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Original Article

Effects of hydrosalpinx on ultrasonographic parameters for endometrial receptivity during the window of implantation measured by power color Doppler ultrasound

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Abstract: Aim: The aim of this study is to assess the effects of hydrosalpinx on ultrasonographic parameters for endometrial receptivity during the window of implantation, as measured by power color Doppler ultrasound. Methods: The women recruited to this study included sixty with unilateral or bilateral hydrosalpinx and fifty-seven prior to in vitro fertilization-embryo transfer (IVF-ET) or artificial insemination (AI) performed due to male infertility. Ultrasonographic parameters for endometrial receptivity were measured during the window of implantation by power color Doppler ultrasound. Results: The proportion of triple-line endometrial pattern and uterine contractions from the cervix to the fundus were significantly lower in the hydrosalpinx group than in the control group. In addition, patients in the hydrosalpinx group had a significantly lower pulsatility index (PI) of uterine spiral arteries. There were no significant differences in endometrial thickness, subendometrial blood flow distribution pattern, uterine artery PI, uterine artery resistance index (RI), or uterine spiral artery RI between the two groups. Conclusions: Hydrosalpinx may be involved in the regulation of endometrial receptivity through its influence on endometrial pattern, uterine contractions, and PI of the uterine spiral arteries.

Keywords: Hydrosalpinx, endometrial and subendometrial blood flow, power color Doppler ultrasound, endometrial receptivity

Introduction

Hydrosalpinx is a common gynecological disease resulting from a series of pelvic inflammatory disorders, which can lead to pelvic pain and infertility. About 30% of infertile women undergoing IVF-ET treatment have hydrosalpinges diagnosed by ultrasound scanning or hysterosalpingography (HSG) [1]. Previous studies have demonstrated the embryotoxic influence of hydrosalpinx fluid [2]. However, IVF-ET treatment of women with hydrosalpinx has also been associated with reduced pregnancy rates [3]. These results suggested that hydrosalpinx fluid can exert detrimental effects on the embryo and endometrium simultaneously.

Successful embryo implantation requires a receptive endometrium. In each menstrual cycle, the human endometrium exhibits a limited period in which to accept the implanting embryo, known as the “window of implantation” (day 21-24 of the menstrual cycle) [4]. During this period, the endometrium undergoes a complex series of distinct cellular and molecular changes initiated by ovarian steroid hormones, which render it receptive to conception [5]. Many studies have focused on the morphological and biochemical characteristics of the receptive endometrium. At the morphological level, endometrial receptivity can be evaluated by histopathological investigation or the appearance of pinopod structures [6]. The biochemical

