ANTI-MÜLLERIAN HORMONE (AMH) AS ENDOCRINE MARKER FOR EMBRYO PRODUCTION IN SUPEROVULATED FRIESIAN COWS

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SUMMARY

The present study aimed to evaluate follicular dynamics, yield, quality and stage of embryos in superovulated Friesian cows with high and low anti-Müllerian (AMH) levels. A total of 10 Friesian cows synchronized with prostaglandin F2α (PGF2α) to bring them in heat before start superovulation protocol. On day 10 post oestrus, cows were injected with 2500 IU pregnant mare serum gonadotrophin hormone (PMSG), then after 48h cow were injected with 3 ml PGF2α and with 5 ml gonadotrophin releasing hormone (GnRH) to induce ovulation on day 14. Cows were artificially inseminated twice at 12 and 24 h after GnRH. Embryos were collected after 7 days from artificial insemination (AI). To measure AMH concentration, 1 blood sample was collected at the beginning of second follicular wave according to ultrasonography. Results showed that numbers of antral follicles and total follicles were significantly greater in high AMH level than in low AMH level donors. Differences in CL numbers of cows in high AMH level was significantly greater than in low AMH level on flushing day. AMH concentration had a significant positive correlation with antral follicles number pre-treatment, total CL number on flushing day, and ovulation rate. The average number and recovery rate of recovered embryos from donor cows with high AMH level were significantly greater than those recovered from cows in low AMH level. Parameters of yield, quality, and stage of embryos had highly significant and positive correlation with AMH level.

In conclusion, circulating AMH concentration, as endocrine marker, is highly associated individually with superovulatory response and embryo production potential in cows.

Keywords: Anti-müllerian (AMH), antral follicles, superovulation, embryo, cow

INTRODUCTION

Recent advances in bovine biotechnology, such as commercially available genomics testing, have allowed for the identification of animals with superior genetics. However, cost-efficient propagation of these superior genetics has been hampered by high variability between animals in response to embryo production techniques, such as superovulation (Bó and Mapletoft, 2014; Hasler, 2014). The ability of a female to produce an oocyte and successfully sustain an embryo is the primary factor that reduces the efficiency of cow–calf production. Ovarian dynamics of cattle are becoming increasingly relevant as indicators of fertility. The concept of the ovarian reserve has been established to both quantify and qualify the female gonad (Ireland *et al*., 2011); it is the number of morphologically healthy follicles contained in the ovary (Ireland *et al*., 2009) and is associated with fertility in cattle (Jimenez-Krassel *et al*., 2009).

With the use of serial ultrasonography, it is possible to measure the follicular population on the ovary and to determine antral follicle count (AFC), which could be used as an indicator of ovarian reserve (Ireland *et al*., 2008). The AFC is the total number of follicles ≥ 3 mm in diameter per pair of ovaries (Jimenez-Krassel *et al*., 2009) and is positively correlated to the ovarian reserve. With establishment of a correlation between AF count and pregnancy in beef heifers (Cushman *et al*., 2009) and dairy cows (Mossa *et al*., 2010).

Anti-Müllerian hormone (AMH) or Müllerian Inhibiting Substance (MIS) is a homodimeric disulfide-linked glycoprotein belonging to the transforming growth factor-β family, with a molecular weight of 140 kDa (Josso *et al*., 2001). In the female, AMH is also produced by the granulosa cells of pre-AF or early-AF on the ovary in cycling females (Monniaux *et al*., 2008; Rico *et al*., 2011). Secretion of AMH is greatest in 2 to 5 mm follicles but smaller follicles also may contribute to serum AMH concentrations (Van Rooij *et al*., 2002). Expression of AMH decreases as follicles grow and enlarge; expression is essentially lost when follicles reach 8 mm diameter or larger (Weenen *et al*., 2004). AMH has been shown to modulate early follicular growth and may thereby inhibit excessive number of follicles from entering the growing follicle pool, preventing premature depletion of the ovarian follicle reserve (Durlinger *et al*., 2002; Monniaux *et al*., 2012). Multiple studies have found that circulating blood AMH concentrations is a reliable endocrine marker of the size of the AF population, which exhibits a link to fertility. (Monniaux *et al*., 2012; Souza *et al*., 2015). A single measurement of AMH concentration in serum is a useful tool to determine the size of the ovarian reserve of follicles in cattle

(Ireland *et al*., 2008). Cattle that have low follicle numbers have diminished ovarian function, poor oocyte quality and suboptimal fertility (Ireland *et al*., 2009). Measurement of circulating AMH concentrations may be the most reliable method for predicting antral follicle numbers (Monniaux *et al*., 2012; Batista *et al*., 2014).

AMH has also been classified as a good predictive marker of the ovarian response to follicular stimulation for oocyte retrieval and in vitro embryo production (Muttukrishna *et al*., 2004; 2005), and it has also been classified as an endocrine marker that could help predict superovulatory responses administered to cows for embryo production (Rico *et al*., 2011). A combination of these variables may make it more difficult to reliably select the cows with the greatest capacity for production of embryos under field conditions. Laboratory methods that reliably predict AF numbers and response to superovulation could have substantial value for selection of cows for use in biotechnology protocols or for genomic selection of cows with greater reproductive capacity (Souza *et al*., 2015).

