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RESEARCH ARTICLE

The Role of Antral Follicle Count in OPU/IVEP Donor Selection in Holstein Cattle

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ABSTRACT

The aim of this study was to determine the effect of antral follicle number on oocyte yield and IVEP in Holstein breed donors. The study groups were constituted following ultrasonographic examinations conducted on 23 Holstein heifers at one-week intervals. The Low-AFC group (n = 8) comprised animals with an average antral follicle counts in the ovary of ≤ 15, as determined by ultrasonographic examination. The High-AFC group (n = 9) comprised animals with an average antral follicle number of ≥30. Oocytes were collected from animals in both groups four times, one week apart, any day of the estrous cycle. The oocytes were classified according to their morphological characteristics and subsequently incorporated into the in vitro embryo production process. The total and viable oocyte numbers were found to be significantly greater in the High-AFC group than in the Low-AFC group ($P < 0.01$). The number of blastocysts obtained per session was also greater in the High-AFC group ($P < 0.01$). No statistically significant difference was observed between the blastocyst and viable oocyte rates in the two groups. Consequently, oocytes and blastocysts were obtained from Holsetin donors with high antral follicle counts in greater numbers. It was thought that the number of antral follicles in Holstein breed donors could be employed as a parameter in donor animal selection to increase the success of OPU/IVEP.

Key Words: Antral follicle counts, Donor selection, OPU/IVEP.

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Holstein Sığırlarda OPU/IVEP için Donör Seçiminde Antral Folikül Sayısının Rolü

ÖZ

Bu çalışmada Holstein ırkı donörlerde antral folikül sayısının oosit verimine ve in vitro embriyo üretimine etkisinin belirlenmesi amaçlandı. Çalışma grupları 23 baş Holstein ırkı düveye 1 hafta arayla yapılan ultrasonografik muayeneler sonrasında oluşturuldu. Ultrasonografik muayene sonrasında ovaryumundaki ortalama antral folikül sayısı ≤ 15 olan hayvanlar Düşük-AFS grubuna (n=8) alındı. Ortalama antral folikül sayısı ≥ 30 olan hayvanlar ise Yüksek-AFS grubuna (n=9) alındı. Her iki gruptaki hayvanlardan östrüs siklusunun rastgele bir gününde birer hafta arayla 4 kez oosit toplandı. Toplanan oositler morfolojik özelliklerine göre sınıflandırılarak in vitro embriyo üretim sürecine alındı. Toplam ve in vitro embriyo üretimine uygun oosit sayısı Yüksek-AFS grubunda Düşük-AFS grubundan yüksek bulundu ($P < 0.01$). Seans başına elde edilen blastosist sayısı da Yüksek-AFS grubunda daha fazlaydı ($P < 0.01$). Blastosist oranı ve in vitro embriyo üretimine uygun oosit oranı iki grup arasında istatistiksel farklılık göstermedi. Sonuç olarak Holstein ırkı donörlerde yüksek antral folikül sayısına sahip hayvanlardan daha fazla sayıda oosit ve blastosist elde edildi. Holstein ırkı donörlerde antral folikül sayısının OPU/IVEP başarısını artırmak için donör hayvan seçiminde bir parametre olarak kullanılabileceği düşünüldü.

Anahtar Kelimeler: Antral folikül sayısı, Donör seçimi, OPU/IVEP

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INTRODUCTION

Reproductive biotechnology methods are often used in cattle breeding to increase the number of animals with superior genetic characteristics (Berglund 2008). Embryo technologies make it possible to rapidly change the genetic composition of a herd by reducing the generation interval (Mapletoft and Hasler 2005). Bovine embryos are produced in vivo and in vitro. In recent times, there has been a gradual decline in the number of embryos produced in vivo, while there has been a corresponding increase in the number of embryos produced in vitro (Watanabe et al. 2018; Viana 2022). The advent of the OPU (ovum pick-up) technique has been a significant factor in the increased production of embryos in vitro (IVEP). The OPU technique allows oocytes to be retrieved from highly productive donors on multiple occasions within a short period of time (Pieterse et al. 1988; Boni 2012).
A number of studies are currently being conducted in different areas with the objective of increasing the oocyte yield obtained from cattle (Demissie et al. 2022; Saleem et al. 2022). The selection of donor animals is of significant importance in order to collect more oocytes in a short time. To increase the number of oocytes retrieved, donors with a high oocyte yield should be used. In embryo production, criteria such as genetics and productivity characteristics are generally focused on for donor animal selection (Mebratu et al. 2020). However, genetically superior donors may not have high oocyte yields. Therefore, evaluating parameters related to oocyte yield in donor selection for OPU/IVEP may increase success rates. It has been found that the anti-Müllerian hormone (AMH) level and the antral follicle count (AFC) provide an indication of the oocyte yield of donor animals (Garcia et al. 2020; Feres et al. 2024). AMH and AFC are positively correlated, making them useful criteria for donor selection (Ireland et al. 2010; Zangirolamo et al. 2018). There is considerable variation in AFC and serum AMH levels between cattle, but these reproductive parameters are highly consistent within the same animal. While AMH measurement requires lab analysis, AFC can be evaluated through ultrasound of both ovaries by a experienced operator (Silva-Santos et al. 2014; Morotti et al. 2018). For this reason, AFC is a more economical parameter for donor animal selection. The AFC represents the number of follicles with a diameter of at least 3 mm that can be observed in the ovary (Morotti et al. 2018). The primary source of oocytes in OPU applications is follicles with a diameter of 3–8 mm (Dieleman et al. 2002, Hendriksen et al. 2004). Accordingly, it is postulated that the oocyte yield of animals with a high AFC will be higher. Therefore, AFC emerges as a critical parameter in the selection of donors for OPU/IVEP procedures (Lollato et al., 2022).

