—Research Note—

Relationship between Ovarian Weight and Follicular Population in Heifers

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Abstract. The size of the ovary varies substantially among cattle. This variation may influence the potential of the ovary to produce follicles. In the present study, we examined whether a relationship exists between the weight of the ovary and the number of antral follicles ≥ 1 mm. Paired ovaries were obtained from Holstein x Japanese Black F1 heifers. Follicles were classified into three size categories (small: 1.0 – < 5.0 mm, medium: 5.0 – < 8.5 mm and large: $\geq$ 8.5 mm), and the number of follicles in each category was recorded. Large variations in the weight of ovaries and the number of follicles were observed among animals. Significant positive correlations ($r \geq 0.4$, $P < 0.001$) were found between the weight of intact ovaries and the number of follicles in all three categories for the ovary contralateral to CL (OCC) and in the small follicles for the ovary ipsilateral to CL (OIC). Significant positive correlations ($r > 0.4$, $P < 0.0001$) were also observed between the weight of ovaries devoid of CL and follicles and the number of small and medium follicles in both OIC and OCC, indicating that the correlation is not due to the increase in ovarian weight associated with the increase in follicular number. Paired ovaries contained a similar number of small and medium follicles, and significant positive correlations were observed between them ($r > 0.6$, $P < 0.0001$). There were significant positive correlations between the weight of OCC and the number of small and medium follicles in paired ovaries ($r > 0.4$, $P < 0.0001$). These results suggest that 1) the weight of an ovary reflects the potential of the ovary to produce antral follicles, and 2) a rough estimation of follicular population might be possible by using the weight of the ovary contralateral to CL in heifers.
Key words: Cattle, Follicular development, Follicular population, Ovarian weight

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A though little scientific attention has been given to it, it is well recognized that ovarian size varies considerably among cattle [1, 2]. This variation is generally considered to be due to the presence of the corpus luteum (CL) and large preovulatory follicles [2], and has been utilized for detecting animals with less or little follicular

activity. However, whether this variation in ovarian size also reflects to the number of follicles is not known. Recently, it has been suggested in humans that the size of the ovary before IVF treatment can be used to predict outcomes of the treatment in term of the number of responded follicles and recovered oocytes [3]. These reports imply that a similar relationship between ovarian size and follicular population may also exist in cattle. Thus, in the present study, we have

investigated the relationship between ovarian weight and the number of visible antral follicles in Holstein × Japanese Black F1 heifers.

Material and Methods

Collection of ovarian samples

The ovaries used in the present study were collected from Holstein × Japanese Black F1 heifers aged 21–26-month-old at a local slaughterhouse in April 2003. Only ovarian pairs with a CL and apparently normal follicles were used in the present study. Each ovarian pair was placed in a fabric paper bag, frozen at –20 C and transported to the laboratory. At the laboratory, the ovaries were thawed at room temperature and weighed to 0.01 g. The position of the CL and the number of follicles were recorded for each ovary. The CL was dissected out of the ovaries and weighed. Since estimation of follicular diameter from ovarian surface tends to give inaccurate values [4], we used the weight of the follicular fluid (FF) to determine the follicular diameter. To establish the relationship between the weight of the FF and the follicular diameter, a series of follicles were dissected and the mean of three perpendicular diameters and the weight of the FF were measured (3.1–16.5 mm, 0.01–2.07 g, n=44). Accordingly the following equation was obtained: $y=12.96x^{0.31}$, where y=the diameter of follicles (mm) and x=the weight of the FF (g), r=0.99. The weight of follicular wall was also calculated using the same set of follicles: $y = -0.0212x^2 + 0.1415x + 0.0061$, where y = the weight of the follicular wall (g) and x = the weight of the FF (g), r=0.99. The follicular fluid was aspirated from follicles and weighed individually while the FF from follicles less than 5 mm was pooled and weighed. The weight of ovaries without a CL and follicles was obtained by subtracting the weight of the CL, aspirated FF, and follicular walls of follicles greater than 5 mm from the total ovarian weight. No attempt was made to adjust for the weight of the follicular wall from small follicles.

Classification of follicles

In the present study, follicles were classified into three categories: small follicles (1 – < 5 mm: SF), medium follicles (5 – < 8.5 mm: MF), and large follicles (≥ 8.5 mm: LF). This classification is based on the functional status of follicles. Small follicles are not dependent on the acute support of gonadotropins [5]. Medium follicles are gonadotropin-dependant follicles that participate in follicular waves and FSH support is necessary for their growth and survival [5, 6]. Large follicles are follicles that have been selected for dominance and that can ovulate in response to the LH-surge [7]. Under the present experimental conditions, the physiological status of follicles could not been assessed. Thus each group contains atretic follicles as well as healthy follicles.