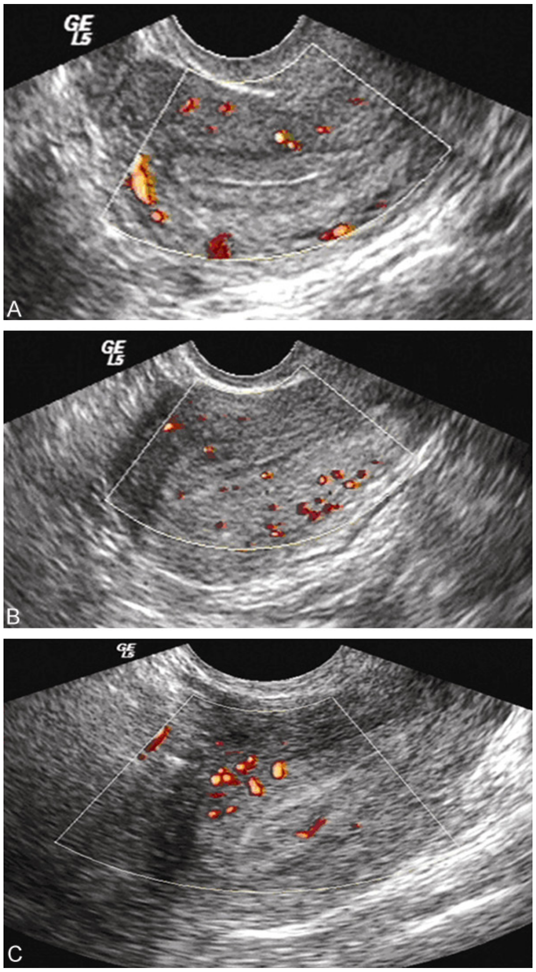

Figure 1. Classification of endometrial-subendometrial blood flow distribution pattern determined by color Doppler ultrasound. A. Vessels penetrate the outer hypoechogenic area surrounding the endometrium but not entering the hyperechogenic outer margin. B. Vessels penetrate the hyperechogenic outer margin of the endometrium but do not enter the hypoechogenic inner area. C. Vessels enter the hypoechogenic inner area.

markers of receptive endometrium include estrogen receptors (ERs), progesterone receptors (PRs), leukemia inhibitory factor (LIF), interleukins (ILs), glycodelin, vascular endothelial growth factor (VEGF), MUC1, and integrins [7]. However, these methods are usually invasive, hysteretic and unacceptable to patients, especially in those who are unwilling to damage the endometrium and prefer to become pregnant in the current cycle [8]. Therefore, it is necessary to develop a non-invasive and reliable method to evaluate endometrial receptivity.

With the advance of diagnostic ultrasonography, it has been widely used for endometrium assessment and measurement of endometrial blood flow. Ultrasonographic technology has gained increasing attention because of the advantages of non-invasiveness, real-time monitoring and predictability it provides. Numerous studies have proposed that endometrial thickness, pattern and endometrial and subendometrial blood flow, as measured by ultrasound scanning, may be related to endometrial receptivity [8, 9]. However, the effects of hydrosalpinx on these ultrasonic parameters, especially during the window of implantation, are not well understood. Therefore, in this study, we aimed to investigate the influence of hydrosalpinx on endometrial and subendometrial blood flow during the window of implantation using power color Doppler ultrasound, through which we hope to evaluate the effects of hydrosalpinx on endometrial receptivity.

Materials and methods

Subjects

This study was approved by the Ethics Committee of the First Affiliated Hospital, Xingjiang Medical University, China. One hundred and seventeen infertile women attending the Assisted Reproductive Unit were recruited after giving written informed consent between June 2009 and February 2011. All subjects had regular menstrual cycles, no history of smoking, and had not received any hormone treatments for the previous 6 months. None of the women had organic diseases of the uterus or ovaries, endometriosis, hyperprolactinemia, or polycystic ovary syndrome. These women were allocated to two groups. In the hydrosalpinx group, 60 women had unilateral or bilateral hydrosalpinx diagnosed by hysterosalpinogram (HSG) and transvaginal ultrasound scan. The diameter of the hydrosalpinx site was approximately 10-65 mm. In the non-hydrosalpinx group, there were 57 women prior to in vitro fertilization-embryo transfer (IVF-ET) or artificial insemination (AI) treatment that underwent IVF-ET or AI because of male infertility.

Ultrasound measurement

An LH peak was determined by the presence of LH in urine samples using a rapid semi-quantitative self-test (Kunming Yunda Bio-tech Co., Ltd., Kunming, China) every day starting on the day in which a leading follicle was more than 14 mm in diameter by transvaginal ultrasound. All women had an ultrasound scan on Day LH+6

Table 1. Demographic characteristics of the hydrosalpinx and non-hydrosalpinx groups

Group	Hydrosalpinx group (n = 60)	Non-hydrosalpinx group (n = 57)	*P* value
Age of women (y)	30.87 ± 4.02	29.73 ± 4.27	NS
Infertility duration (y)	3.47 ± 2.39	2.67 ± 1.95	NS
Body mass index (Kg/m^2)	21.34 ± 0.29	21.41 ± 0.57	NS
Basal FSH level (mIU/mL)	7.82 ± 0.65	8.04 ± 0.91	NS
Basal LH level (mIU/mL)	4.47 ± 1.31	4.15 ± 1.09	NS
Basal E2 level (pg/mL)	43.34 ± 15.29	45.19 ± 14.32	NS
Basal T level (ng/mL)	0.38 ± 0.01	0.49 ± 0.15	NS
Basal PRL level (ng/mL)	19.27 ± 1.51	20.14 ± 2.21	NS

Note: Data given as mean ± standard deviation (SD). NS = not significant.

using power color Doppler ultrasonic diagnostic equipment with a vaginal probe of 5.0-7.0 MHz (GE Medical Systems, Wisconsin, WI, USA) at approximately 7 to 9 PM after they had emptied their bladder. The ultrasound measurements were performed as previously described [10]. All ultrasound measurements were performed by the same person.

