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Relationship between heart girth, serum progesterone and superovulation response of donor Holstein cows

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ABSTRACT

Objective: To determine effects of variations of heart girth, volume trait on embryo quality grade, and to explore the relationship between heart girth and circulating progesterone and correlations of circulating progesterone with embryo quality grade in superstimulated donor Holstein cows.

Methods: Nineteen cows were subjected to a standard superstimulation protocol using follicle stimulating hormone. Blood samples were collected before superovulation, at insemination and at collection of embryos for progesterone analysis. Embryo quality grades were compared between high and low heart girth donors. Moreover, Pearson's correlations were determined between heart girth, progesterone and embryo quality.

Results: Variation of heart girth was not associated with significant differences in embryo quality grade between high and low heart girth donor cows (P>0.05). However, we observed a significantly higher percentage of the third-grade embryo (24.09%) in low heart girth donors compared to 9.64% in high heart girth donors. Moreover, the percentage of the transferable embryo was numerically higher and that of the degenerated embryo was numerically lower in low heart girth cows. Donor cows with low response to superovulation (total structures $\leqslant$ 3) expressed numerically higher mean heart girth, compared to donors with high (total structures >15) and medium (total structures = 4-15) superovulation. Heart girth and body weight of donor cows were moderately correlated (r=0.45, P<0.05), but none was correlated with circulating progesterone at different sampling times except for a moderate correlation between body weight and progesterone at embryo collection (r=0.54, P=0.02). Circulating progesterone before superovulation was moderately correlated to the second-grade embryo (r=0.46, P<0.05) and to the third grade (r=0.52, P<0.05) embryo.

Conclusions: Volume traits heart girth may influence the response of Holstein cows to superovulation; however, future studies with a higher number of cows are warranted to clarify significant influences.

1. Introduction

Conformation traits related to milk production in dairy cattle have been widely investigated in genetic selection programs for cattle without emphasis on functional traits as fertility[1]. It has been stated that genetic progress through selection of the low heritability traits as nonreturn rate, days open and calving interval is difficult[2]. Establishment of relationships between conformation traits and fertility might be an indirect way for improving fertility in dairy cows and this can be achieved by selection for certain traits[3]. Linear type traits can be defined as a variety of observable

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conformation features of an animal[4]. Volume traits of dairy cows have been a subject of extensive research in recent years to determine response to various hormonal manipulations during the regular dairy herd reproductive management programs because of the significant associations of these traits with fertility[5]. Inefficient response with subsequently reduced synchronization rates and pregnancy had been attributed to variations of body mass where larger cows achieved lower fertility rates. Heart girth is one of the volume traits associated with variations of reproductive responses of dairy cows submitted to different hormonal treatments[6]. Lower pregnancy rates and more services per conception were observed in taller, wider, deeper and more angular cows in the study carried out by Berry *et al*[4]. Those results contradicted the findings of Royal *et al*[3] who found decreased interval to the first postpartum ovulation in smaller cows and better fertility in larger herd-mates. Moreover, longer calving intervals have been observed in taller cows[7]; however, in that study, narrow-chested cows achieved lower conception rates. Pryce *et al*[8] studied the genetic correlations between body volume traits and fertility in dairy cows and found that chest width (r=0.28), body depth (r=0.26) and stature (r=0.33) were positively correlated with calving interval. Most of the literature determining effects of volume traits on fertility in dairy cows utilized the traditional reproductive measures which may be biased or inaccurate[6]. In addition, relationships between heart girth as a volume trait and endocrine reproductive measures have not been well-established and need further investigation.

Multiple ovulation embryo transfer has been a well-established reproductive biotechnology in dairy cattle. Nevertheless, the variability of the superovulation response of donor cows is still representing the greatest challenge. To the best of our knowledge, no studies have related circulating progesterone levels and superovulation outcomes to volume traits as heart girth in dairy cows.

The aim of the present study was to determine 1) effects of variations of heart girth on the quality grade of *in vivo* produced embryos, and 2) relationship between heart girth and circulating progesterone; correlations of circulating progesterone with embryo quality grade in superstimulated donor Holstein cows.

2. Materials and methods

2.1. Animals

A total of 19 multiparous Holstein cows (second to forth parity, mean 2.7±0.47; 3 to 6 years of age) belonging to a private dairy herd in Northwestern Egypt were included in the study. The farm strategy was planned to improve genetic composition of the herd through superovulation of excellent performing cows. In this regard, multiparous cows were selected as embryo donors based on genetic superiority which was confirmed *via* examination of records related to previous lactations.

