FULL PAPER *Theriogenology*

The Effects of Twisting and Type of Aspiration Needle on the Efficiency of Transvaginal Ultrasound-Guided Ovum Pick-Up in Cattle

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ABSTRACT. The effects of twisting and type (single- or double-lumen) of aspiration needle on the efficiency of transvaginal ultrasound-guided ovum pick-up (US-guided OPU) were investigated in cattle. The first study using slaughterhouse ovaries revealed that twisting of the needle during follicle aspiration improved the oocyte recovery rate without deleterious effects on the attachment of cumulus layers. Vacuum pressure affected the oocyte recovery and cumulus attachment, regardless of the needle type. The needle type did not affect the oocyte recovery or cumulus attachment with an optimized vacuum pressure. In the second study, US-guided OPU was performed in live cows using two types of needles with a vacuum pressure of 75 mmHg. The needle type did not affect the oocyte recovery or cumulus attachment of the recovered oocytes. The results revealed that twisting of the needle is effective in follicle aspiration, and suggested that a single-lumen needle is as useful as a double-lumen needle for US-guided OPU in cattle.

KEY WORDS: cattle, needle twisting, needle type, oocyte, ovum pick-up.

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The transvaginal ultrasound-guided ovum pick-up (US-guided OPU) in cattle reported by Pieterse *et al.* [16] has been widely utilized as a new technique which enables repeated retrieval of oocytes from live cows in various conditions, including milking [5], dry [5], pregnant [15], prepubertal [4, 8] and infertile cows [10]. Several factors, such as hormonal stimulation [8, 14], time of US-guided OPU within the estrous cycle [17] and frequency within a certain period [9], have been reported to affect the efficiency of recovery of oocytes and quality of cumulus-oocyte-complexes (COCs). However, even if many follicles can be recruited *in vivo* by these modifications, a reliable follicle aspiration system is required. Nevertheless, there are few reports of attempts to improve the follicle aspiration system.

Recently, follicle aspiration experiments using slaughterhouse ovaries showed that vacuum pressure [2], and the diameter [2, 7] and bevel [3] of aspiration needles affected the oocyte recovery rate, cumulus attachment and quality of the oocytes. A double-lumen needle allows simultaneous flushing and aspiration of follicles, overcoming the potential problem of failure to retrieve oocytes by only a single aspiration. However, few reports [6] have demonstrated the superiority of double-lumen needles to single-lumen needles in live cows.

Although follicle aspiration with needle twisting has been carried out to retrieve follicular oocytes from live cows [16, 19], the effects of needle twisting on the oocyte recovery rate and the quality of recovered oocytes have not been determined.

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The aim of this study was to investigate the effects of twisting and type (single- or double-lumen) of aspiration needle on the efficiency of oocyte retrieval using slaughterhouse ovaries and live cows.

MATERIALS AND METHODS

Follicle aspiration system: A single-lumen needle (18 gauge, 60-cm long; Fujihira Industries, Tokyo, Japan) was connected to a 50-m*l* plastic conical tube (Falcon 2098; Becton Dickinson Labware, Lincoln Park, NJ, U.S.A.) with a silicone tube (100-cm long, 1-mm internal diameter). The conical tube was joined to a vacuum pump with a foot-pedal switch (PRO-PUMP; Pioneer Medical Inc., Madison, CT, U.S.A.). When a double-lumen needle (17 gauge, 60-cm long: K-OPSD-1760; Cook, Queensland, Australia) was used, the aspiration line was connected to a 50-m*l* conical tube joined to the vacuum pump, and a flushing line was connected to a Perista pump (MICRO TUBE PUMP MP-3; Tokyo-Rikakikai Co., Tokyo). US-guided OPU from live cows was conducted using a single or double-lumen needle and an ultrasound machine (EUB-405; Hitachi, Tokyo) with a 6.5-MHz fingertip probe (Model EUP-F-331; Hitachi) which was equipped with a handmade probe-carrier (50-cm long).

