The effect of Anti-Mullerian hormone on yield of oocytes recovered by ovum pick-up (opu) in heifers

Research Article

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ABSTRACT

The aim of this study was to determine the relationship between the oocyte yield by the ovum pick-up (OPU) technique with the Anti-Mullerian Hormone (AMH) concentrations of the donors. Ten healthy Holstein heifers aged 12 to 15 months were included in the study. AMH measurements were performed with Bovine VIDAS® Anti-Mullerian Hormone kits (Biomeriux, Marcy l'Etoile, France) using the Mini Vidas device. A total of 67 OPU sessions were performed on a random day of the cycle. Oocytes were classified according to their quality, and viability evaluation of oocytes was made according to the cell layer number and cumulus integrity in the cumulus-oocyte complex (COC), the homogeneity of their cytoplasm. The average oocyte yield in OPU sessions per animal was range from 4–8. There was an significant negative correlation between the collected oocyte numbers and plasma AMH levels. In conclusion, it was observed that AMH concentration did not affect the number of viable and the quality oocytes collected in weekly OPU administration in animals. It was thought that OPU applications performed without knowing the day of the cycle did not provide the expected correlation with AMH data.

Keywords: AMH, heifer, oocyte, ovum pick-up

INTRODUCTION

Assisted reproductive technologies, such as in vitro embryo production, (IVEP) have been widely practiced in the recent years for the purpose of genetic improvement (Guerreiro et al., 2004). In 2019 IVEP accounted for 72.68% (1.031.567) of the total embryo production (1.419.336) in cattle breeding worldwide. The vast majority of embryos (1.010.680) were produced from OPU (Ovum Pick-Up) while few (20.887) were obtained through slaughterhouses (Viana, 2020).

The success of oocytes obtained by the OPU technique for IVEP is associated with physiological characteristics, such as the number of antral follicles in the ovary and oocyte developmental competence. Recent studies have shown that Anti-Mullerian hormone (AMH) can be used as a biomarker to identify factors including selection of donors with superior production traits for IVEP, estimation of ovary reserves, future fertility characteristics, milk production level, and longevity in herd (Guerreiro et al., 2014; Viana, 2020).

Antral Follicle Count (AFC) is a reliable phenotypic biomarker positively associated with fertility, ovarian reserve, ovarian function, superovulation response, in vitro blastocyst production, transferable embryo production/count, and offspring birth weight (Ireland et al., 2008; Sabuncu et al., 2019).

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Circulating AMH concentration is reported to have a positive correlation with total AFC in mice, human, Bos taurus, and Bos indicus cattle (Guerreiro et al., 2014). AMH production begins with the onset of follicular wave in the ovary and peaks in the primordial, primary, and secondary follicles. AMH production decreases after the selection of the dominant follicle and is not secreted from atretic follicles (Lussier et al., 1987). Studies have shown that the circulating AMH is highly associated with AFC. It has been suggested that determining the level of AMH can be an endocrine marker to define the best donors in IVEP, regardless of the genotype and age of the animal (Broer et al., 2011; Baruselli et al., 2018; Maculan et al., 2018).

Possible functions of AMH in the ovary include inhibition of follicular activation and growth, FSH-stimulated growth, granulosa cell growth, and aromatase (Poole et al., 2016). In the absence of AMH, the follicles develop faster and the ovarian follicular reserves deplete sooner (Umer et al., 2019). Since the AMH concentration in cows remains almost unchanged after puberty, heifers who reach puberty with a low ovarian reserve are at risk for faster depletion of their ovarian reserves and a shorter reproductive life (Pankhurst, 2017; Alward ve Bohlen, 2020).

It is reported that in cattle, circulating AMH concentration can be used as a possible marker for veterinarians to predict AFC in ovaries as well as to predict superovulation response and IVEP performance (Mossa et al., 2012; Ribeiro et al., 2014). A recent study investigating the possibility of producing embryos from young female calves has found that AMH concentration was a very useful marker to predict IVEP performance (Batista et al., 2014). It is reported that the determination of circulating AMH concentration in very young calves can be an important biomarker in selecting the best donors for in vitro embryo production because ultrasound examination of ovaries in this age group is difficult and impractical (Armstrong et al., 1992). Given the assumption that AMH concentration is not affected by factors, such as period of cycle, age, or nutrition, and remained almost constant throughout the life of the cow, the present study aimed to determine the relationship between the oocyte yield collected by the OPU technique with the AMH concentrations of the donors.

