Difficulty of embryo-transfer (ET) and pregnancy rate based on the uterocervical angle

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Summary

When the angle formed by the uterine body and cervical axes (uterocervical angle) was less than 115°, a catheter for embryo transfer could not be smoothly inserted into the uterine body, and so a hard catheter was used, which significantly reduced the pregnancy rate and implantation rate. When the uterocervical angle measured before embryo transfer by ultrasonography is less than 115°, careful preparation, such as catheter selection for embryo transfer and the setting of a longer operation time, is necessary.

Key words: Uterocervical angle; Embryo transfer; Pregnancy rate; Implantation rate.

Introduction

The success rate of IVF-ET is dependent on the quality of the sperm and ova of the couple and therapeutic techniques [1]. For the latter, stimulation of the ovary, ovum pickup, culture, and embryo transfer (ET) are necessary. ET is the last stage of all IVF-ET procedures, and its technology has progressed. Ultrasound-guided ET [2-8] is currently the main method because the pregnancy rate is higher than that obtained by blind ET [2]. It has been reported that ultrasound-guided ET on days 3 and 4 increased the pregnancy rate compared to that on day 5 [7], and catheters used for ET were related to the pregnancy rate, showing the importance of catheter selection [9, 11]. Furthermore, performing a mock ET as a trial shortened the operation time of real ET and increased the pregnancy rate [12]. Various modifications have been made for ET as described above, but failure of these actions wastes the long-term IVF-ET treatment. Thus, ET should be very carefully performed. A catheter for ET cannot be easily inserted into the uterus in some cases in which ET is difficult and the pregnancy rate may be low. As we repeatedly performed ET, it was clarified that the angle formed by the uterine body and cervical axes was small in cases with difficulty in ET catheter insertion. Catheter insertion for ET was easier as the angle formed by the uterine body and cervical axis was close to 180° (linear), and soft catheters could be used, which may have increased the pregnancy rate. When the angle was small (close to 0°), catheter insertion was difficult, and a hard catheter was necessary, or a catheter was inserted after the angle was expanded by traction of the ectocervical region using Martin forceps in some cases. The pregnancy rate may decrease as this angle decreases, but the clinically problematic angle has not yet been identified.

In this study, we retrospectively investigated the angle, smaller than that whereby catheter insertion for ET becomes difficult, and the pregnancy rate is reduced. Measurement of this angle and careful preparation before ET are clinically significant.

Materials and Methods

Patients

The subjects included 102 patients (162 cycles) who underwent in vitro fertilization/fresh or frozen ET between September 2005 and December 2006. The mean patient age was 35.7 (25-45) years, and the mean frequency of assisted reproductive technology (ART) was 1.4. The mean BMI of the patients was 20.9 (17.4-33.3), the mean endometrial thickness at the time of ET was 12.2 (6.3-23.2) mm, and the distance between the catheter tip for ET and the fundus of the uterus was 5.8 (0.0-17.2) mm.

Ovarian stimulation protocol

For ovarian stimulation, the long protocol was mainly used. FSH (300 units) was intramuscularly injected for two days from day 3, followed by intramuscular injection of 150 units of hMG for several days, and ova were collected 35 hours after hCG injection. The luteal phase was supported by IM injection of 125 mg of progesterone (3 times every other day).

Measurement of the uterocervical angle

Measurement of the uterocervical angle was performed using transvaginal ultrasound sonography immediately before embryo transfer. A transvaginal ultrasound scan was then performed and a mid-plane longitudinal section obtained. The uterine body and cervical axes were traced. The uterocervical angle was measured on the ultrasonogram using a protractor.

The uterocervical angle is the angle between a line joining the external cervical os and internal cervical os, and a line joining the internal cervical os and uterine fundus (Figure 1).

