RESEARCH ARTICLE

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Relationship Between Puberty and Anti-müllerian Hormone in Simmental Heifers

Ali Tekce[1a], Omer Korkmaz[1b*]

[1] Harran University, Veterinary Faculty, Department of Obstetrics and Gynecology, Şanlıurfa, Turkey

[a]ORCID: 0000-0002-1626-6626; [b]ORCID: 0000-0001-5179-2181

*Corresponding Author
E-mail: ahuramazda@harran.edu.tr

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Abstract

The aim of the study is investigate the relationship between puberty and plasma Anti-Müllerian hormone (AMH) levels in Simmental heifers from six months to puberty. The heifers were selected through random sampling and their body weight, heart-girth circumference, hip height and body condition score were regularly recorded every month from six months of age (Group 1, n=10) until twelve months. Their blood samples were taken for AMH measurement. First data from six-month old heifers were classified in Group 1, the 7th month in Group 2, the 8th month in Group 3, the 9th month in Group 4, the 10th month in Group 5, the 11th month in Group 6 and the 12 months in Group 7. Two of the Simmental heifers in the study exhibited symptoms of estrous cycle in the 7th month, 2 in the 8th month, 1 in the 9th month, 2 in the 10th month and 3 in the 11th month. We calculated that the difference between all data except the AMH value was statistically significant in the first months before and after the onset of estrous cycle ($P<0.01$). In conclusion, we found that AMH decreases before puberty, and proceeds at a stable value afterwards in Simmental heifers. We also found that AMH levels are correlated with hip height, heart-girth circumference and body weight during puberty. The study suggests that the differences in body condition score and body weight are significant ($P<0.05$) in the month of estrous cycle onset and the following months.

Keywords: Body weight, Cattle, Heart-girth circumference, Hip height, Puberty.

INTRODUCTION

Puberty in cattle is the period in which genital system organs begin to develop and ends with the first ovulation in the fertile estrous cycle (Gupta et al., 2016). Oogenesis, which begins in the prenatal period, is concluded in this process (Day and Anderson, 1998; Dodson et al., 1989). Puberty in cattle, as standardized by Day and Anderson (1998) consists of 4 phases: infertile phase in the first 2 months, development phase up to six month, stationary phase between 6 and 10 months, and onset of puberty after 10 months. Calves initially have 75-100 thousand primary or primordial follicles in their ovaries. This number decreases in the prepubertal stage, falls to 60 thousand in adults, and is reduced to a few thousand as age increases (Noakes et al., 2009).

Anti-Mullerian hormone (AMH) is a dimeric glycoprotein secreted from the gonads and is included in the TGF-β (Transforming growth factor-beta) group. Origins of AMH synthesis are granulosa cells in healthy antral and preantral follicles of sufficient size in the ovarium (Korkmaz et al., 2016). The antral follicles count (AFC) in heifers is used to determine puberty status and estrous cycles, and as an indicator about the future fertility and fertile life of the animal. In heifers, it is also reported that ovarium diameter, peripheral progesterone amount, genital tissue development, uterus thickness and secretions increase in parallel with AFC. The usability of this method is low because transrectal ultrasonography for AFC determination requires expertise (Ireland et al., 2009). Studies report that plasma AMH levels are correlated with AFC in heifers (Pfeiffer et al., 2014; Batista et al., 2016). In light of this information, it is possible that AMH plays an important role in heifers' puberty and reproduction biology and may act as an indicator of ovarium reserves like AFC.

Studies on AMH plasma levels in heifers from birth to puberty are limited. This study aims to measure serum AMH levels in heifers up to puberty, and to demonstrate its relationship with the process and development of puberty in heifers.

MATERIALS AND METHODS

Animal Material

This study was conducted with HRÜ-HADYEK's ethics board decision dated 03.07.2019 no. 20197004 01-14. The study material consisted of 10 Simmental heifers selected from six-months old, non-problematic healthy heifers with normal development (based on clinical examination) from identical accommodation and nutrition conditions.