MATERIAL and METHOD

Ten healthy Holstein heifers aged 12 to 15 months were included in the study. Heifers were selected all their genomic features were examined and among with high AMH levels (>300 pg/mL). Before OPU applications, blood samples were taken from the jugular vein into heparinized tubes for once. Blood samples were centrifuged at 5000 rpm for 10 min and serum samples were stored at -20°C until the time of measurement. AMH measurements were performed with Bovine VIDAS® Anti-Mullerian Hormone kits (Biomeriux, Marcy l'Etoile, France) using the Mini Vidas device (Biomeriux, Marcy l'Etoile, France).

A total of 67 OPU sessions were performed on a random day of the cycle at intervals of minimum one and maximum two weeks without any hormone administration. The OPU was done using Esaote MyLab TwiceVet ultrasonography device and a compatible intravaginal OPU probe, catheter and aspiration device combination (Esaote 5001) were used. For OPU, animals had lower epidural anesthesia (4–6 cc of local anesthetic, Adokain, Sanovel, İstanbul, Turkey). After cleaning the rectum, the perineal region was cleansed. Rectal palpation of the ovary was performed through the rectum Special convex vaginal probe (4.0–9.0 MHz probe and catheter with 20 G needle at the tip) was inserted into the vagina and directed to the right fornix of the vagina for aspiration from the right ovary and fixed. The right ovary was manually brought into the probe's scanning surface through the rectum and the ovary was fully visualized. The ovaries were examined before aspiration and follicle numbers were recorded. The ovaries were manually rotated from the rectum, and each follicle was positioned sequentially on the needle drilling line and all follicles with a diameter of 3-8 mm were aspirated. The follicle fluid was aspirated using vacuum (80–90 mm/Hg) with the aspiration pump installed in the system immediately after the performance of puncture of each follicle. After the aspiration process was completed, the needle apparatus of the probe was removed and washed, allowing the oocytes that might remain in the system to be taken into the commercial OPU medium (IVF Bioscience, Denmark). The same procedure was repeated for the left ovary.

The collected OPU fluid was transferred to the petri dishes and the oocytes were screened (1.6X magnification) in stereomicroscope (Leica S8 APO, Wetzlar, Germany). The retrieved oocytes were taken into the washing solution (BO-WASH, IVF Bioscience, Denmark). At this stage, oocytes according to their quality were classified as A, B, C, and D quality under an inverted microscope (Leica DM IL, Germany). Collected COCs were evaluated according to:

A Quality: It has > 5 compact cell layers and homogeneous cytoplasm (Image 1),

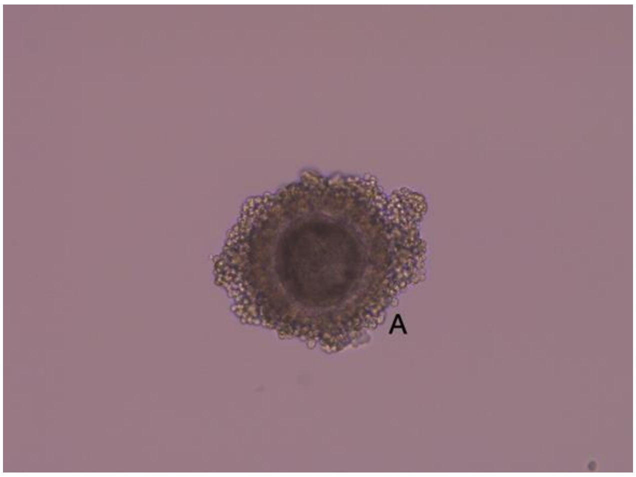

Image 1. A quality oocyte. A: A quality oocyte

B Quality: It has 3-5 layers of compact cells and few inhomogeneous areas in the cytoplasm or >5 layers of compact cells and dense inhomogeneous areas (Image 2),

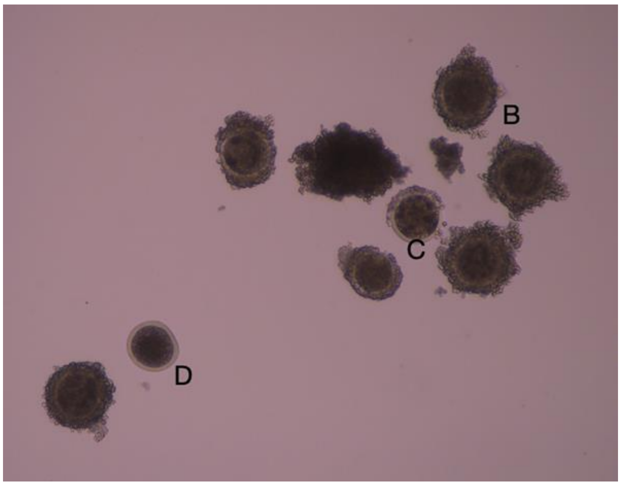

Image 2. B, C and D quality oocytes. B: B quality oocyte, C: quality oocyte, D: D quality oocyte.