Culture

For the culture medium, universal IVF medium (Medicult) or 10% SSS-HTF (Irvine Scientific) was used until confirmation of fertilization, and Blast Assist System 1 (Medicult), 10% SSS-

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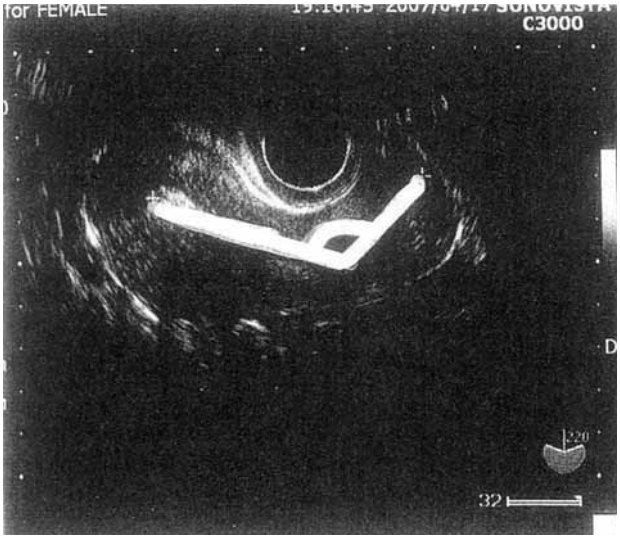


Figure 1. — Vaginal ultrasound showing the uterocervical angle.

early cleavage medium, or complete early cleavage medium (Irvine Scientific) was used for two days after confirmation of fertilization. For freezing/thawing in the pronucleus phase, vitrification kits VT101 or VT102 (Kitazato Supply) were used. Embryos were cultured at 37°C in 5% CO_2, 5% O_2, and 90% N_2.

Embryo transfer procedure

One to three embryos were transferred two or three days after ova pickup. ET catheter insertion into the uterus was tested in the follicular phase of the previous cycle before ET. For the ET catheter, a soft (Kitazato Supply, Tokyo, Japan) was used first, and a hard style-attached Flespout catheter (Kitazato Supply) was used when the insertion was difficult. When insertion remained difficult, a hard Wallace embryo replacement catheter (code no. mantle: 1816NST, tube: 1816, Smith Medical) was used. The catheter for the actual ET was selected based on this trial. In actual ET, the intravaginal region was washed with physiological saline after urination, and the mantle was inserted into the uterus. With transvaginal ultrasonic guiding, an ET catheter was inserted into the uterus, and embryos were transplanted by placing the catheter tip 10 mm from the uterine fundus.

In all cases, the catheter was then checked under a dissecting microscope for retained embryos. If these were found, they were reloaded and transferred again. The patients were asked to remain in bed for two hours after the procedure.

Judgment of clinical pregnancy

Clinical pregnancy was judged positive when urinary hCG was positive at two weeks after ET, and the gestational sac was observed by transvaginal ultrasonography at three weeks after ET. The pregnancy rate for ET cycles was investigated.

Data analysis

Statistical analysis was performed using the Microsoft software package. The Student's t-test, multiple mean comparison and chi-square test were used with $p < 0.05$ being considered as significant.

Results

Patient profile

In Table 1 the profiles of the three patient groups are reported. No statistically significant differences were found among the three study groups. The groups were homogeneous for patient age, number of previous ETs, estradiol level, number of oocytes retrieved, fertilization rate, number of good embryos, and number of transferred embryos.

Table 1. — *Patient profile*.

Uterocervical angle (°)	0-114	115-129	130-	p
No. of patients	42	44	76	–
Age (years)	36.1 ± 5.4	35.6 ± 5.1	35.5 ± 4.5	NS
No. of previous ETs	1.4 ± 0.5	1.5 ± 0.8	1.4 ± 0.6	NS
Estradiol level (pg/ml)	2646 ± 3011	2261 ± 1356	2385 ± 1616	NS
No. of oocytes retrieved	8.5 ± 7.2	8.2 ± 5.1	9.6 ± 6.2	NS
Fertilization rate	58.1 ± 23.0	60.0 ± 23.2	56.4 ± 25.4	NS
No. of G1, G2	0.9 ± 1.0	0.9 ± 0.8	0.9 ± 0.9	NS
No. of G1, G2, G3	1.9 ± 1.1	1.7 ± 0.9	1.9 ± 0.8	NS
No. of transferred embryos	2.4 ± 0.7	2.5 ± 0.7	2.3 ± 0.7	NS

Values are mean ± SD. NS: not significant.

Resistance due to the uterocervical angle in embryo transfer

Catheter insertion into the uterus for ET was not smooth in 43.2% (70/162) of all patients, and a longer time was necessary because of resistance to insertion. Regarding the uterocervical angle, insertion was difficult in 76.2% (32/42) of cases with an absolute angle value smaller than 115°, 31.8% (14/44) of cases with an angle between 115° and 129°, and 30.3% (23/76) of cases with an angle larger than 130°, respectively (Figure 2). The number of cases with insertion resistance was significantly increased in cases with an angle smaller than 115° compared to that in cases with larger angles ($p < 0.01$).