The maximum thickness of endometrium on both sides of the midline was measured in a longitudinal plane. The endometrium presented as a triple-line pattern (hyperechogenic outer lines and a central echogenic line) or a homogeneous pattern (homogeneous endometrium). The endometrial-subendometrial blood flow distribution pattern was determined by demonstrating pulsatile color signals in the subendometrial and endometrial regions. The distribution pattern was based on Applebaums' criteria, summarized as follows: 1, vessels penetrating the outer hypoechogenic area surrounding the endometrium but not entering the hyperechogenic outer margin; 2, vessels penetrating the hyperechogenic outer margin of the endometrium but not entering the hypoechogenic inner area; 3, vessels entering the hypoechogenic inner area (**Figure 1**) [11]. Uterine contractions were divided into five types: CF, propagating from the cervix to the fundus; FC, propagating from the fundus to the cervix; OPP, propagating from the fundus and cervix at the same time; RA, propagating from a random site of endometrium; or NA, no significant movement in endometrium [12].

Using color Doppler imaging, flow velocity waveforms were obtained from the ascending main branch of the uterine artery on the right and left sides of the cervix in the longitudinal plane before they entered the uterus. Endometrial spiral artery blood flow was detected intra-endometrial or in the adjacent sub-endometrial regions within 10 mm of endometrial echogenic borders. The Doppler gate was positioned when a vessel with good color signals was identified on the screen. An average of three to five cardiac cycles was selected for pulsatility index (PI), resistive index (RI), maximum peak systolic blood flow velocity (Vmax), and time-averaged peak systolic blood flow velocity (Vmean). Mean levels were used for analysis.

Statistical analysis

The values are presented as mean ± standard deviation (SD). Measurement data were assessed by independent sample t-test. Before statistical analysis, and the data were tested for normal distribution by applying the one-sample Kolmogorov-Smirnov test. The homogeneity of variances was evaluated by Levene's test. Numeration data were assessed using a chi-squared test. Statistical analyses were performed using SPSS software, version 17.0 (SPSS, Chicago, IL, USA). A *P* value of < 0.05 was regarded as statistically significant.

Results

Between June 2009 and February 2011, 117 patients attending the Assisted Reproductive Unit were recruited: 60 of them had unilateral or bilateral hydrosalpinx; 57 of them underwent IVF-ET or AI treatment due to male infertility. There were no significant differences with regard to the age of the women, duration of infertility, body mass index or basal hormone levels between the hydrosalpinx and non-hydrosalpinx groups (**Table 1**).

No significant differences were observed in endometrial thickness or endometrial-subendometrial blood flow distribution pattern in the peri-implantation phase between the hydrosalpinx and non-hydrosalpinx groups (**Table 2**). The proportion of homogeneous pattern of endometrium was significantly higher in the hydrosalpinx group (26/60) than in non-hydrosalpinx group (10/57) (**Table 2**). Uterine contractions in a random pattern were not detected in either group. Uterine contractions showed a predominance of FC patterns in the hydrosal-

Table 2. Comparison of ultrasound parameters of endometrial receptivity during the window of implantation in the hydrosalpinx and non-hydrosalpinx groups