It has been demonstrated that the AFC is one of the most effective techniques for evaluating reproductive efficiency in Bos taurus cattle breeds. Although AFC reveals individual differences between animals, it is of great importance in assessing the future reproductive performance of the same animal (Burns et al. 2005). In addition, there is research indicating that the effect of AFC on reproductive efficiency differs between Bos taurus and Bos indicus cattle breeds. Therefore, further studies are needed to determine the suitability of AFC for the selection of donors for OPU/IVEP in a variety of bovine breeds (Dos Santos et al. 2016, Garcia et al. 2020).
The aim of this study was to determine the effect of AFC on the yield of oocytes and blastocysts in Holstein breed heifers.

MATERIALS and METHODS

The study animals were a selection of 23 Holstein heifers from a farm that had genomic selection in place. The animals were between 14 and 18 months old and exhibited body condition scores ranging from 3.0 to 3.5. The animals did not have any health problems. The animals were kept under the same conditions and fed ad libitum. The ration comprised alfalfa silage, hay, corn silage, concentrate feed and alfalfa.

Experimental Design

Ultrasound scans were performed on 23 Holstein heifers 4 times at 1-week intervals to form the study groups. The AFC in the animals' ovaries was recorded during ultrasound scans. Animals with an average number of antral follicles in the ovaries ≤ 15 were included in the low antral follicle count (Low-AFC) donor group. Animals with an average number of antral follicles in their ovaries ≥ 30 were included in the donors with high antral follicles (High-AFC) group. The study excluded other animals.
Oocytes were retrieved from donors in the Low-AFC (n=8) and High-AFC (n=9) groups by transvaginal ultrasound 4 times, starting any day of the estrous cycle, 1 week interval. The experimental design is schematized in Figure 1.

Ovum-Pick Up

For oocyte collection, a catheter-aspiration device (230 V, Minitube), 4.0-9.0 MHz microconvex probe (Esaote, SC3123 VET) and real-time ultrasonography device (Esaote MyLab TwiceVet, 5001) were used. Aspiration vacuum pressure was 80-90 mm/Hg during oocyte retrieval. A catheter and a microconvex vaginal probe was used to aspirate all follicles larger than 2 mm from the ovary (20-gauge needle).