Catheter selection for ET based on the uterocervical angle

The hard catheter (Wallace embryo replacement catheter (Wallace)) was more frequently used in cases with a small absolute angle (Figure 3). The angle was smaller than 100° in 77.8% (7/9) of cases in which Wallace was used, but the Wallace was not used in cases with a 115° or larger angle. The pregnancy rates obtained using the three types of catheters for ET used at our hospital (Flespout catheter: code nos. FRC-3G-1 and FRC-3ST-1, Wallace: code nos. mantle: 1816NST, tube: 1816) were 27.0% (31/115), 26.3% (10/38), and 22.2% (2/9), respectively, showing no significant differences.

Frequency of use of single-hook forceps for embryo transfer in relation to the uterocervical angle

The number of cases in which the Martin forceps were used for ET was investigated in relation to the angle (Figure 4). Martin forceps were used in 19.0% (8/42) of cases with an absolute angle value smaller than 115°, and

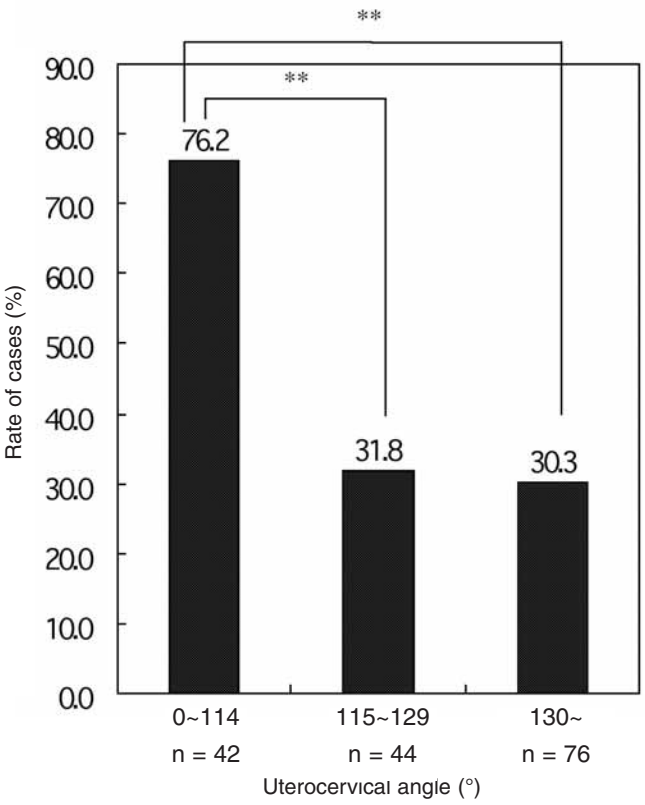


Figure 2. — Rates of cases with resistance to catheter insertion in ET in the uterocervical angle groups are presented. **p < 0.01.

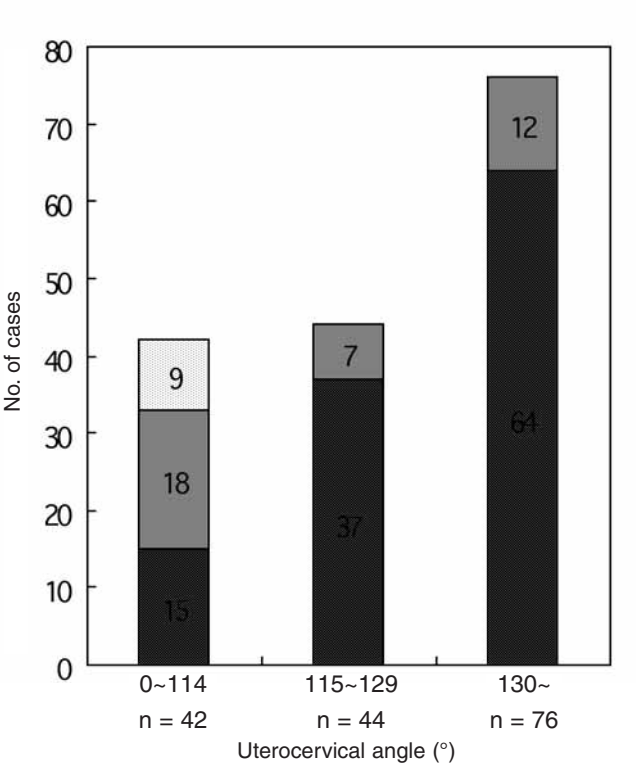


Figure 3. — Catheters for embryo transfer used for various uterocervical angles. Black and dark gray bars represent Flespout retrocatheters, and the light gray bar represents the Wallace embryo replacement catheter.