Oocyte collection and classification: Follicular contents aspirated from slaughterhouse ovaries were pooled in a 50-m*l* plastic conical tube and allowed to settle for 10–15 min. The bottom part was drawn off with a Pasteur pipette into 100-mm plastic dishes (Falcon 1005). The recovered follicular contents from live cows were poured through an Em-Con filter (Immno Systems, Spring Valley, WI, U.S.A.) and the filter was rinsed with 200 to 300 m*l* of washing medium:

Dulbecco's phosphate-buffered saline with 1% calf serum (Gibco BRL, Gland Island, NY, U.S.A.), 0.05 mg/m*l* streptomycin sulfate (Meiji Seika, Tokyo), 100 units/m*l* penicillin G potassium (Banyu Pharmaceutical Co., Tokyo). Retrieved oocytes were examined under a stereomicroscope and graded into 4 classes [13]: I) oocytes with several compact cumulus layers, II) partially denuded oocytes, III) completely denuded oocytes, and IV) oocytes with expanded cumulus layers.

Study 1: *Follicle aspiration from slaughterhouse ovaries*: Bovine ovaries obtained from a local slaughterhouse were kept in physiological saline and used within 4 hr after slaughter. Twenty to thirty follicles ≥ 3 mm in diameter on the surface of ovaries were aspirated per session. The needle was rinsed with washing medium after each session. Using a double-lumen needle, washing medium was sent through the needle by the Perista pump (2–3 m*l*/min), and the medium was aspirated along with the follicular contents.

In the first experiment, the effects of twisting the needle during follicle aspiration on the oocyte recovery rate and cumulus attachment of recovered oocytes were investigated using a single-lumen needle with vacuum pressure of 75 mmHg with or without needle twisting. The needle was twisted two or three times at an angle of 180 degrees around its longitudinal axis to scoop out the entire follicle wall.

In the second experiment, the effects of vacuum pressures of 50, 75, 100, 125 or 150 mmHg on the oocyte recovery rate and cumulus attachment of recovered oocytes were examined using single- and double-lumen needles with needle twisting. The actual aspiration flow at each vacuum pressure was measured by aspirating water for 1 min and was expressed in milliliters of water per minute [2].

Study 2: *US-guided OPU from live cows*: The effects of the type of aspiration needle on the US-guided OPU were examined using two dry Holstein cows. They were injected with 2% xyladine hydrochloride (Celactal; Bayel Japan, Osaka, Japan) and 2% lidocaine hydrochloride (Xylocaine; Fujisawa Pharmaceutical Co., Osaka) to induce sedation and epidural anesthesia, respectively.

US-guided OPU was performed twice a week for 5 weeks (a total of 10 times in each cow). Preceding follicle aspiration, the aspiration needle and line were filled with flushing medium: washing medium supplemented with 10 IU/m*l* heparin sodium (Heparin Upjohn 1000; Pharmacia & Upjohn, Tokyo). All follicles ≥ 5 mm in diameter were aspirated using both types of needles with twisting (4 or 5 times) during collection of the follicular fluid. When a single-lumen needle was used, the vacuum pressure was 75 mmHg (aspiration flow rate: 7.5 m*l*/min) and the lumen of the needles and tubing were flushed with the flushing medium every 2 or 3 follicle aspirations. For the double-lumen needle, the vacuum pressure applied was 75 mmHg (aspiration flow rate: 12.0 m*l*/min).

Statistical analysis: In Study 1, the effects of the needle twisting and the type of the needles with the optimum vacuum pressure on the oocyte collection were analyzed by unpaired *t*-tests. The effects of vacuum pressure using the two types of needles were analyzed by one-way analysis of variance (ANOVA) followed by the Bonferroni/Dunn post-hoc test. The data obtained from Study 2 were compared by unpaired *t*-tests.

RESULTS

Study 1: Needle twisting increased the oocyte recovery rate ($P < 0.05$) and did not decrease the proportion of Class I oocytes (Table 1).

When a single-lumen needle was used, the oocyte recovery rate increased with an increase in vacuum pressure from 50 to 75 mmHg ($P<0.05$). However, a further increase in vacuum pressure did not improve the oocyte recovery rate (Table 2). When a double-lumen needle was used for follicle aspiration, the vacuum pressure did not affect the oocyte recovery rate (Table 3). Vacuum pressures of 50 to 100 mmHg and 50 mmHg resulted in a higher proportion of Class I oocytes ($P<0.05$) for single- and double-lumen needles, respectively. Regardless of the needle type, the proportion of Class III oocytes was increased by increasing the vacuum pressure ($P<0.05$; Tables 2 and 3). Therefore, the optimum vacuum pressure for follicle aspiration estimated from the oocyte recovery rate and the attachment of cumulus cells was 75 mmHg for a single-lumen needle and 50 mmHg for a double-lumen needle. Under these conditions, there were no differences in the oocyte recovery rate or the proportion of the Class I oocytes between the single- and double-lumen needles (Tables 2 and 3).