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) followed by the Steel-Dwass test. Correlations between two variables were analyzed by regression analysis. Values were expressed as mean ± SEM.

Results

Weight of ovaries and number of follicles

Eighty pairs of ovaries in various stages of the estrous cycle were used in the present study. The weight of the ovaries varied considerably among animals and up to a 6 fold difference was observed (Table 1). Between paired ovaries, this difference was much smaller and a significant positive correlation was observed between ovaries ipsilateral (OIC) and contralateral to the CL (OCC: r=0.39, P<0.001). Large variation in ovarian weight was still observed after the removal of the CL and much of the follicles (Table 1). Again, a significant positive correlation was observed between paired ovaries (r=0.83, P<0.0001).

The mean numbers of SF, MF, and LF per ovary/ animal are shown in Table 1. As for the weight of ovaries, large variations were observed among animals. Within animals, the OIC and OCC contain similar numbers of SF and MF, and significant positive correlations were observed between them (SF: r=0.73, P<0.0001, MF: r=0.60, P<0.0001). On the other hand, a negative correlation was observed for LF between the OIC and OCC (r= –0.49, P<0.0001). No correlation was found between the numbers of SF and MF or LF.

Table 1. Weight of ovaries ipsilateral to the CL (OIC) and contralateral to the CL (OCC) and the number of small (S), medium (M), and large (L) follicles

	Weight of ovaries (g)		Number of follicles		
	Intact	Without CL and follicles	S: < 5 mm	M: 5 - < 8.5 mm	L: ≥8.5 mm
OIC (n=80)	10.88 ± 0.27	6.19 ± 0.19	26.5 ± 1.6	5.9 ± 0.5	0.8 ± 0.1
	(6.06–18.08)	(3.41–11.70)	(2–65)	(0–19)	(0–3)
OCC (n=80)	7.02 ± 0.24	5.26 ± 0.19	26.0 ± 1.6	6.4 ± 0.5	1.0 ± 0.1
	(2.28–15.79)	(2.11–12.82)	(0–91)	(0–20)	(0–3)
Paired (n=80)	17.89 ± 0.42	11.45 ± 0.36	52.5 ± 3.0	12.3 ± 0.9	1.8 ± 0.1
	(9.54–33.87)	(5.56–24.52)	(2–150)	(1–33)	(0–4)

Table 2. Correlations between weight of ovary and number of small (S), medium (M), and large (L) follicles

	Class of follicle		
Correlated trait	S: 1 - < 5 mm	M: 5 - < 8.5 mm	L: ≥8.5 mm
Whole ovary ipsilateral to CL (OIC)	r = 0.50	r = 0.20	r = 0.24
	P < 0.0001	NS	P < 0.05
Whole ovary contralateral to CL (OCC)	r = 0.71	r = 0.41	r = 0.40
	P < 0.0001	P < 0.0001	P < 0.001
OIC without CL and follicles	r = 0.56	r = 0.43	r = 0.11
	P < 0.0001	P < 0.0001	NS
OCC without CL and follicles	r = 0.71	r = 0.42	r = 0.03
	P < 0.0001	P < 0.001	NS

NS, not significant (P>0.05).

Relationship between weight of ovaries and number of follicles

Significant positive correlations were found between the weight of the OIC and the number of SF and LF in the OIC (Table 2). Significant correlations were also found in the OCC for follicles of all classes (Table 2). Similarly, significant positive correlations were found between the weight of ovaries without a CL and follicles and the number of SF and MF for both the OIC and OCC (Table 2). Since the weight of the OCC appeared to be a better indicator of follicular population, relationships between the weight of the OCC and the number of SF and MF per animal were analyzed. There were significant positive correlations between the weight of the OCC and the number of follicles per animal for SF (r=0.64, P<0.0001; Fig. 1a) and MF (r=0.46, P<0.0001; Fig1. b).

Discussion

In the present study we found positive correlations between the weight of the ovary and the number of SF and MF in heifers. To our knowledge, this is the first report to demonstrate the relationship between the weight of the ovary and the number of follicles it contains in cattle.

Although little attention has been given to it, it is known that ovarian weight varies considerably among cattle of a similar age and physiological condition [1, 2]. This variation is generally thought to be due to the presence of a CL and large preovulatory follicles [2]. The present results indicate that the weight of the ovary also reflects the number of small and medium follicles in heifers.