The criteria used to select donor cows included productive parameters as 305-day milk yield, peak milk yield, persistence, total solids of milk, milk fat and milk protein as well as incidence of clinical and subclinical mastits. Moreover, the reproductive parameters used to select donors included regularity of cyclicity, inseminations per conception, days open, incidence of abortion and incidence of postpartum infertility problems. The cows were fed a totally mixed ration composed mainly of corn silage and formulated to meet or exceed requirements according to Nutrition Research Council recommendations[9]. Animals were housed in a free stall barn and were kept in a separate group. They were fed three times per day and were milked thrice daily using an automatic milking parlor. All animal handling procedures including injections, samplings, and flushing were carried out in accordance with the guidelines of the Animal Care and Use Committee of Research Institute for Faculty of Veterinary Medicine, Cairo University, Egypt (CU-II-F-16-18).

2.2. Heart girth measurements

At the initiation of superovulation, the thoracic circumference just behind the shoulder, of each donor cow was measured using a specific caliber and the reading was considered an estimate of heart girth. The average heart girth value of the cows under investigation was 207.89 cm. The process was carried out after the morning milking.

2.3. Superovulation protocol

The protocol of superovulation was carried out according to Abdel Aziz *et al*[10]. Briefly, donor cows received controlled internal drug release (CIDR) inset (1.39 g of Progesterone; Pfizer Animal Health, NewYork, USA) on Day 0. Two days later, all cows were administered intramuscularly (*i.m.*) with 12 mg of gonadotropin-releasing hormone agonist (Buserelin, Receptal, MSD Animal Health, New Cairo, Egypt). And superovulation treatment for all animals consisted of twice daily injections of descending doses of porcine follicle stimulating hormone (*i.m.*; Folltropin V, Bioniche Pharma USA, LLC, Lake Forest, Illinois, USA) for four days, starting with 80 mg on Day 4, for a total dose of 400 mg (80-80, 60-60, 40-40 and 20-20 mg pFSH). On Day 7, all donor cows received 500 mg of Cloprostenol (*i.m.*; Estrumate, MSD Animal Health, New Cairo, Egypt) and CIDR inserts were removed. Animals were given second dose of Cloprostenol and Buserelin next day. The cows were observed twice daily for estrus expression. Donor cows were artificially inseminated 2 times with frozen semen 48 and 60 h after CIDR removal.

2.4. Collection and evaluation of embryos

Embryos were collected on day 7 post-insemination. For embryo collection, each horn was flushed separately using 250 mL of Vigro complete flushing medium (Agtech inc, USA). Recovered fluids were collected onto an embryo filter then on to petri-dish. Embryos were immediately searched under a stereomicroscope "×100" (Agtech Inc., USA) and classified using the International Embryo Transfer Society criteria[11].

Embryos were graded from 1 to 4 according to the following criteria: First grade embryo, excellent symmetrical and spherical embryo mass with individual blastomeres uniform in size, color and density. The intact viable embryonic mass respresetns at least 85%. Second grade embryo: fair embryo with moderate irregularities in overall shape of embryonic mass or in size, color and density of individual blastomeres whith 50% of cellular material intact and viable; Third grade embryo: poor embryo with major irregular shape of embryonic mass or in size, color and density of individual cells. At least 25% of cellular material in garde 3 embryo should be intact, viable embryonic mass. Degenerated embryo: dead or degenerated embryo or oocyte, it is not viable.

After flushing, the superovulation response (number of total structures "total ova/embryos"collected) was calculated for each cow using trans-rectal ultrasonography (Linear type 7.5 MHz probe, Sonoscape, China) for counting the number of corpora lutea on the two ovaries. Cows yielding more than 15 total structures per flush were classified as high superovulation cows, those producing 4-15 total structures per flush were classified as medium superovulation cows, meanwhile cows yielding 3 or less total structures were considered as having low superovulation.

2.5. Sampling and progesterone assays

Coccygeal blood samples were obtained from individual donor cows prior to initiation of superovulation, at insemination and at collection of embryos. Blood samples were obtained in vacutainer tubes and were left 12 h at 4 ℃ for separation of serum. Then samples were centrifuged at 3 000 rpm for 30 min and plasma samples were separated and kept deep frozen at -80 ℃ till analysis.