Group	Hydrosalpinx group (n = 60)	Non-hydrosalpinx group (n = 57)	*P* value
Endometrial thickness (mm)	9.87 ± 1.26	9.88 ± 1.43	NS
Endometrial pattern[a]			
Triple-line pattern	34 (34/60)	47 (47/57)	
Homogeneous pattern	26 (26/60)	10 (10/57)	
Zone of vascular penetration[b]			
1	12 (12/60)	4 (4/57)	
2	28 (28/60)	38 (38/57)	
3	20 (20/60)	15 (15/57)	
Uterine movements[c]			
CF	12 (12/60)	30 (30/57)	
FC	36 (36/60)	24 (24/57)	
OPP	2 (2/60)	0 (0/57)	
RA	0 (0/60)	0 (0/57)	
NA	10 (10/60)	3 (3/57)	
Uterine artery			
PI	2.44 ± 0.78	2.46 ± 0.70	NS
RI	0.90 ± 0.31	0.84 ± 0.08	NS
Vmax	32.78 ± 10.53	31.44 ± 12.64	NS
Vmean	6.24 ± 5.32	6.08 ± 2.84	NS
Spiral artery			
PI[d]	0.88 ± 0.28	1.16 ± 0.60	< 0.05
RI	0.51 ± 0.15	0.51 ± 0.16	NS
Vmax	6.46 ± 1.82	7.25 ± 2.66	NS
Vmean	2.71 ± 1.06	3.05 ± 1.66	NS

Note: Data given as mean ± standard deviation (SD). CF = cervix to fundus; FC = fundus to cervix; OPP = opposing; RA = random; NA = no action; RI = pulsatility index; PI = resistive index; Vmax = maximum peak systolic blood flow velocity; Vmean = time-averaged peak systolic blood flow velocity; NS = not significant. [a]$P = 0.030$, by chi-square test; [b]$P > 0.05$, by chi-square test; [c]$P = 0.024$, by chi-square test; [d]$P = 0.012$, by independent sample t-test.

pinx group (36/60). On the contrary, the CF pattern was dominant in the non-hydrosalpinx group (30/57) (**Table 2**).

Table 2 also summarizes the characteristics of uterine artery and spiral artery blood flow. There was no difference in uterine artery PI, RI, Vmax or Vmean in the peri-implantation stage between the hydrosalpinx and non-hydrosalpinx groups. The average value of uterine spiral artery PI in the hydrosalpinx group (0.88 ± 0.28) was significantly lower than that of the non-hydrosalpinx group (1.16 ± 0.60, $P < 0.05$). Uterine spiral artery RI, Vmax and Vmean were similar between the two groups.

Discussion

Endometrial receptivity is critical to the process of embryo implantation and is characterized by the production of numerous cytokines and growth factors [13]. Transvaginal ultrasonography performed with a high-frequency end-fired probe placed in the vagina provides excellent resolution imaging of the uterus and ovaries. In recent years, several parameters have been proposed for assessing endometrial receptivity, including endometrial thickness, morphology and blood flow [14]. In the present study, we show that hydrosalpinx fluid can affect endometrial pattern, contractions, and spiral artery blood flow. These data provide a potential mechanism for the detrimental effects of hydrosalpinx fluid on human endometrium.

Previous studies reported that the ideal range of endometrial thickness for implantation ranges between 9 and 11 mm [15], and it is widely believed that an endometrial thickness that is below 6 mm will reduce the likelihood of pregnancy. However, Sundstrom reported a case of normal pregnancy in which endometrial thickness was no more than 4 mm at the day of oocyte retrieval [14, 16]. Other studies have also reported no relationship between endometrial thickness and pregnancy outcomes [9, 17]. In our study, there was no significant difference in endometrial thickness during the window of implantation between the hydrosalpinx and non-hydrosalpinx groups. Because the current results were not related to pregnancy outcomes directly, we can only conclude that hydrosalpinx fluid has no influence on endometrial thickness. The current data were consistent with a previous finding that endometrial thickness on the day of oocyte retrieval was similar among patients with and without hydrosalpinx during IVF-ET treatment [18].

A triple-line pattern may be the sonographic parameter that most reflects endometrial receptivity, because it is associated more frequently with pregnancy cycles [19]. A previous study reported that of patients in an IVF-ET cycle, 44.8% with a triple-line pattern before hCG injection became pregnant; and 80% with a triple-line pattern on the day of oocyte retrieval became pregnant [20]. In this study, we found that the proportion of a triple-line endometrial pattern during implantation was significantly lower in the hydrosalpinx group than in the non-hydrosalpinx group. These results suggest that hydrosalpinx fluid may influence the endometrial pattern, through which it exerts a detrimental effect on embryo implantation.