Study 2: The oocyte recovery rate and the proportion of recovered oocytes in each class per session did not vary significantly between the two needle types (Table 4).

DISCUSSION

Needle twisting increased the oocyte recovery rate by 30%. Needle twisting during follicle aspiration may detach

Table 1. Effects of needle twisting on the oocyte retrieval from slaughterhouse ovaries using a single-lumen needle

Needle twisting	No. of follicles aspirated (replicates)	% of oocytes recovered	% of recovered oocytes of each class[a)]			
			I	II	III	IV
+	150 (5)	63.7 ± 12.6[b)]	58.4 ± 14.0	15.5 ± 6.8	17.9 ± 12.6	8.2 ± 7.4
–	150 (7)	33.1 ± 6.7[c)]	62.8 ± 18.0	12.5 ± 12.2	18.8 ± 14.4	6.0 ± 10.5

a) Values are based on the total number of oocytes recovered.
b,c) Values (means ± SD) with different superscripts differ significantly ($P<0.05$).

Table 2. Effects of vacuum pressure on the oocyte retrieval from slaughterhouse ovaries using a single-lumen needle

Vacuum pressure (mmHg)	Aspiration flow rate (ml/min)	No. of follicles aspirated (replicates)	% of oocytes recovered	% of recovered oocytes of each class[a)]			
				I	II	III	IV
50	5.0	80 (4)	50.0 ± 4.1[b)]	75.2 ± 12.8[b)]	7.3 ± 9.5[b,d)]	7.6 ± 5.1[b)]	10.0 ± 14.1
75	7.5	105 (5)	66.2 ± 16.1[c)]	68.9 ± 3.9[b)]	14.7 ± 9.1[b,c,d)]	7.0 ± 6.7[b)]	9.4 ± 6.5
100	10.5	105 (5)	71.0 ± 6.5[c)]	65.5 ± 9.9[b)]	16.8 ± 7.3[b,c)]	13.4 ± 6.6[b)]	4.4 ± 6.5
125	13.0	105 (5)	75.4 ± 8.4[c)]	49.4 ± 11.6[c)]	18.5 ± 7.8[c)]	24.5 ± 12.6[c)]	7.6 ± 5.5
150	15.5	105 (5)	72.8 ± 10.6[c)]	35.1 ± 3.6[d)]	5.0 ± 2.8[d)]	50.4 ± 4.7[d)]	8.9 ± 4.0

a) Values are based on the total number of oocytes recovered.
b-d) Values (means ± SD) within the same columns with different superscripts differ significantly (P<0.05).

Table 3. Effects of vacuum pressure on the oocyte retrieval from slaughterhouse ovaries using a double-lumen needle

Vacuum pressure (mmHg)	Aspiration flow rate (ml/min)	No. of follicles aspirated (replicates)	% of oocytes recovered	% recovered oocytes of each class[a)]			
				I	II	III	IV
50	8.0	100 (5)	65.0 ± 10.0	83.8 ± 7.5[b)]	9.7 ± 4.9[b,d)]	3.2 ± 4.4[b)]	3.4 ± 4.6[b)]
75	12.0	100 (5)	65.0 ± 16.6	70.2 ± 12.2[c)]	16.7 ± 14.6[b,c)]	3.6 ± 5.2[b)]	9.4 ± 6.8[b,c)]
100	16.0	100 (5)	69.0 ± 12.5	48.6 ± 8.3[d)]	22.8 ± 4.2[c)]	14.0 ± 5.7[c)]	9.9 ± 10.9[b,c)]
125	20.0	100 (5)	74.0 ± 6.5	59.4 ± 7.8[c,d)]	5.4 ± 3.0[d)]	16.4 ± 4.1[c)]	17.5 ± 6.0[c)]
150	25.0	100 (5)	66.0 ± 5.5	28.7 ± 11.2[e)]	25.6 ± 7.7[c)]	30.5 ± 9.7[d)]	12.0 ± 3.8[b,c)]

a) Values are based on the total number of oocytes recovered.
b-e) Values (means ± SD) within the same columns with different superscripts differ significantly (P<0.05).

