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Conception rate in Holstein dairy cows having both normal sized follicles and cystic follicles at estrus

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ABSTRACT

Objective: To investigate the conception rate when cows showing clear signs of estrus and having both normal sized follicles and cystic follicles were artificially inseminated. **Methods:** Thirty parous Holstein cows showing clear signs of estrus and having both normal sized follicles (diameter <25 mm) and cystic follicles (diameter ≥ 25 mm) simultaneously in their ovaries were artificially inseminated. Changes in the ovaries were observed using real-time ultrasonography for 25 d after artificial insemination. The concentration of plasma progesterone (P_4) was measured simultaneously to monitor the function of the corpus luteum. **Results:** The normal sized follicles ovulated in 21 out of 30 cows (ovulation rate: 70.0%), and 11 of these cows successfully conceived (conception rate: 36.7%) (Group C) while 10 cows did not conceive (Group NC). The maining 9 cows, normal sized follicles did not ovulate (Group NO). The change in P_4 in cows that ovulated was similar to that in normal cows. Although there were no significant differences in median number and size of cystic follicles among the groups, all cows in Group C only had one cystic follicle. **Conclusion:** These results support artificially inseminated of cows that show clear signs of estrus even if they have both cystic follicles and normal sized follicles, and especially when the cows have only one cystic follicle.

1. Introduction

Cows with cystic ovarian disease (COD) have abnormally large follicles (cystic follicles) with a diameter of at least 25 mm that persist for more than 10 d. These cystic follicles are steroidogenic and secrete estradiol or progesterone. As a result, they interfere with normal ovarian cyclicity [1–3]. Cows with COD are generally infertile as long as the cystic follicles remain steroidogenic [3]. However, COD can resolve spontaneously [4–6], and some cystic follicles become non-steroidogenic even if the structures remain [3,7,8].

Although non-steroidogenic cystic follicles are hormonally inactive and do not influence the normal estrous cycle [3,9–13], it is impossible to determine whether the cystic follicles are steroidogenic or non-steroidogenic by rectal examination or real-time ultrasonography. In the non-steroidogenic case, estrus occurs even if cystic follicles are present [1]. It is therefore possible that normal follicles and non-steroidogenic cystic follicles coexist, and cows may be able to conceive if they are inseminated before the normal follicles ovulate.

However, there are no reports about the reproductive performance of cows having both normal follicles and cystic follicles. Cows that are confirmed to have cystic follicles by rectal examination or real-time ultrasonography will be diagnosed as having COD, and are normally given hormonal treatment but are not artificially inseminated [14]. As a result, these cows, which have non-steroidogenic cystic follicles, have extended calving intervals [2,15–17]. The most important parameter for determining the economic losses due to COD is the sum of days from calving to insemination [18]. The primary objective of this study was, therefore, to investigate the conception rate when cows showing clear signs of estrus and having both normal sized follicles and cystic follicles were artificially inseminated. Ovarian dynamics and blood progesterone concentration (P_4) after artificial insemination (AI) were also monitored.

2. Material and methods

2.1. Animals

Cows showing clear signs of estrus and found to have both normal sized follicles and cystic follicles in their ovaries by rectal examination were selected for this study. In accordance

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with the definition of COD [2], follicles with a diameter <25 mm were considered normal sized follicles, and those with a diameter ≥25 mm were considered cystic follicles. Thirty parous Holstein cows between 2 and 6 years of age were selected. Although the cows were kept at 9 different farms, they were all housed in a free stall barn, fed a total mixed ration and had free access to water. Their body conditioning score was 2.5–4.0, and their milk volume was 25–40 kg/d. The signs of estrus included sniffing the genitalia of other animals, discharge of cohesive strands of mucus from the vulva, increased bellowing, mounting, standing, and chin resting. The cows were artificially inseminated when they showed signs of estrus, and this was considered Day 0. Cows with a history of treatment for ovarian or uterineabnormalities (COD, persistent ovarian follicles, endometritis, and pyometra) were excluded from this study.