Since the weight of follicles itself contributes to the total ovarian weight, the variation in ovarian weight might simply be a reflection of the variation in the number of follicles. This is certainly the case for LF, i.e. significant correlations between the weight of intact ovaries and the number of LF, and the removal of the CL and follicles eliminated this correlation. Nevertheless, large variation in ovarian weight was still apparent after the removal of the CL and follicles, and significant positive
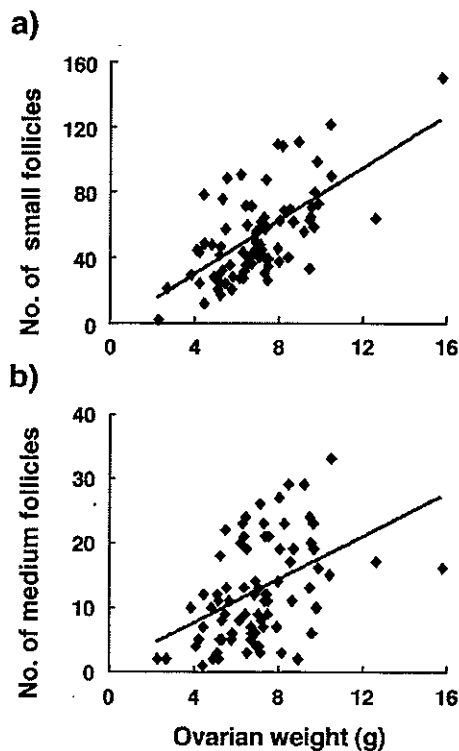
a)

b)

Fig. 1. Relationship between weight of the ovary contralateral to the CL and total number of small (a) and medium (b) follicles in paired ovaries (n=80). Significant correlations were observed for both small ($r=0.64$, $P<0.0001$) and medium ($r=0.46$, $P<0.0001$) follicles.

correlations between the weight of these ovaries and the number of SF and MF were observed. These results indicate that the variation in ovarian weight is not only due to the weight of antral follicles and CL, but also the weight of the ovary devoid of these temporal surface structures. Recently, Cushman *et al.* [8] demonstrated that the number of microscopic follicles correlates well with the number of antral follicles in cattle. Based on this finding, we suggest that the heavier ovaries observed in the present study contain more microscopic follicles, and these follicles at least partially contributed to the weight of the ovary. A pair of ovaries in young cattle contains, on average, over 100,000 primordial follicles [9]. Primordial follicles enter the growth phase at a certain rate, and increase their size by multiplying granulosa

cells and differentiating surrounding stromal cells into theca cells. The ovaries contain several hundred of these early developing follicles [9]. Thus, these primordial and early developing follicles can be a considerable source of ovarian weight. In this context, the weight of the ovary may also reflect the number of microscopic follicles it contains. If this is the case, the size of the ovary and its ability to produce antral follicles in adult life might be at least partially determined during the prenatal period when the size of the follicular reserve is set [9]. To examine this hypothesis, however, laborious histological work is needed.

The present results implicate the possibility of estimating an approximate number of antral follicles from the size of the ovary in heifers. In humans, the volume of the ovary measured using ultrasound has been advocated as a predictor for response to superovulation [3]. In cattle, one of the problems associated with superovulation is the difficulty to predict its outcome due to a large variation in superovulatory response [10]. To date, some attempts have been made to solve this problem [11, 12]. These researchers demonstrated that the superovulatory response could be predicted from the number of small to medium follicles prior to treatment by ultrasonography. Ultrasonographic counting of follicles, however, requires skilled personnel, as well as expensive equipment. In the present study, a significant positive correlation between the weight of the OCC and the number of SF per animal was observed. Since the number of SF that are recruited in a follicular wave appears to be the key to determine the output of superovulatory treatment [8], evaluation of the OCC by conventional palpation per rectum can be employed to roughly estimate the number of follicles when ultrasonographic evaluation is not available. However, further work is needed to confirm this possibility.

It must be noted that the results obtained in the present study were from relatively uniform animals, i.e. 21–26-month-old heifers with apparently healthy ovarian activity. Several factors are likely to affect the ovarian size. For example, small inactive ovaries that are largely devoid of surface follicles are often found in cattle with reproductive disorders. Once the problem is solved, follicular development resumes, and the size of the ovary increases. It was also shown that the weight of the ovary tends to increase in aged,

yet reproductively active cows despite of a drastic reduction in the number of follicles [1]. The number of follicles is also influenced by the stage of the estrous cycle [13]. For example, the number of medium follicles changes considerably during the estrous cycle, while that of small follicles is largely unchanged [13]. This may be one of the reasons why no correlation was found between the numbers of SF and MF in the present study. Therefore, a cautious approach must be taken to establish the relationship between the number of follicles and the size of ovaries.

In conclusion, the present study suggests that 1) the weight of an ovary reflects the potential of the ovary to produce antral follicles, and 2) a rough estimation of follicular population might be possible by using the weight of the ovary contralateral to CL in heifers.

Acknowledgements

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