C Quality: It has several cell layers (>3) and few inhomogeneous areas in the cytoplasm or no regional cumulus, and has a little homogeneous area (Image 2),

D Quality: It has completely bare, small, granular, inhomogeneous cytoplasm (Image 2) (Petyim et al., 2003).

Viability evaluation of oocytes was made according to the cell layer number and cumulus integrity in the COC, the homogeneity of their cytoplasm, and the absence of degenerated areas. Non-cumulus cells and degenerated oocytes were considered as quality D (dead). In bovine oocytes, A quality oocyte has compact cumulus layers and a translucent ooplasm. B quality oocyte has less compact cumulus layers and dark ooplasm, and C quality oocyte has expanded cumulus cells and dark ooplasm D quality oocyte hasn't cumulus layer and has dark ooplasm. SPSS-Statistics-22 package program (IBM, Richmond, USA) was used in the statistical analysis of the results. A correlation test was performed between blood AMH results and collected oocytes counts and qualities (P<0,05). The data were presented as the mean ± SD.

RESULTS

The data on the ratio of quality grades of oocytes, AMH values, and average antral follicle counts after 67 OPU applications in 10 Holstein heifers are given in Table 1 and 2. The average oocyte yield in OPU sessions per animal was range from 4–8. The mean number of follicles aspirated per OPU was 14.44, the number of oocytes collected per OPU was 6.1, and the mean recovery rate was 49.81%. The averages of oocyte quality were determined as A quality 22.37%, B quality 23.45%, C quality 28.61%, and D quality 25.68% in total OPU applications.

Table 1. Oocyte qualities, total oocytes counts, AMH concentration, and correlation

Animals	A quality (%)	B quality (%)	C quality (%)	D quality (%)	Total oocytes count	AMH value pg/ml	Average number of antral follicles (3-8 mm)
1	34.38	28.13	21.88	15.63	34.38	839.11	17.43
2	15.79	23.68	39.47	21.05	15.79	818.92	16.17
3	19.57	21.74	32.61	26.09	19.57	696.55	15.40
4	19.44	27.78	22.22	30.56	19.44	683.26	17.67
5	12.50	28.57	23.21	35.71	12.50	604.14	12.43
6	28.00	12.00	28.00	32.00	28.00	460.97	13.17
7	10.42	22.92	33.33	33.33	10.42	418.53	11.14
8	31.71	26.83	24.39	17.07	31.71	396.98	14.71
9	25.00	17.86	32.14	25.00	25.00	382.72	13.50
10	26.92	25.00	28.85	19.23	26.92	332.31	12.25
Average%	22.37	23.45	28.61	25.58	22.37		
Correlation	-0.233	0.053	-0.086	-0.092	-0.122		
MEAN	1.63	1.49	1.78	6.84	38.89	563.35	14.62
SD	0.08	0.06	0.06	0.07	9.99	180.61	2.25
SEM	0.03	0.02	0.02	0.02	3.33	60.20	0.75

Table 2. Aspirated follicle counts, collected oocyte counts, recovery rate, viable and degenerated oocyte counts.

Parameters	Results
Total 3 mm follicle	488
Total 3-8 mm follicle	928
Total ≥8 mm follicle	157
Number of aspirated follicles	803
Number of oocytes collected	400
Average recovery rate (%)	49.81
Average AMH (pg / ml)	563.35
Viable oocyte rate (%)	75
Degenerate oocyte rate (%)	25
Average number of antral follicles (3-8 mm) per OPU	14.44
Average collected oocytes per OPU	6.1
Total oocytes per OPU section	57.43

DISCUSSION

Among the existing reproductive technologies, IVEP is an important tool for reproduction of superior quality genetic material (Lohuis, 1995; Camargo et al., 2005). However, oocyte and antral follicle population competence is one of the most important limiting factors encountered during in vitro embryo production (Taneja et al., 2000; Pontes et al., 2011; Bó et al., 2012). AFC has minimal lifetime variability for an animal; similarly, AMH also varies little during the estrous cycle (Sabuncu et al., 2019). Therefore, AMH concentration and AFC are positively correlated with each other and the ovarian reserve (Burns et al., 2005; Ireland et al., 2007).