2.2. Ultrasound scanning

Ovaries were examined on Days 0, 1, 10, and 20 using an ultrasonograph (Model SSD-500; Aloka, Tokyo, Japan) equipped with a 5-MHz transrectal linear transducer. The presence of normal sized follicles was confirmed on Day 0, and the number of cystic follicles was counted. The size of follicles was expressed as an average of a minor axis and the major axis. Ovulation of normal sized follicles was checked on Day 1, and ovarian dynamics were monitored on Days 10 and 20. Pregnancy was confirmed at 30 d after AI by ultrasonography.

2.3. Blood sampling

A vacuum-type heparinized tube was used to collect blood samples from the tail vein on Days 0, 5, 10, 15, 20, and 25. Plasma was separated by centrifugation (2 000× g for 10 min) and stored at −80 °C until the determination of P_4.

2.4. Hormone analysis

P_4 was measured using a commercial radioimmunoassay kit for progesterone (Diagnostic Products, Los Angeles, CA, USA), which has been previously validated for cattle [19]. Intra- and inter-assay coefficients of variation were 8.8% and 9.7%, respectively, and the average sensitivity of the assay was 0.02 ng/mL.

2.5. Statistical analysis

The cows were assigned to three groups: Group C (normal sized follicles ovulated on Day 1, and cows successfully conceived), Group NC (normal sized follicles ovulated on Day 1; however, cows did not conceive), and Group NO (normal sized follicles did not ovulate). Among the groups, the number and size of cystic follicles were analyzed using a Mann–Whitney U test. Results are reported as mean ± standard deviation (SD) when normally distributed and as median (minimum–maximum) when skewed. The fate of cystic follicles was analyzed using a Chi-square test followed by Fisher's exact test. The P_4 data were analyzed using repeated measures analysis of variance (treatment × time) followed by Fisher's protected least significant difference test. P values ≪0.05 were considered statistically significant difference.

3. Results

3.1. Ovulation of normal sized follicles and conception rate

Ovulation of normal sized follicles occurred in 21 of 30 cows (70.0%) on Day 1, and 11 of these cows (36.7%) successfully conceived (Group C), while 10 cows did not conceive (Group NC). None of the nine cows with normal sized follicles that did not ovulate on Day 1 (Group NO) conceived Tables 1 and 2.

3.2. Change in P_4

The change in P_4 in the 11 cows in Group C was similar to that in cows with normal estrus cycle [20]. P_4 increased considerably after AI, reached >1 ng/mL on Day 5, and remained at this level throughout the observation period. P_4 increased to >1 ng/mL on Day 5 and remained at this level until Day 15 in cows in Group NC, but it decreased on Day 20 and dropped to ≤1 ng/mL on Day 25. P_4 did not increase in cows in Group NO and remained approximately 1 ng/mL throughout the entire observation period (Figure 1).

3.3. Number and size of cystic follicles

The median number (minimum–maximum) of cystic follicles was 1 (1–1) in Group C, 1.8 (1–3) in Group NC, and 1.6 (1–2) in Group NO. The number of cows with multiple cystic follicles was 0 (0%) in Group C, 4 (40.0%) in Group NC, and 4 (44.4%) in Group NO. The median cystic follicle size was 34.9 (27–50) mm in Group C, 30.6 (25–45) mm in Group NC, and 36.1 (25–50) mm in Group NO. There were no significant differences in these parameters among the groups.

3.4. Ovarian dynamics

A corpus luteum (CL) developed after ovulation of normal sized follicles in all cows in Group C and Group NC even when

Table 1
The state of cystic follicles.

	Mean number of CF (range)	The number of cows having plural CF	Mean size of CF (mm) (range)
Group C (n = 11)	1^a	0 (0%)	34.9 (27–50)
Group NC (n = 10)	$1.8 (1–3)^b$	4 (40.0%)	30.6 (25–45)
Group NO (n = 9)	$1.6 (1–2)^b$	4 (44.4%)	36.1 (25–50)

CF: cystic follicles, the values in the same column with different letters as superscripts are significantly different ($P \leq 0.05$).

Table 2
The fate of cystic follicles.