Table 4. Effects of needle type on the efficiency of US-guided OPU in live cows

Needle type	Vacuum pressure (mmHg)	Aspiration flow rate (ml/min)	No. of follicles aspirated	% of oocytes recovered	% of oocytes of each class[a,b)]			
					I	II	III	IV
Single-lumen	75	7.5	95	33.9 ± 8.8	59.2 ± 30.4	21.8 ± 16.4	17.3 ± 15.8	1.7 ± 5.3
Double-lumen	75	12.0	89	31.5 ± 24.6	60.8 ± 9.3	28.3 ± 11.0	10.9 ± 10.7	0

Values are means ± SD of 10 sessions.
a) Values are based on the total number of oocytes recovered.
b) Three sessions, in which no oocytes were recovered, were excluded from the data for the double-lumen needle.

the COC from the follicular wall by curettage of the follicular wall and improve the oocyte recovery rate. Oocytes with a number of compact cumulus layers have higher developmental competence than those with fewer layers of an incomplete or expanded cumulus [1]. Accordingly, no matter how high the recovery rate is, manipulations that result in tearing the cumulus layers off the COCs should not be performed. In the present study, the cumulus cells were not detached from the oocytes by twisting the needle.

In the present study using slaughterhouse ovaries, the optimum vacuum pressures (aspiration flow rates), judging from the oocyte recovery rate and the cumulus attachment, were 75 mmHg (7.5 ml/min) and 50 mmHg (8.0 ml/min) for single- and double-lumen needles, respectively. Increasing the vacuum pressure was reported to improve the oocyte recovery rate [1, 7]. In the present study, however, the oocyte recovery rate was not increased, when more than 75 mmHg of vacuum pressure was applied to the single-lumen needle. The reason for this discrepancy is not clear. We aspirated follicles ≥ 3 mm including those ≥ 10 mm in diameter in the present study. It is likely that the walls of large follicles (≥ 10 mm in diameter) will fold around the needle tip when negative pressure is applied, resulting in COCs staying in the follicles behind the folded follicular wall [17]. Thus, an excessive vacuum pressure will not increase the oocyte recovery rate beyond a certain level (about 75%) and may increase the detachment of cumulus cells from the oocytes by increasing the flow speed of follicular fluid containing oocytes in the needle and tubing [2, 3, 7, 10].

No beneficial effect of follicle flushing during US-guided OPU has been reported using double-lumen needles [12, 18] when *in vivo* matured oocytes were retrieved from human preovulatory follicles, where the junction between the cumulus cells and follicular wall is loose. Bovine oocytes are usually aspirated from small antral follicles in US-guided OPU. The oocytes in the small antral follicles are attached to the follicular wall very tightly, and therefore follicle flushing may improve the oocyte recovery rate. In the present study, however, follicle flushing using a double-lumen needle did not improve the oocyte recovery rate in slaughterhouse ovaries. This result was in agreement with a previous report [7], in which follicles ≥ 2 mm in diameter on

the surface of slaughterhouse ovaries were aspirated at 50 mmHg vacuum pressure using single- or double-lumen needle (17 gauge, 35 cm).

For OPU with the double-lumen needle in Study 2, the vacuum pressure was applied at 75 mmHg, because no oocytes were recovered in our preliminary experiments performed at the most suitable vacuum pressure (50 mmHg) for slaughterhouse-derived ovaries. The oocytes may be lost by follicle irrigation under a decreased vacuum pressure caused by incomplete insertion of the needle opening into follicles in live cows. The opening of the irrigation channel is most likely inserted into follicles since it opens near the tip of the bevel. The vacuum pressure may be lost at the part of the opening freed to the abdominal cavity.

The oocyte recovery rate and the proportion of the Class I oocytes retrieved per session were not improved by using a double-lumen needle. Although Fry *et al.* [6] reported that the oocyte recovery rate was significantly higher using a double-lumen compared to a single-lumen needle, in a later report [7], they concluded that there was no such increase. These studies suggest that the needle type (single- or double-lumen) does not affect the efficiency of US-guided OPU in cattle. When follicle aspiration was carried out in live cows, we often observed an inflow of blood into the follicular fluid. Aspirated blood coagulates in the needle and tube, and blocks the aspiration line during follicle aspiration [10]. US-guided OPU with a single-lumen needle more easily induces this blockage of aspiration compared with that of a double-lumen needle, in which the needle and tube are irrigated continuously. However, this problem of the single-lumen needle can be overcome by frequent flushing of the needle and tube. Since double-lumen needles are expensive, single-lumen needles are more economical than double-lumen needles for commercial US-guided OPU in cattle.

In conclusion, the present study demonstrated that twisting of the needle is effective for follicle aspiration, and we suggest that a single-lumen needle is as useful as a double-lumen needle for US-guided OPU in live cattle.

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