (9.52%)	0.006
Stage III	24 (88.89 %)	3 (11.11%)	0.004
Stage IV	16 (80.0%)	4 (20.0%)	0.002

Data are presented as n (%).

Table 2
Comparison of intrauterine insemination cycles with peritoneal fluid detected by transvaginal ultrasound and those not detected before laparoscopy.

	Cycles with fluid ($n = 49$)	Cycles without fluid ($n = 223$)	p
Age (y)	33.37 ± 0.54	33.81 ± 0.23	0.912
Duration of infertility (y)	3.22 ± 0.27	3.37 ± 0.16	0.083
Gonadotrophins dose (IU)	1350 ± 75	1275 ± 75	0.701
E_2 (pg/mL) on hCG day	831.00 ± 80.4	800.8 ± 42.2	0.104
Mean follicles (n)	3.2 ± 0.4	3.4 ± 0.4	0.112
P (ng/mL) on hCG day	0.74 ± 0.09	1.17 ± 0.38	0.376
No. of washed sperm (10^6/mL)	42.43 ± 3.88	43.96 ± 1.96	0.702
Motility of washed sperm (%)	93.6 ± 0.35	93.8 ± 0.28	0.693
Endometrial thickness (mm)	11.8 ± 0.42	11.6 ± 0.41	0.382
Peritoneal fluid volume (mL)	15.14 ± 0.63	8.54 ± 0.39	<0.001
No. of pregnancies, n (%)	8 (16.32 %)	70 (31.39%)	0.035
No. of multiple pregnancies, n (%)	1 (12.5%)	6 (8.5%)	0.215
No. of ectopic pregnancies, n (%)	1 (2.0 %)	2 (0.8 %)	0.312
No. of abortions, n (%)	1 (12.5%)	6 (8.5 %)	0.221

Data are presented as n (%) or mean ± standard deviation.
E_2 = estradiol; hCG = human chorionic gonadotropin; P = progesterone.

groups with reddish and yellowish peritoneal fluid (28.5% vs. 30.0%, $p = 0.865$).

Discussion

The reasons for subfertility in patients with pelvic endometriosis remain unclear. Previous studies have suggested that 25–50% of infertile women have pelvic endometriosis, and that 30–50% of women with endometriosis are infertile [12,13]. Several mechanisms have been proposed to elucidate the association of endometriosis and infertility [2,13]. In the past decade, several studies have demonstrated that women with endometriosis have an increased volume of peritoneal fluid, increased concentration of activated macrophages, and increased peritoneal fluid concentrations of prostaglandins, interleukin-1, tumor necrosis fluid factor, and proteases. Peritoneal fluid from women with endometriosis reportedly contains an ovum capture inhibitor that prevents normal cumulus–fimbrial interactions [14]. These alterations may adversely affect the functions of the oocytes, sperm, embryos, or the fallopian tubes, causing subsequent implantation disturbances. Terrance et al [9] proposed that the volume of peritoneal fluid is positively correlated with the severity of endometriosis. However, the volume of peritoneal fluid was not significantly positively correlated with the stage of pelvic endometriosis in our data.

Table 3
Analysis of the pregnancy rates of intrauterine insemination cycles correlated with the stage of pelvic endometriosis and the color of peritoneal fluid.

	Reddish color ($n = 242$)	Yellowish color ($n = 30$)	p
Stage I	50/164 (30.5%)	7/19 (36.8%)	0.157
Stage II	9/38 (23.6 %)	1/4 (25.0%)	0.125
Stage III	4/24 (16.6 %)	1/3 (33.3%)	0.121
Stage IV	6/16 (37.5 %)	0/4 (0.0%)	0.223

Data are presented as n (%).

The endometriosis patients who achieved pregnancy had a significantly lower mean of fluid volume than those who did not become pregnant during a 2-year follow-up period in the study of Syrop et al [1]. In their study, patients with a peritoneal fluid volume <12 mL achieved pregnancy in nearly half the time as those with a volume >12 mL. In our study, patients with a mean peritoneal fluid volume of 15.1 mL, detected using transvaginal ultrasound, had lower pregnancy rates after IUI treatment. All of our cases of pelvic endometriosis with peritoneal fluid within the cul-de-sac were disclosed by laparoscopic intervention, but only when the amount of peritoneal fluid was ~15 mL could it be detected using the sagittal view of transvaginal ultrasound. This indicates that small amounts of peritoneal fluid within the cul-de-sac cannot be detected using transvaginal ultrasound. However, our data showed that greater volumes of peritoneal fluid may have more negative effects on pregnancy outcomes.

Recent studies have suggested that menstrual effluent contains factors that induce the local destruction of the peritoneal mesothelium, thereby creating adhesion sites for endometrial cells. Van et al [15] found higher levels of hemoglobin in the peritoneal fluid of patients with endometriosis. This hypothesis is based on the possibility that hemoglobin, when released into the peritoneal cavity following red blood cell lysis, may activate these processes. All products of hemoglobin catabolism are biologically active, including heme, which is cleaved by heme oxygenase into iron, carbon monoxide, and biliverdin, which is further converted into bilirubin. Various colors of peritoneal fluid in endometriosis patients may represent different statuses. Heme may cause a reddish color, and bilirubin may cause a yellowish color. However, no differences in color distribution were observed among the stages of pelvic endometriosis according to our statistical results. Therefore, we could not determine the metabolic pathway of endometriosis because of the complex interactions between pelvic endometriosis and peritoneal fluid.

The color of peritoneal fluid had a significant relationship with pregnancy outcomes. The pregnancy rates with yellowish fluid in Stages I–III were better than those with reddish fluid in the same stages. However, no pregnancies occurred with yellowish fluid in Stage IV. This shows that changes in the peritoneal cavity may be more crucial for implantation than peritoneal fluid. As a result of the small number of severe endometriosis cases in this study, we did not emphasize the effect of peritoneal fluid on the severity of endometriosis.

Peritoneal fluid has not been placed in the revised classification of endometriosis by the American Society of Reproductive Medicine [16] since 1996. Most previous studies have revealed that peritoneal fluid has a detrimental effect on *in vivo* and *in vitro* model assays [6,10,17–20]. This study recommends that peritoneal fluid be included in the AFS scoring system because of its negative effect on the pregnancy outcomes of infertile patients. In conclusion, when peritoneal fluid is detected by transvaginal ultrasound in infertile women with pelvic endometriosis, we suggest that patients receive laparoscopic intervention or aspiration out of the peritoneal fluid using ultrasound guidance prior to IUI treatment.

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