Estimation of serum progesterone was carried out using an active radioimmunoassay kit (Immulite, Siemens, Germany) according to manufacturer instructions. The intra- and inter-assay coefficients of variation were 1.60%, 0.12% and 6.02%, 2.91% for high reference and low reference samples, respectively. The limit of sensitivity was 0.007 ng/mL.

2.6. Statistical analysis

Data were statistically analyzed using SPSS software (SPSS version 19). The differences in the median embryo quality grades between groups were analyzed using the non-parametric independent samples test. Meanwhile the percentages of embryo quality grades were compared statistically using *Chi* square test.

Relationships among body weight, heart girth at superovulation and circulating progesterone levels as well as correlations between circulating progesterone concentrations before initiation of superovulation, at estrus and at collection of embryos and embryo quality were tested using Pearson correlations. A P value less than 0.05 was considered statistically significant.

3. Results

3.1. Variation of superovulation response between small and large donor Holstein cows

Effects of heart girth on superovulation and embryo quality from donor Holstein cows were provided in Table 1. Variation of heart girth did not affect superovulation in donor Holstein cows (P>0.05). Total structures, the first-grade embryo, the second-grade embryo, the third-grade embryo, degenerated embryo, and total transferable embryo did not differ between high and low heart girth donors(P>0.05).

3.2. Variation of percentage of embryo quality grades between small and large donor Holstein cows

Percentage of embryo quality grades in high and low heart girth donor Holstein cows was shown in Table 2. Apart from significantly higher (P<0.05) third-grade embryo percentage (24.09% *vs.* 9.64% for low and high heart girth donors, respectively), no significant differences were observed in percentages of embryo quality grades between high and low heart girth donor cows. Low heart girth donors produced numerically higher percentage of the total transferable embryo and numerically lower degenerated embryos.

Table 1. Embryo quality grades obtained from donor Holstein cows with high and low heart girth measurements at superstimulation.

Heart girth	Total structures	Transferable embryos			Degenerated embryos
		First grade	Second grade	Third grade	
High (n=11)	9.0(2.25, 16.25)	1.5(1.00, 6.75)	1.0(1.00, 3.50)	0.5(0.00, 1.00)	1.0(0.25, 7.50)
Low (n=8)	9.0(2.00, 10.00)	3.0(0.00, 3.00)	0.0(0.00, 2.00)	1.0(0.00, 4.00)	2.0(0.00, 5.00)
P value	0.658	0.650	1.000	0.177	0.650

Data are expressed as median (interquartile range). High heart girth: donors in which heart girth measurements were higher than average of all cows (>207.89 cm); Low heart girth: donors with lower heart girth than average of all cows (<207.89 cm).

Table 2. Percentage of embryo quality grades in superstimulated donor Holstein cows according to heart girth measurements.

Heart girth	Transferable embryo percentage (%)			
	First grade	Second grade	Third grade	Total transferable
High (n=11)	30.12	18.07	9.64	57.83
Low (n=8)	27.71	13.25	24.09	65.05
P value	0.43	0.26	0.02	0.21

High heart girth: donors in which heart girth measurements were higher than average of all cows (>207.89 cm); Low heart girth: donors with lower heart girth than average of all cows (<207.89 cm).

3.3. Heart girth measurements in donor cows with high, medium and low superovulation response

Heart girth measurements were similar among high, medium and low superovulation donor cows in the present investigation (P>0.05). Low superovulation donors exhibited the highest heart girth measurements; however, the difference was not significant (Table 3).

Table 3. Heart girth measurements of high, medium and low response donor Holstein cows submitted to superstimulation.

Response to superstimulation	Heart girth (cm)	F value	P value
High (n=7)	208.86 ± 10.08		
Medium (n=6)	205.17 ± 2.40	0.53	0.60
Low (n=6)	209.50 ± 8.69		

Data are expressed as mean±SD.

3.4. Correlation coefficient of body weight, heart girth and circulating progesterone concentrations in donor Holstein cows

Relationships among the linear traits of heart girth and body weight and circulating progesterone in 19 donor Holstein cows were shown in Figure 1. Heart girth was moderately correlated to body weight (r=0.45, P<0.05). Circulating progesterone concentrations before superovulation, at estrus and at collection of embryos were not correlated to heart girth of donor cows (P>0.05). On the other hand, circulating progesterone levels at embryo collection were moderately correlated with body weight of donor cows (r=0.54, P<0.05).