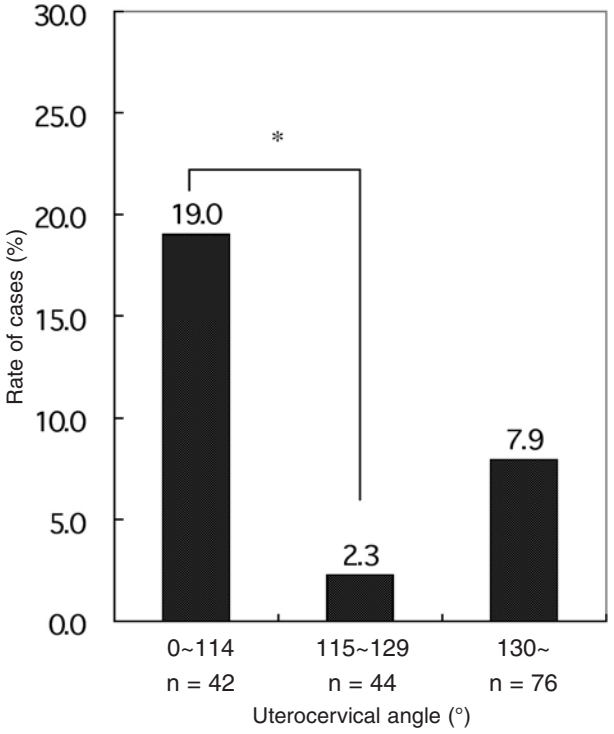


Figure 4. — Rates of Martin forceps usage in embryo transfer in relation to the uterocervical angle. The rate of cases in which Martin forceps were used in ET in each angle group is presented. *p < 0.05.

2.3% (1/44), and 7.9% (6/76) of cases with an angle between 115° and 129°, and larger than 130°, respectively. The frequency of Martin forceps usage was significantly higher in cases with an angle smaller than 115° than in cases with an angle between 115° and 129° ($p < 0.05$). The pregnancy rates in cases with and without the use of the Martin forceps were 26.7% (4/15) and 26.5% (39/147), respectively, showing no significant differences.

Relationship between the uterocervical angle and clinical pregnancy rate

The overall pregnancy rate to embryo transfer cycles was 26.5% (43/162). The uterocervical angle and pregnancy rate are shown in Figure 5. The pregnancy rates were 16.7% (7/42), 31.8% (14/44),and 28.9% (22/76) in cases with an absolute angle smaller than 115°, between 115° and 129°, and larger than 130°, respectively. The pregnancy rate was significantly lower in cases with an absolute angle smaller than 115° than in cases with an angle between 115° and 129° and larger than 130° ($p < 0.05$ and $p < 0.05$).

Relationship between the uterocervical angle and implantation rate

The overall implantation rate to embryo transfer cycles was 13.4% (52/388). The uterocervical angle and implantation rate are shown in Figure 6. The implantation rates