Ultrasound can provide uterine contraction information, which offers a useful parameter for the study of uterine physiology [12]. It had been reported that uterine contractions predominantly propagate from the fundus to the cervix (FC) during the follicular phase of the menstrual cycle and contractions from the cervix to the fundus (CF) predominated during the estrogen-dominant phases of the cycle. Abnormal contractions of the uterus have been observed in specific clinical conditions [21]. Our results indicate that uterine contractions during the window of implantation in the non-hydrosalpinx group were of the CF pattern. However, the FC pattern was dominant in the hydrosalpinx group. A previous study showed that retrograde uterine contractions were most frequent at mid-cycle and might facilitate sperm transport and embryo implantation [21]. Therefore, we speculated that the uterine contractions with an FC pattern in the patients of the hydrosalpinx group are necessary to expel hydrosalpinx fluid from the uterine cavity to maintain the microenvironment and homeostasis, and produce an unfavorable effect on embryo implantation.

Sufficient endometrial blood supply is usually considered an essential requirement for implantation [22]. When vessels enter the hypoechogenic inner area of the endometrium, the blood supply is much better than the other two patterns observed. Reportedly, no conception was achieved without subendometrial blood flow. But there was no close relationship between the patterns of blood distribution and pregnancy cycles [11]. In this study, we found that the zones of vascular penetration were similar between these two groups. These results suggest that hydrosalpinx fluid probably has no influence on blood flow distribution.

We found that the parameters of the uterine artery were similar between the hydrosalpinx and non-hydrosalpinx groups. Numerous studies have shown that uterine artery blood flow can be used to evaluate uterine receptivity [9]. Other studies have also reported no relationship between uterine artery blood flow and pregnancy outcomes [14]. Therefore, its value in predicting endometrial receptivity remains uncertain. Because uterine arteries have many branches in the womb and are the major blood supply of the myometrium, we postulated that it is difficult to predict endometrial receptivity by uterine artery blood flow. Spiral arteries are the terminal branches of the uterine artery and the major nutrition source of the endometrium. Previous studies showed that spiral artery blood flow reflects the blood perfusion of the endometrium and is an ideal parameter for evaluating endometrial receptivity [23]. Our results showed that the values of the uterine spiral artery PI in the hydrosalpinx group were significantly lower than those in non-hydrosalpinx group. A previous study also demonstrated that endometrial blood flow in a hydrosalpinx group was significant reduced compared to a non-hydrosalpinx group at the time of oocyte retrieval during IVF-ET treatment [18]. These results indicate that hydrosalpinx fluid may be involved in the influence of uterine spiral artery blood flow.

In conclusion, hydrosalpinx fluid may be involved in the regulation of endometrial pattern, uterine contractions and spiral artery blood flow during the window of implantation. We suggest this as a possible mechanism by which hydrosalpinx fluid produces a detrimental effect on endometrial development. Transvaginal ultrasonography may provide a non-invasive method for physicians to evaluate endometrial receptivity during the window of implantation in patients with hydrosalpinx.

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Disclosure of conflict of interest

None.

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References

[1] Strandell A, Waldenstrom U, Nilsson L, Hamberger L. Hydrosalpinx reduces in-vitro fertilization/embryo transfer pregnancy rates. Hum Reprod 1994; 9: 861-863.

[2] Leese HJ, Tay JI, Reischl J, Downing SJ. Formation of Fallopian tubal fluid: role of a neglected epithelium. Reproduction 2001; 121: 339-346.

[3] Katz E, Akman MA, Damewood MD, Garcia JE. Deleterious effect of the presence of hydrosalpinx on implantation and pregnancy rates with in vitro fertilization. Fertil Steril 1996; 66: 122-125.

[4] Revel A. Defective endometrial receptivity. Fertil Steril 2012; 97: 1028-1032.

[5] Zhu HX, Zhang WW, Zhuang YL, Huang LL. Mifepristone as an anti-implantation contraceptive drug: roles in regulation of uterine natural killer cells during implantation phase. Am J Reprod Immunol 2009; 61: 68-74.

[6] Achache H, Revel A. Endometrial receptivity markers, the journey to successful embryo implantation. Hum Reprod Update 2006; 12: 731-746.