	Maintain	Luteinize	Disappear	Develop
Group C	10	0	1	0
Group NC	15	1	2	3
Group NO	13	0	1	2

CF: cystic follicles; Maintain: CF was maintained; Luteinize: CF luteinized; Disappear: CF disappeared; Develop: new CF developed.
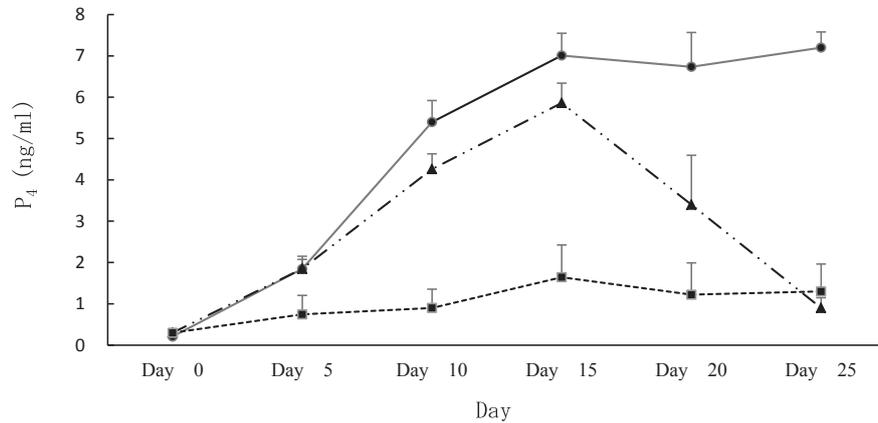
Figure 1. Plasma P$_4$ concentration between Day 1 and 25 in each group. Group C (●) (n = 11); Group NC (▲) (n = 10); Group NO (■) (n = 9).

cystic follicles were present (Figure 2). However, all CL of cows in Group NC had regressed by approximately Day 20. There were three outcomes for cystic follicles present on Day 0. They were maintained during the observation period (10 in Group C, 15 in Group NC, and 13 in Group NO), luteinized (one in Group NC), or they regressed and disappeared (1 in Group C, 2 in Group NC, and 1 in Group NO). New cystic follicles also developed during the observation period (3 in Group NC and 2 in Group NO). However, there were no significant differences in these parameters among the groups.
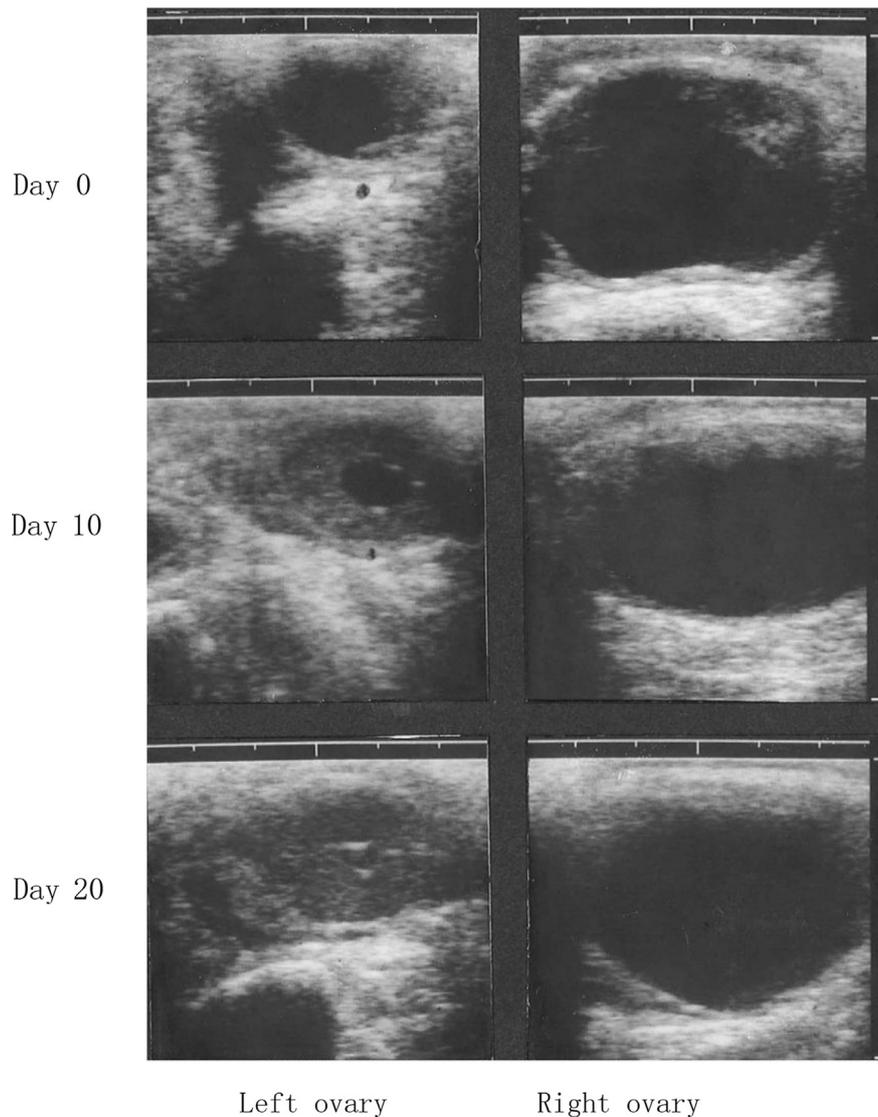