3.5. Correlation coefficient between circulating progesterone concentrations and superovulation response in donor Holstein cows

Moderate correlations were observed between circulating progesterone before superovulation and the second-grade embryo (r=0.46, P=0.03), and the third-grade embryo (r=0.52, P=0.03). No significant correlations could be detected between progesterone level at estrus and embryo quality. Meanwhile, the third-grade embryo was moderately correlated with circulating progesterone concentrations at collection of embryos (r=0.48, P=0.04).

4. Discussion

The current study was designed to investigate the effects of heart girth as body volume trait on the response of donor Holstein cows to superovulation. We studied the correlations between heart girth and circulating levels of progesterone at three-time points during the protocol. Moreover, correlations between heart girth and superovulation were also determined. To the best of our knowledge, the effects of heart girth on the response of donor cows to superovulation have not been searched.

Although embryo quality grades did not differ between high and low heart girth donors, percentages of embryo quality grades varied numerically. The percentage of the third-grade embryo

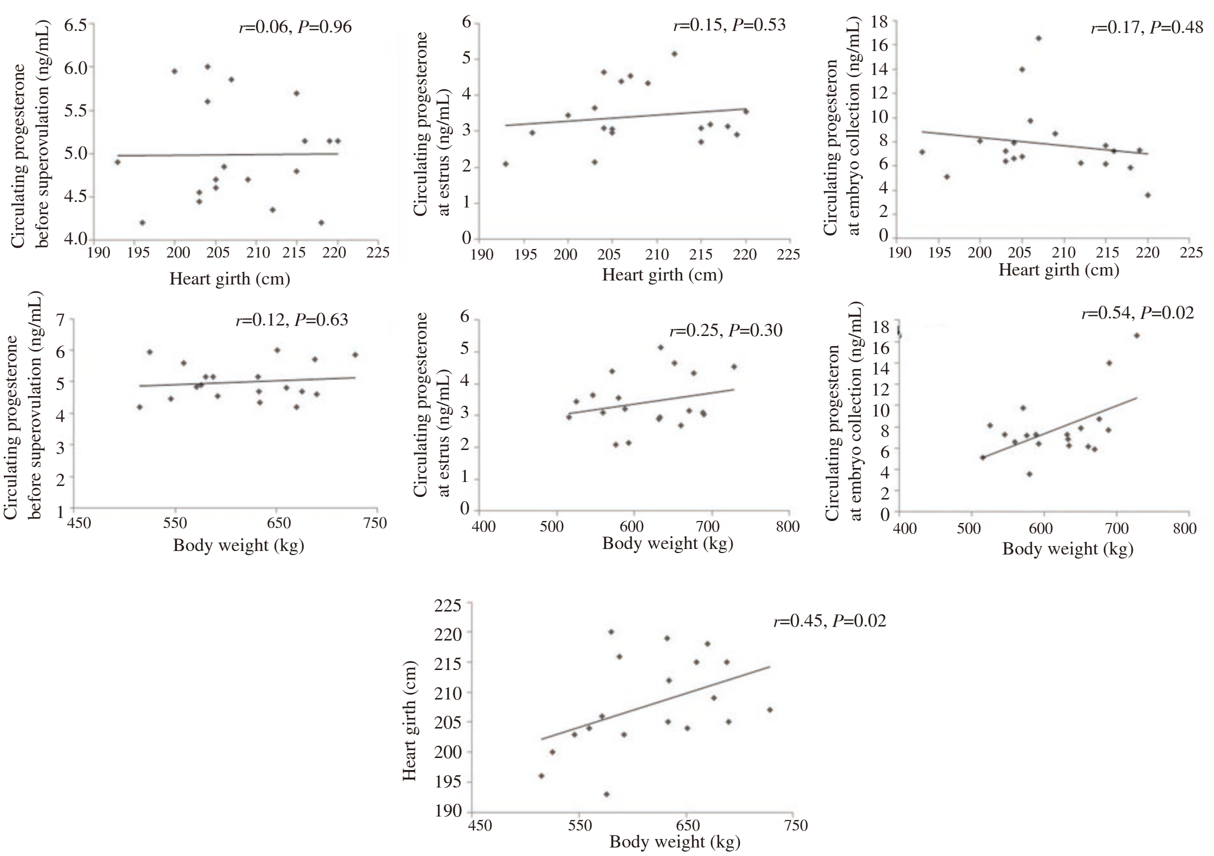

Figure 1. Relationships among linear traits of heart girth and body weight and circulating progesterone in donor Holstein cows.