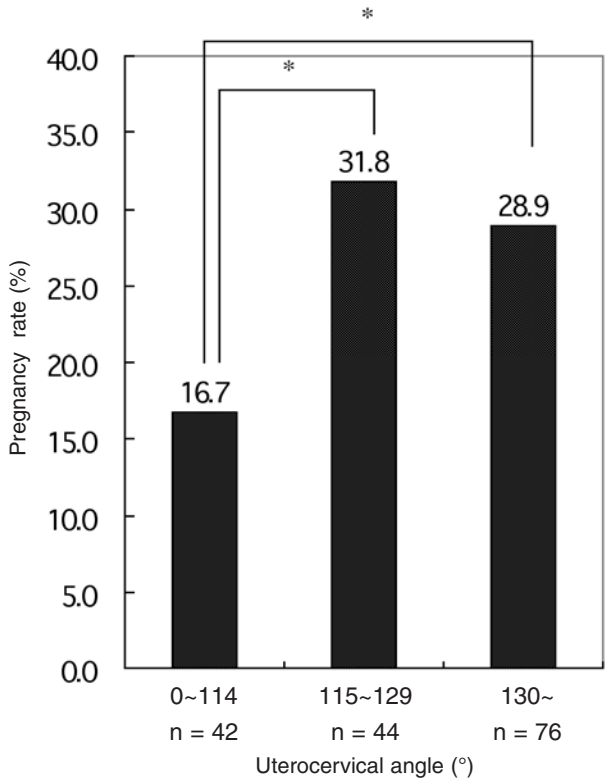


Figure 5. — Influence of the uterocervical angle on pregnancy rate. The rate of cases with successful pregnancy in each uterocervical angle group is presented. *p < 0.05.

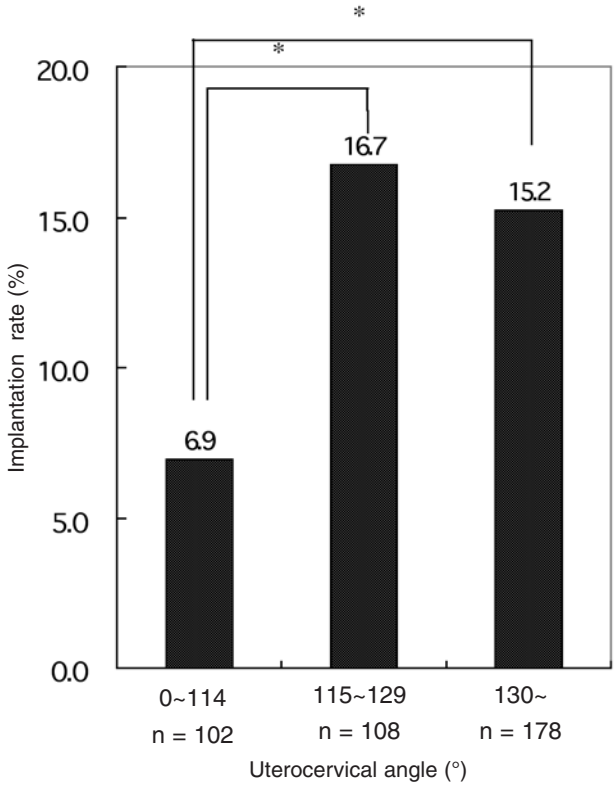


Figure 6. — Influence of the uterocervical angle on the implantation rate. The rate of cases with successful implantation in each uterocervical angle group is presented. *p < 0.05.

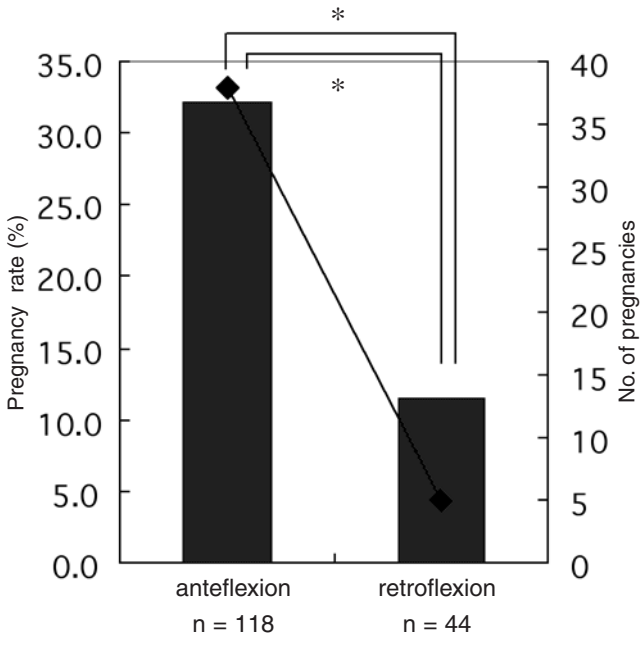


Figure 7. — Influence of differences in uterine morphology between anteflexion and retroflexion on the pregnancy rate. The left and right axes represent the pregnancy rate and the number of pregnancies, respectively. Black bar represents the pregnancy rate and square dot represents number of pregnancies. *p < 0.05.

were 6.9% (7/102), 16.7% (18/108),and 15.2% (27/178) in cases with an absolute angle smaller than 115°, between 115° and 129°, and larger than 130°, respectively. The implantation rate was significantly lower in cases with an absolute angle smaller than 115° than in cases with an angle between 115° and 129° and larger than 130° ($p < 0.05$ and $p < 0.05$).