Study Organization and Grouping

Body weight increase, heart-girth circumference, hip height and body condition score values were recorded regularly every month as reported by Kaya (2019) for heifers selected through random sampling (n=10) between six and twelve months of age. Furthermore, blood samples were collected from vena jugularis into non-anticoagulant vials on the day of measurement for the relevant month. Blood samples were centrifuged for 15 minutes at 3000 rpm. Plasma samples were stored at -20 °C until analysis. Plasma AMH levels were determined using ELISA (DRG Instruments Elisa Mat 2000) commercial kits (Beckman Coulter, AMH Gen II., USA). The heifers were subjected to transrectal ultrasonographic ovarian activity examinations (SIUI, CTS-800, linear probe, 5 MHz, Guangdong, China) to note the onset of ovarium activity. In addition to ultrasonographic examination, criteria such as vulva swelling, increased mobility, tendency to mount on other animals, vaginal discharge were monitored and the first ovulating estrous cycle was recorded.

Each month in which measurements were taken and AMH was analyzed was grouped to compare data. Group 1 is the group recording the data from 6-months old Simmental heifers (n=10). The data for the same heifers was included in Group 2 when they reached 7 months of age, Group 3 by 8, Group 4 by 9, Group 5 by 10, Group 6 by 11 and Group 7 by 12 months of age.

Statistical Analysis

Statistical analysis for the data was carried out using Statistical Package for Social Sciences (SPSS for Windows; version 22.0) software package. Variable conformity to normal distribution was investigated using visual (histogram and probability graphs) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk tests). Descriptive analyses are given as mean ± standard error for variables with normal distribution. Since the relevant data was found to exhibit normal distribution, it was evaluated using the one-way ANOVA test. Variance homogeneity was determined with Levene's test. Cases where the P value was below 0.05 were considered statistically significant results. In cases where there was a significant difference between the groups, they were compared with the post-hoc Tukey test. Correlation coefficients and statistical significance between AMH and other parameters were calculated by Pearson test.

RESULTS

In the present study, data at 6, 7, 8, 9, 10, 11, 12 months for heifers hip height (cm), heart-girth circumference (cm), body condition score, body weight (kg) and plasma AMH(ng/ml) values is presented in detail in Table 1 as mean ± standard error for easy interpretation.

Table 1: Mean and standard error values of the Simmental heifers's Hip height (HH), Heart-girth circumference (HGC), Body Condition Score (BCS), Body weight (BW) and AMH values for each month

GROUPS	**HH (cm)**	**HGC (cm)**	**BCS**	**BW (kg)**	**AMH (ng/ml)**
6 months (GROUP I)	92.7±6.68	105.4±6.867	3.25±0.35	95.5±13.2	0.159±0.0116
7 months (GROUP II)	96.8±6.647	111±7.56	3.45±0.28	117.2±7.584	0.148±0.021
8 months (GROUP III)	99±6.9	113±7.53	3.4±0.3	124±5.9	0.137±0.015
9 months (GROUP IV)	102±7.1	117±9.11	3.5±0.4	140±9.3	0.143±0.031
10 months (GROUP V)	104±8.3	121±9.43	3.5±0.3	156±9	0.125±0.022
11 months (GROUP VI)	109±6.4	136±12.5	3.063±0.177	175±9.7	0.156±0.036
12 months (GROUP VII)	116±5	141±12.3	3±0.164	192±14	0,176± 0,036

In our study, 2 of the 10 experimental Simmental heifers exhibited symptoms of estrous cycle and ovulation started in the 7th month, 2 in the 8th month, 1 in the 9th month, 2 in the 10th month and 3 in the 11th month. We observed that hip height, heart-girth circumference, body condition score, body weight and plasma AMH (ng/ml) values were statistically significantly (P<0.01) different in the months before and after the first ovulating estrous cycle for all parameters except for the AMH value (Table 2).

Table II: Hip height (HH), Heart-girth circumference (HGC), Body Condition Score (BCS), Body weight (BW) and AMH values before the first estrus (BO), first estrus (O) and after the first estrus (AO) in Simmental heifers.

	HH (cm)	**HGC (cm)**	**BCS**	**BW (kg)**	**AMH (ng/ml)**
BO	96.35±2.52^{a}	109.82±3.08^{a}	3.34±0.08^{a}	112.96±5.62^{a}	0.25±0.10
O	104.50±3.19^{ab}	121.30±4.68^{ab}	3.30±0.11^{a}	145.10±8.94^{b}	0.13±0.01
AO	110.87±2.84^{b}	135.07±4.77^{b}	2.88±0.10^{b}	178.93±5.70^{c}	0.16±0.004
P value F value	P<0.01 F: 6.434	P<0.01 f: 8.834	P<0.01 f: 6.640	P<0.001 f: 22.62	P>0,05 f: 1.215

a, b, c: Different letters in the same column represent statistical difference. **: p<0.01.