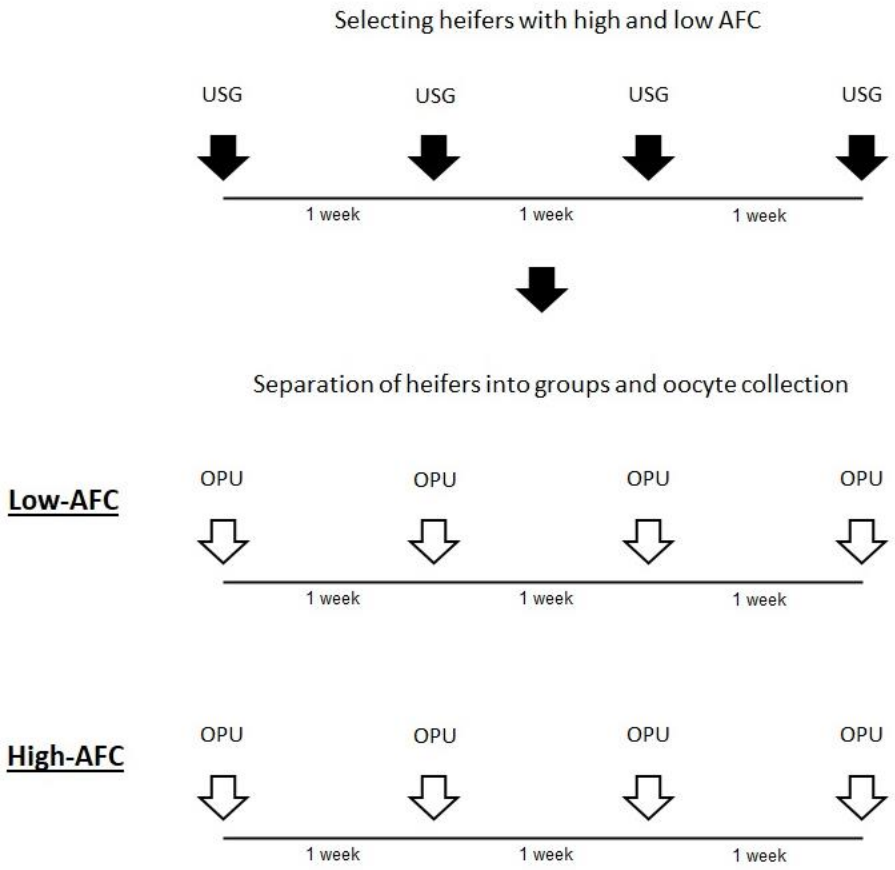

Figure 1. Separation of the heifers into groups and design of the experiment (AFC: Antral Follicle Count, USG: Ultrasound-Guided Antral Follicle Counting, OPU: Ovum - Pick Up).

Cumulus Oocyte Complex Classification

Cumulus oocyte complexes (COCs) were examined under a stereomicroscope (S ApoE, Leica). Morphological features were used to classify COCs. COCs were evaluated for morphological characteristics, including cytoplasm homogeneity, number of cumulus cell layers, and cumulus cell density. COCs were classified according to their quality. It was rated as very good, good, fair, or poor (respectively Grade A, B, C and D) (Petyim et al. 2003; Gordon 2003). A, B and C quality oocytes were included in the IVEP process. COCs with homogeneous cytoplasm, uniform appearance and at least one layer of compacted cumulus cells were classified as viable (Hayden et al. 2022).

In vitro Embryo Production

In vitro embryo production was performed using media produced by a commercial company (IVF Bioscience). After oocyte collection, the COCs that were suitable for IVEP were washed three times in the oocyte washing medium (BO-Wash). The COCs were then incubated in an in vitro maturation medium (BO-IVM) at 38.5 °C and 5.5 % CO_2 for 20 - 22 hours. For in vitro fertilization (IVF), semen from the same sire was used throughout the experiment. Sperm washing was performed with semen preparation medium (BO-SemenPrep). The number of spermatozoa was calculated as 1 million per mL. Spermatozoa were then added to in vitro fertilization medium containing COCs (incubated at 38.5°C, 5% CO_2 for 20 hours). After IVF, prospective zygotes were vortexed and transferred to in vitro culture medium (BO-IVC) coated with mineral oil (BO-Oil). It was cultured in culture medium at 38.5°C, 6% CO_2 and 6% O_2 for 7 days. Embryo quality and developmental stages were assessed according to the International Embryo Transfer Society (IETS) (Bó and Mapletoft 2013; Alkan et al. 2023).

Statistical Analysis

The data were analysed with the SPSS 25.0 statistical software (IBM Corp. Released 2017. IBM SPSS Statistics for Windows). *Shapiro–Wilk* test was used for the prerequisites of homogeneity of variances and normality of variables. The mean ± standard deviation (SD) of normally distributed variables was presented. The variables were evaluated with t-test. Blastocyst rate were analysed by Chi-square test. At the 95% confidence level ($p<0.05$), the differences were considered statistically significant.

RESULTS

Oocyte and blastocyst yield from donors with high and low AFC are shown in Table 1. The oocytes counts of the highest quality (Grade A) obtained following oocyte retrieval was significantly greater in the High-AFC group than in the Low-AFC group ($P < 0.01$). Furthermore, the number of oocytes exhibiting quality grades B, C, and D was also greater in the High AFC group ($P < 0.01$). Additionally, the study revealed that the number of viable oocytes suitable for IVEP was statistically higher in the High AFC group than in the Low AFC group. Nevertheless, no statistically significant difference was observed in the rate of viable oocytes between the two groups. Oocytes counts of all qualities was found to be statistically higher in the High-AFC group, as demonstrated by the results of the study. Accordingly, the total number of oocytes obtained from animals with high AFC was also higher ($P < 0.01$). Blastocysts counts obtained per OPU was approximately twice as high in the High-AFC group as in the Low-AFC group ($P < 0.01$). No statistically significant difference was observed in the blastocyst rate between the two groups.