Figure 2. Ovarian dynamics of one cow in Group C. The normal sized follicle in the left ovary on Day 0 ovulated, and the corpus luteum developed on Day 10 and was maintained until Day 20. In the right ovary, a cystic follicle was present throughout the observation period. Scale bars: 10 mm.

4. Discussion

In this study, ovulation of normal sized follicles occurred in 70.0% of all cows, and the conception rate was 36.7%. Furthermore, although 10 cows did not conceive after ovulation of normal sized follicles, CL developed and secreted P_4 successfully. This confirmed previous reports that some normal sized follicles that coexist with cystic follicles can ovulate normally [9,12], Hatler and others (2003). Cows with COD recover spontaneously in some cases [4–6], and steroidogenic cystic follicles become non-steroidogenic during this recovery [3,7,8]. Non-steroidogenic cystic follicles are hormonally inactive and do not influence the normal estrous cycle [3,9–13]. In these cases, the ovaries have normal function and cyclicity, and normal estrus occurs even when cystic follicles are present [1].

In this study, cystic follicles of cows in Group C and Group NC were considered to be non-steroidogenic, because normal sized follicles ovulated and CL developed. On the other hand, normal sized follicles of cows in Group NO did not ovulate, and there was no significant increase in P_4 during the observation period. Furthermore, new cystic follicles developed in two cows. These results suggest that the cystic follicles of cows in Group NO might have been steroidogenic. Moreover, three new cystic follicles developed in cows in Group NC after ovulation of normal sized follicles. This indicates that endocrinological abnormalities might have also persisted in some of the cows in Group NC.

It is essential to differentiate between steroidogenic and non-steroidogenic cystic follicles when cows show clear signs of estrus and have both normal sized and cystic follicles. The median number and size of cystic follicles did not differ among the groups in this study. Consequently, the number and size of cystic follicles could not be used as indicators of whether cystic follicles were steroidogenic or non-steroidogenic. Even cows with steroidogenic cystic follicles exhibit periods of follicular growth that are associated with increased estradiol secretion [9,21]. Therefore, it is difficult to determine whether cystic follicles are steroidogenic or non-steroidogenic. However, all cows in Group C had only one cystic follicle. This might suggest that normal sized follicles are normal preovulatory follicles when there is only one coexisting cystic follicle.

Ovulation of normal sized follicles occurred in two-thirds of cows in the present study, and the conception rate was 36.7%. This rate is close to the reported conception rate of 34.0% for cows in Japan after the first AI [22]. This suggests that to shorten the parturition interval it is worth artificially inseminating cows that show clear signs of estrus even if the cows have both cystic follicles and normal sized follicles. Because a gonadotropin-releasing hormone analog induces a surge of luteinizing hormone that is similar to the preovulation surge [23], prescribing gonadotropin-releasing hormone to cows that show signs of estrus and have both normal sized and cystic follicles at AI may be recommended for inducing ovulation of normal sized follicles and for treatment of COD.

Conflict of interest statement

There are no conflicts of interest.

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