[7] Meng CX, Andersson KL, Bentin-Ley U, Gemzell-Danielsson K, Lalitkumar PG. Effect of levonorgestrel and mifepristone on endometrial receptivity markers in a three-dimensional human endometrial cell culture model. Fertil Steril 2009; 91: 256-264.

[8] Alcazar JL. Three-dimensional ultrasound assessment of endometrial receptivity: a review. Reprod Biol Endocrinol 2006; 4: 56.

[9] Wang L, Qiao J, Li R, Zhen X, Liu Z. Role of endometrial blood flow assessment with color Doppler energy in predicting pregnancy outcome of IVF-ET cycles. Reprod Biol Endocrinol 2010; 8: 122.

[10] Raine-Fenning NJ, Campbell BK, Kendall NR, Clewes JS, Johnson IR. Quantifying the changes in endometrial vascularity throughout the normal menstrual cycle with three-dimensional power Doppler angiography. Hum Reprod 2004; 19: 330-338.

[11] Applebaum M. The uterine biophysical profile. Ultrasound Obstet Gynecol 1995; 5: 67-68.

[12] Bulletti C, de Ziegler D. Uterine contractility and embryo implantation. Curr Opin Obstet Gynecol 2006; 18: 473-484.

[13] Salamonsen LA, Nie G, Hannan NJ, Dimitriadis E. Society for Reproductive Biology Founders' Lecture 2009. Preparing fertile soil: the importance of endometrial receptivity. Reprod Fertil Dev 2009; 21: 923-934.

[14] Schild RL, Knobloch C, Dorn C, Fimmers R, van der Ven H, Hansmann M. Endometrial receptivity in an in vitro fertilization program as assessed by spiral artery blood flow, endometrial thickness, endometrial volume, and uterine artery blood flow. Fertil Steril 2001; 75: 361-366.

[15] Isaacs JD Jr, Wells CS, Williams DB, Odem RR, Gast MJ, Strickler RC. Endometrial thickness is a valid monitoring parameter in cycles of ovulation induction with menotropins alone. Fertil Steril 1996; 65: 262-266.

[16] Sundstrom P. Establishment of a successful pregnancy following in-vitro fertilization with an endometrial thickness of no more than 4 mm. Hum Reprod 1998; 13: 1550-1552.

[17] Ng EH, Chan CC, Tang OS, Yeung WS, Ho PC. Factors affecting endometrial and subendometrial blood flow measured by three-dimensional power Doppler ultrasound during IVF treatment. Hum Reprod 2006; 21: 1062-1069.

[18] Ng EH, Chan CC, Tang OS, Ho PC. Comparison of endometrial and subendometrial blood flows among patients with and without hydrosalpinx shown on scanning during in vitro fertilization treatment. Fertil Steril 2006; 85: 333-338.

[19] Friedler S, Schenker JG, Herman A, Lewin A. The role of ultrasonography in the evaluation of endometrial receptivity following assisted reproductive treatments: a critical review. Hum Reprod Update 1996; 2: 323-335.

[20] Jarvela IY, Sladkevicius P, Kelly S, Ojha K, Campbell S, Nargund G. Evaluation of endometrial receptivity during in-vitro fertilization using three-dimensional power Doppler ultrasound. Ultrasound Obstet Gynecol 2005; 26: 765-769.

[21] Bulletti C, de Ziegler D, Polli V, Diotallevi L, Del Ferro E, Flamigni C. Uterine contractility during the menstrual cycle. Hum Reprod 2000; 15 Suppl 1: 81-89.

[22] Ng EH, Chan CC, Tang OS, Ho PC. Comparison of 2-dimensional, 3-dimensional, and vascular ultrasonographic parameters for endometrial receptivity between 2 consecutive stimulated in vitro fertilization cycles. J Ultrasound Med 2007; 26: 931-939.

[23] Merce LT, Barco MJ, Bau S, Troyano J. Are endometrial parameters by three-dimensional ultrasound and power Doppler angiography related to in vitro fertilization/embryo transfer outcome? Fertil Steril 2008; 89: 111-117.