AMH controls the number of follicles and the selection of dominant follicles during the follicular wave. It prevents premature depletion of ovarian follicular reserve by regulating the selection of 4–6 small antral follicles from the pool in each follicular wave and their entry into the cycle (Umer et al., 2019). If the antral follicle pool of the donor animal is large and 50% of the oocytes in this antral follicle pool are viable, all oocytes in the selected preovulatory follicles may be viable (Pankhurst, 2017). This indicates that cows with a larger antral follicle population have much higher AMH concentrations than cows with a smaller antral follicle population. Therefore, AMH measurements are considered an independent indicator of the follicle reserve in cows (Ireland et al., 2008; Monniaux et al., 2012; Baruselli et al., 2018).

It has been reported that a positive correlation was observed between plasma AMH concentration and follicle number, total COC, viable COC number, and IVEP in the study of 59 Holstein cow performing OPU. In addition, greater number of COCs and higher viability rates of the collected COCs have been reported in cows with high AMH concentrations compared to those with low AMH levels (Guerreiro et al., 2014).

The measurement of circulating AMH concentrations helps to predict superovulation response for in vivo embryo production and IVEP in cattle (Batista et al., 2014; Gamarra et al., 2015; Vernunft et al., 2014). Heifers with low AMH concentrations were found to have lower pregnancy rates, greater probability of removing from a herd after birth of first calf, and shorter herd life than those with higher AMH concentrations (Ireland ve Mossa, 2018). In a study conducted with 1200 cows, AMH levels ranged from 10 to 3.198 pg/ml, and the mean concentration was 320.3 ± 251.1 pg/ml. When these animals were grouped by AMH level as the top 20% and the lowest 20%, the average of the intermediate group, which constitutes 60% of the animals, was reported as 263 pg/ml (141 to 450 pg/ml) (Ribeiro et al., 2014). However, studies show that normal ranges of AMH are between 0.01–400 pg/ml, and very few animals reached levels above 400 pg/ml (Rico et al., 2009; Souza et al., 2015). The average plasma AMH concentrations of the animals used in our study were found to be 563.35 pg/ml. It was considered that the absence of statistical differences between numbers and quality of oocyte obtained from animals could be due to the high concentration of AMH in all animals (Table 1). It was thought that the fact that the animals used as donors in the study were selected from a facility where genomic selection was performed may have minimized the individual differences.

The viability of collected oocytes had been reported to 77.7% and 96.7% after estrus stimulation (Machatkova et al., 2004; Beck, 2014). In the present study, it was determined that 22.25% of the oocytes obtained were quality A, 24% quality B, and 28.75% quality C. It was determined that 75% of the oocytes obtained were alive. In cattle, it has been reported that the time interval between individual OPU applications has a molecular effect on the quality of oocytes and embryos (Hanstedt et al., 2009; Wrenzycki, 2018). It is believed that an increase in blood flow to individual follicles is associated with follicular growth rates, while the decrease is associated with follicular atresia. It was observed that the timing of OPU sessions has an impact on the quality of viable oocytes obtained and 75% viability rates were at an acceptable level. Given that AMH concentration is correlated with AFC, high AMH concentration provides insight about the abundance of AFC (Acosta et al., 2003; Acosta, 2007).

Due to the fact that OPU applications were performed on random days and at 1-2 week intervals, no individual difference was detected in the number of oocytes collected in repeated OPU applications. It was thought that the lack of statistical difference between the number of oocytes collected and AMH concentrations in the present study may be related to the selection of animals with high AMH levels during donor selection or the AMH level being above the average values. It is known that AMH concentration can be used as an important biomarker in biotechnology studies. During the selection of animals to be used in biotechnology studies, it is reported that AMH concentration is a parameter that should be evaluated in addition to breed, yield, or genomic characteristics.

CONCLUSION

In conclusion, it was observed that AMH concentration did not affect the number of viable and the quality oocytes collected in weekly OPU administration in animals. It was considered that AMH, which was reported to be used to determine the duration of ovarian reserve and uses of donors, may be effective in the number of embryos per OPU. As a result of the presented study, it was thought that OPU applications performed without knowing the day of the cycle did not provide the expected correlation with AMH data.

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Ethical approval: The study was conducted with the approval of Selcuk University Veterinary Faculty Experimental Animals Production and Research Center Ethics Committee, Turkey (SÜVDAMEK) (2019/28).

Conflict of interest: The authors declare that they have no competing interests.

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