Table 1. Post-OPU oocyte and blastocyst data of Holstein heifers with high and low AFC (mean ± standard error of mean, Viable oocyte rate: viable oocyte number/total oocyte number, Blastocyst rate: blastocyst number/viable oocyte number, OPU: Number of OPU sessions).

Variables	Low-AFC	High-AFC	P Value
Grade A oocytes / %	1.28 ± 0.24 / 21.13	3.58 ± 0.49 / 24.42	< 0.0001
Grade B oocytes / %	1.50 ± 0.25 / 24.74	3.19 ± 0.52 / 21.61	0.007
Grade C oocytes / %	1.75 ± 0.29 / 28.86	3.66 ± 0.38 / 24.81	< 0.0001
Grade D oocytes / %	1.53 ± 0.29 / 25.25	4.33 ± 0.63 / 29.32	< 0.0001
Viable oocytes/OPU	4.53 ± 0.46	10.52 ± 1.06	< 0.0001
Viable oocytes rate (%)	74.74	70.67	-
Total oocytes/OPU	6.06 ± 0.51	14.86 ± 1.52	< 0.0001
Blastocysts/OPU	1.34 ± 0.18	2.58 ± 0.29	0.001
Blastocyst rate (%)	29.65	24.46	-

DISCUSSION

Counts and quality of collected oocytes affect IVEP success rates. Increasing oocyte yield increases the number of blastocysts obtained per OPU. Oocyte yield varies considerably even within the same breed. It is therefore crucial to select suitable donors in order to increase the success of OPU/IVEP. One of the most practical methods for evaluating the oocyte yield AFC was higher when synchronization was applied prior to OPU. In a separate study examining the impact of AFC on oocyte yield, it was demonstrated that there was a positive correlation between the total and viable oocyte numbers and AFC (Dos Santos et al. 2016). In a comparable study, Monteiro et al. (2017) of donor animals is antral follicle count. A study investigating the association between AFC and reproductive efficiency found a positive correlation between the two variables (Mossa et al. 2012). García et al. (2020) found in their study that the total and viable oocyte numbers obtained from donors with high AFC were higher. Furthermore, the study revealed that the oocyte yield of donors with a high reported that they collected a greater number of oocytes from donors with a high AFC. Viana et al. (2003) posited that ovarian damage and the formation of scar tissue may be heightened in donors with a high AFC due to the increased number of follicle punctures. It has been stated that this situation may negatively

affect oocyte yield in animals with high AFC in repeated OPU applications. The results of the presented study indicate that a greater number of total and viable oocytes were obtained from Holstein breed heifers than from donors with a high AFC. Furthermore, oocytes counts of all qualities in the high AFC group was observed to be higher than in the low AFC group. The results were in line with those of previous studies, which indicated that a greater number of oocytes could be harvested from donors with a higher AFC.

For IVEP, blastocysts counts obtained per OPU and the blastocyst rate are as important as oocyte yield. A study evaluating the effect of antral follicle population on in vitro embryo production (IVEP) found that the number of blastocysts obtained per OPU from donors with a high AFC was higher than from donors with a low AFCAdditionally, the study demonstrated that there was no statistical difference in the rates of oocytes collected from animals with high and low AFC reaching the blastocyst (Guerreiro et al. 2014). Monteiro et al. (2017) reported that the antral follicle population influences the number of blastocysts obtained per OPU. The study by Silva-Santos et al. (2014) exhibited that the number of embryos obtained per OPU and vitrifiable embryos were greater in donors with high AFC. Furthermore, the study demonstrated that a greater number of blastocysts were obtained in the group with a high AFC, yet the cleavage rate remained comparable. Ireland et al. (2007) observed that embryos obtained from animals with a high AFC was four times greater than that obtained from animals with a low AFC. In the presented study, a higher number of blastocysts per OPU was obtained in the high AFC group. But there was no statistical difference in blastocyst rates between the groups. It was thought that this discrepancy was due to the fact that, although a greater number of viable oocytes were collected in the high AFC group, the rate of viable oocytes was similar.

CONCLUSION

Antral follicles counts affected the oocyte yield of donor animals. In OPU applications, more oocytes were collected from donors with high AFC. Viable oocytes counts collected in the high-AFC group was also greater. However, viable oocyte rate and blastocyst rate did not show any statistical difference between the two groups according to AFC. It was thought that using of donors with high AFC could increase the success of OPU/IVEP. It was concluded that AFC in Holstein breed donors can be used as a parameter in donor animal selection.

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Ethics approval and consent to participate: This study was approved by the Local Ethics Committee (Selcuk University Local Ethics Committee for Animal Experiments, Approval Number: 2024/02/30). All applied methods are in accordance with the ARRIVE guidelines.

Competing interests: None of the authors have any conflict of interest to declare.

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