was significantly higher in low heart girth donors. Additionally, total transferable embryo percentage was numerically higher in low heart girth donors, meanwhile, the percentage of degenerated embryo was numerically higher in high heart girth donors. Further, donor cows with low superovulation (total structures collected $\leqslant$3) had numerically higher heart girth than medium and high superovulation cows. These findings substantiate that differences in body volume traits of donor cows submitted to superovulation may affect their responses. Findings from previous studies in single ovulating Holstein cows showed body volume traits as body depth and strength were associated with low fertility in primiparous and multiparous cows[4,6]. It is presumable that the few numbers of donor cows included in our study might have causeda lack of statistical significance between large and small superstimulated donor groups. Controversially, smaller framed cows with larger body capacities had shorter calving interval and age at the first calving[12].

Lower fertility of large cows (heart girth >207.89 cm) was attributed to incorrect dosages of different hormonal manipulations[6]. According to Kron[6], effective dosages for small cows (heart girth <207.89 cm) might be insufficient for large cows. These findings substantiate that further research is warranted to examine the effects of different doses of follicle stimulating hormone, gonadotropin-releasing hormone, and prostaglandin F2alpha (PGF2 alpha), based on body volume traits, on the response of donor cows to superstimulation. Cows with incomplete luteal regression after PGF2 alpha administration will have greater circulating progesterone concentrations than cows with complete luteolysis. Recently, it wasreported that subluteal progesterone concentrations were associated with better superovulation in Holstein donor cows superstimulated after dominant follicle ablation[13]. On the other hand, decreased total quality embryos and decreased number of embryos were recorded in donors with low concentrations of circulating progesterone[14,15]. The present study could not find any significant correlations between serum progesterone and heart girth. The discrepancies among different studies regarding the effects of subluteal levels of progesterone during multiple ovulation embryo transfer might be due to different follicular wave number at initiation of superovulation. Indeed, future research should be planned to investigate the ideal concentrations of progesterone which would permit the optimal superovulation in donor cows.

Circulating progesterone concentrations at initiation of superovulation, at estrus and at collection of embryoswere not significantly correlated with heart girth of donor cows in the current study, menwhile a significant moderate correlation was observed between circulating progesterone levels at embryo collection and body weight of donor cows. Kron[6] reported that circulating progesterone concentrations per corpus luteum volume were highly insignificant in relation to the size of cow at administration of PGF2 alpha.

Our study revealed weak to moderate correlations between progesterone levels at initiation of superovulation and embryo quality grades 2 and 3. Higher serum progesterone levels during superovulation were associated with greater numbers of embryo quality grades classified as 3 and 4 when compared to low progesterone donors[13]. It is plausible that higher progesterone levels caused suppression of luteinizing hormone pulses leading to inefficient follicle maturation and poorer quality of resulting embryos. Inconsistency between our findings regarding higher percentage of grade 2 embryo might be due to different experimental design. Wiley *et al*[13] planned to have a group of donors with low progesterone (subluteal levels) by administering PGF2 alpha at ablation of dominant follicles. In addition, those authors measured progesterone daily, while we measured progesterone at three specific time points.

Noticeably, circulating progesterone at estrus following superovulation was not correlated with quality of *in vivo* produced embryos in the current study. The pattern of periovulatory progesterone milieu seems more important than progesterone level itself. Follicular growth in lower progesterone milieu is associated with increases in luteinizing hormone pulses, higher estradiol concentrations and larger diameters of mature follicles[16]. Collectively, these events eventually lead to decreased fertility in beef and dairy cattle.

Conceivably, the findings from the present study revealed significantly different percentages of grade 3 embryoand numerically different percentage of transferable and degenerated embryos between superstimulated donor Holstein cows with high and low heart girth values. In addition, heart girth was not correlated with circulating progesterone levels, while high progesterone levels before superovulation were correlated with the second and third-grade embryos.

Conflict of interest statement

The authors declare there is no conflict of interest.

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