Our study found a significant (P<0.05) negative correlation between serum AMH levels and hip height (r=0.0698), hearth-girth circumference (r=-0.727) and body weight (r=0.671) in Simmental heifers (Table 3).

Table 3: Correlation of AMH with Hip height (HH), Heart-girth circumference (HGC), Body Condition Score (BCS), and Body weight (BW) in Simmental heifers.

Pearson Correlation	AMH (ng/ml)	HH (cm)	HGC (cm)	BCS	BW (kg)
AMH (ng/ml)	1	-.698*	-.727*	-.073	-.671*
		.025	.017	.840	.034
HH	-.698*	1	.917**	.204	.872**
	.025		.000	.571	.001
HGC	-.727*	.917**	1	-.073	.973**
	.017	.000		.841	.000
BCS	-.073	.204	-.073	1	-.116
	.840	.571	.841		.750
BW	-.671*	.872**	.973**	-.116	1
	.034	.001	.000	.750	

* Correlation is significant at 0.05 level
** Correlation is significant at 0.01 level.

DISCUSSION AND CONCLUSION

This is the first study that monitored the monthly plasma AMH levels in Simmental heifers between 6 and 12 months of age, and investigated the correlation of these values with findings such as body weight, hip height, heart-girth circumference and body condition score. The study findings indicate that the difference between the heifers's body weight in the non-estrous period, first estrous cycle period and the period after the first cycle is significant. This data emphasizes once again the relationship between body weight and puberty, and suggests that the scope of these studies may be expanded.

The aim for puberty is to promote puberty in an earlier period within the reproductive limits. Regular puberty is also seen as an indicator of good fertility. If a significant correlation is found between puberty and AMH in species with economic value as modern livestock, we will gain a biomarker that will predict good fertility at an early age. In this and similar studies, the aim is to use the changes in AMH levels as a biomarker. Hannah et al., (2016) reported that the level of AMH in dairy cattle is generally higher than that of beef cattle, and dairy heifers enter puberty earlier, suggesting a relationship between AMH and puberty in heifers.

Although the number of primordial follicles decreases between the prepubertal ages of 7-9 in humans, the number of active follicles increases (Kelsey et al., 2011), it was reported that AMH production in the ovaries is high at the onset of puberty, and a decrease in plasma AMH levels is observed due to the rate and number of antral follicles transform into non-AMH producing antral follicles (Lashen et al., 2013). In this study, we observed that AMH levels in heifers decreased before puberty, similar to the findings in humans. The explanation for the lower AMH values at the onset of puberty compared to the previous month may be the transformation of small follicles in the ovarium into graff follicles, which produce less AMH, as reported by Lashen et al. (2013). Another study (Korkmaz et al., 2016) found that AMH value increases with the number of ovarian secondary follicles, while it decreases with the number of graff follicles, supporting this hypothesis.

A study monitoring AMH levels in heifers from birth to puberty (Lashen et al., 2017), reported that AMH increased in the first ten weeks and then decreased at the onset of puberty. Our data is consistent with this study, the level of AMH in heifers at the onset of puberty was lower than the period before puberty. These findings support the hypothesis that AMH level decreases, in parallel with the transformation of secondary follicles, which are the main source of AMH in puberty, into graff follicles, where AMH synthesis is lower.

A study (Sabuncu et al., 2019) reports that AMH levels vary within the same species, depending on by diverse factors such as nutrition. Furthermore, infectious diseases and endocrine disorders are also noted to affect AMH synthesis by affecting the ovarian reserve. (Mossa et al., 2015; Mossa et al., 2017). Indeed, it was reported that ovarian reserve in pregnant cattle is negatively affected by nutritional deficiency (Mossa et al., 2010; Mossa et al., 2013). In this study, we ensured that all subjects were of the same breed, at the same age and subject to the same feeding conditions to avoid these factors.

In conclusion, AMH decreases before the onset of puberty in Simmental heifers. We also found that AMH levels are correlated with hip height, herth-girth circumference and body weight during puberty. The study suggests that the differences in body condition score and body weight are significant in the month of estrous cycle onset and the following months. This study suggests that species-specific, comprehensive and expansive studies are necessary in this field.

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Conflict of Interest

The authors declare that there is no conflict of interest in the content of the article

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