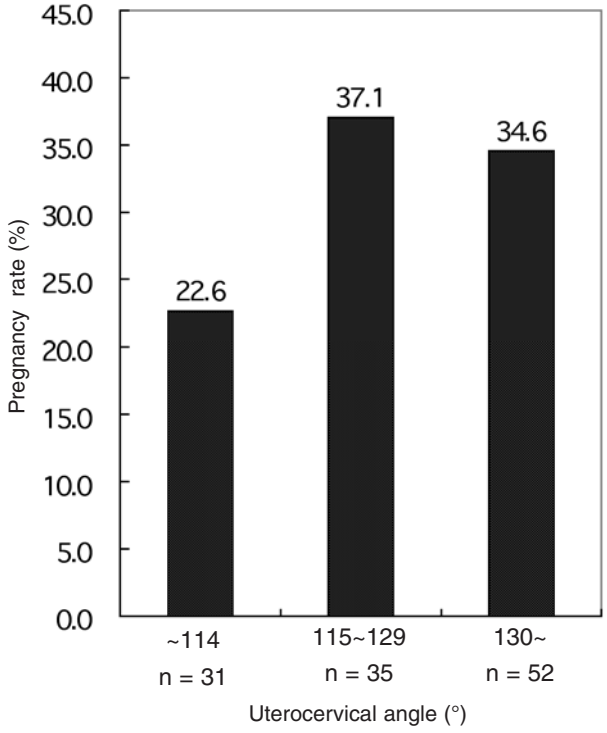
Pregnancy rate in relation to differences between ante- and retroflexion of the uterus

Differences in the pregnancy rate between ante- and retroflexion of the uterus were investigated. The pregnancy rates in cases with ante- and retroflexion of the uterus were 32.2% (38/118) and 11.4% (5/44), respectively. Both the number of pregnancies and pregnancy rate were significantly lower in cases with retroflexion ($p < 0.05$ and $p < 0.05$) (Figure 7).

The pregnancy rate was investigated in relation to the angle in ante- and retroflexion of the uterus. The rate decreased with a reduction in the angle in both ante- and retroflexion of the uterus, and the decrease was more marked in retroflexion (not significant) (Figure 8A/B).

Discussion

The uterocervical angle changed depending on the degree of urinary bladder filling. Sallam *et al*. measured the uterocervical angle with the urinary bladder filled immediately before ET by transabdominal ultrasonogra-



Figue 8A. — Pregnancy rates in relation to the uterocervical angle of anteflexion are presented (not significant).

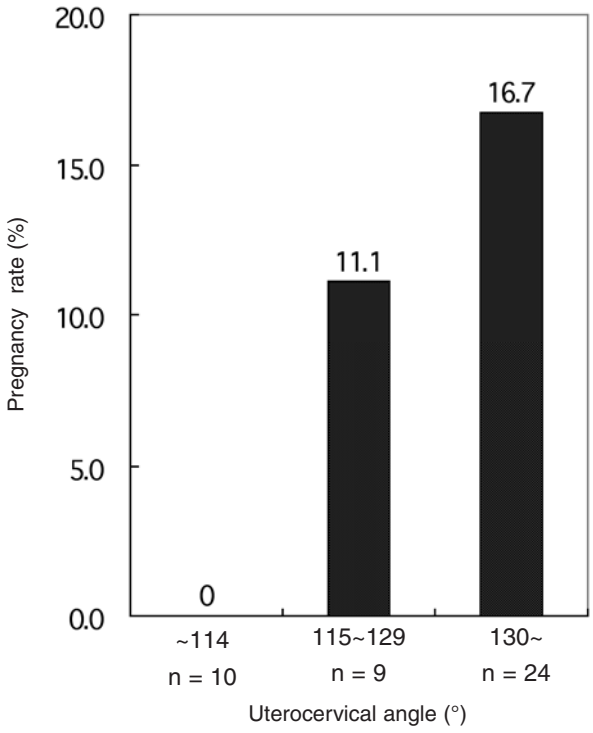


Figure 8B. — Pregnancy rates in relation to the uterocervical angle of retroflexion are presented (not significant).

phy to obtain the accurate uterocervical angle in ET [13]. We froze the sagittal view of transvaginal ultrasonography immediately after urination, not with a full urinary bladder and measured the uterocervical angle by tracing the uterine body and cervical axes on the image. ET was also performed immediately after the measurement of the uterocervical angle with transvaginal ultrasound guidance. As a rule, we performed transvaginal ultrasound-guided ET after urination because the resolution of transvaginal ultrasonography was better than that of transabdominal ultrasonography in not only retroflexion but also anteflexion of the uterus, allowing reliable ET at 10 mm from the fundus of the uterine body. When the angle of anteflexion is sharp, filling of the urinary bladder increases the uterocervical angle, making catheter insertion easier. Thus, transabdominal ultrasound-guided ET after filling the urinary bladder is also useful [13]. However, when the angle of retroflexion of the uterus is sharp, there is no stimulation-free method to increase the angle.

We retrospectively investigated the angle formed by the uterine body and cervical axis, which is involved in the clinical success of ET, expecting an increase in the success rate of ET by careful preparation for ET in cases with a clinically problematic small angle.

On investigation of the pregnancy rate in relation to the angle formed by the uterine body and cervical axis (uterocervical angle), the pregnancy rate was significantly lower in cases with an angle smaller than 115° than in cases with 115° or larger, suggesting that ET is difficult in cases with an angle smaller than 115°. Sallam *et al*. reported that the pregnancy rate significantly decreased when the angle was larger than 60° (smaller than 120° in our study), compared to that with 0 degrees [13], which is consistent with our findings.

Regarding the difference in the pregnancy rate between cases with ante- and retroflexion of the uterus, the rate was significantly lower in cases with retroflexion. ET was more difficult in retroflexion than in anteflexion, suggesting that the difference in uterine morphology between anteflexion and retroflexion affects the pregnancy rate.

The pregnancy rate was investigated in relation to the uterocervical angle in ante- and retroflexion of the uterus. The pregnancy rate decreased as the angle reduced in both ante- and retroflexion, and the decrease was more marked in retroflexion.

When the uterocervical angle was less than 115°, the ET catheter could not be smoothly inserted, and a hard catheter was used, or the ectocervical region was pulled using Martin forceps.

Accordingly, when the angle measured by ultrasonography before ET is less than 115°, careful preparation, such as selection of the ET catheter, use of the Martin forceps, and setting a longer operation time, is necessary before the execution. Sallam *et al*. modified the catheter

to adjust it to the cervical angle, and obtained good outcomes [13].

The pregnancy rates obtained using the three types of ET catheters with different degrees of hardness were 27.0% (31/115), 26.3% (10/38), and 22.2% (2/9), respectively, showing no significant differences. The pregnancy rates in cases with and without the use of single-hook forceps were 26.7% (4/15) and 26.5% (39/147), respectively, showing no significant differences. The catheter was changed, or single-hook forceps were used in cases with difficulty in ET, and these actions did not reduce the pregnancy rate, i.e., no significant difference was noted in the rate between cases with and without difficulty in ET [14, 15]. These findings indicate that adequate performance of ET avoids reduction of the pregnancy rate.

However, the pregnancy rate decreased when the angle was less than 115°, although an appropriate catheter was selected, and preparation for embryo transfer was carefully set, suggesting that the usefulness of catheter selection and use of single-hook forceps was limited when the angle was less than 115°, and ET into the uterine body was not reliable. For cases of repeated ET failure with a small uterocervical angle for which a hard catheter has been used, transcervical ET into the uterine body should be avoided, and consideration of zygote intrafallopian transfer (ZIFT) a transvaginal transmyometrial embryo transfer (Towako method), in which embryos are transferred to the endometrium by transvaginal puncture of the myometrial layer under ultrasound guidance, but not using a catheter [16], is necessary.

Conclusions

When the angle formed by the uterine body and cervical axes was less than 115°, a catheter for ET could not be smoothly inserted, and a hard catheter was used. The pregnancy rate and implantation rate by ET was significantly lower when the angle was less than 115°. When the angle is less than 115° on ultrasonography before ET, careful preparation, such as catheter selection and setting a longer operation time, is necessary before ET execution.

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