The present study aimed to evaluate follicular dynamics, yield, quality and stage of embryos in superovulated Friesian cows with high and low AMH levels.

MATERIALS AND METHODS

Animal care, housing and feeding:

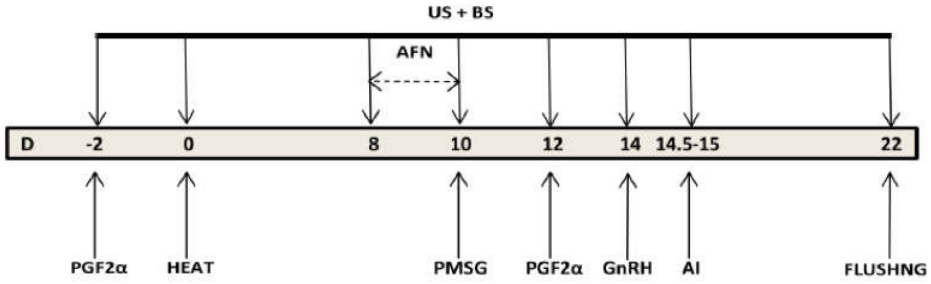
Cows were taken from Sakha Animal Production Research Station and embryo processing was carried out at Embryo Transfer lab. of International Livestock Management training Center - Sakha (ILMTC) belonging to Animal Production Research Institute (APRI), Agricultural Research Center, Ministry of Agriculture, Egypt, during the period from October 2018 to April 2019.

A total of 10 Friesian cows having live body weight (LBW) of 450-550 kg within the 2nd parity were used as embryo donors. Donor cows were subjected to clinical examination of the ovaries and reproductive tract to exclude any abnormalities of the reproductive organs to make sure that all donor cows were cyclic, fit and free of diseases before superovulation protocol.

Experimental cows were fed and cared under the same conditions applied in Sakha Animal Production Research Institute Station. Animals were housed in a semi-open shaded yard and fed based on the recommendation of the Ministry of Agriculture. During winter and spring months, animals were fed on berseem (*Trifolium alexandrinum*), concentrate feed mixture (CFM) and rice straw (RS), while during summer and autumn months, animals were fed the same CFM, berseem hay (BH), corn silage and RS to cover their nutritional requirements according to their LBW and milk production.

Superovulation protocol:

All cows were synchronized with prostaglandin F2α (Estrumate, containing 263 µg Cloprostenol Sodium BP (Vet) equivalent to 250 µg Cloprostenol; Friesoythe,Germany) at a level of 3 ml/cow to bring them in heat before start superovulation protocol (Day 0). On day 10 post oestrus, each cow was i.m. injected with a dose of 2500 IU Pregnant Mare Serum Gonadotrophin (PMSG, Folligon, Intervet International B.V., Boxmeer, Netherlands). Each cow was i.m injected with 3 ml PGF2α on day 12 post oestrus, and with 5 ml GnRH (1 ml contains 0.0042 mg buserelin acetate equivalent to 0.004 mg buserelin; Receptal® VET.; MSD Animal Health) after 2 days (Day 14) to induce ovulation. Cows were artificially inseminated twice at 12 and 24 h after GnRH with frozen semen (15 × 106 sperm/0.25 ml straw) by the same bull's semen and inseminator., as shown in the diagram.



US: Ultrasonography, BS: Blood Samples, AFN: Antral Follicles Number
Diagram showing superovulatory protocol used in the present study.

Ultrasonography examination:

Cows in all protocols were subjected to ultrasonography device (ESAOTE Pie Medical Aquila Pro Vet + Probe 8.0 Mhz LA Rectal Veterinary Transducer) during protocol days for counting the number of total follicles and corpora lutea, and estimating the diameter of follicles on the ovarian surface on day 0, PMSG, PGF2α, AI and flushing. However, count of antral follicles (2-5 mm in diameter) was recorded on day 8-10 of oestrus, as shown in the diagram. Ovulation rate (OR%) was calculated according to the following equation:

OR(%)= (Number of CLs on day of flushing/number of follicles on day of AI) x 100.

Embryo collection and evaluation:

All cows had embryo recovery performed by one specialist. Seven days after AI, CL structures from both ovaries were counted by ultrasound to estimate superovulation response to the protocol as shown in the diagram. Shortly after the ovarian ultrasound evaluation, technique of non-surgical flushing was followed using the closed system. It was done according to the method described for cattle by (Newcomb et al., 1978). Lidocaine spinal anesthesia was used (lidocaine hydrochloride injectable 2%, 5 ml/dose; Phoenix Pharmaceutical Inc., St. Joseph, MO). y-tubing (Y-Junction Tubing), catheters (Rubber ET catheter, 2-way Foley, 20-mL balloon), medium for embryo recovery (Phosphate Buffer Saline (Dulbecco A) 10 tablets/1L, OXOID). The pH value of the medium was adjusted to be in the range between 7.25-7.50, while osmolarity level ranged between 260-310 mOsm/kg. One percent of fetal or estrus cow serum was added to Modified Dulbecco's PBS for flushing.

Collected embryos were searched under a stereo microscope (x 20-80), and classified for quality as 1 (excellent), 2 (good), 3 (fair), 4 (poor), or 5 (dead or degenerating) and for stages as 1 (morula), 2 (compact morula), 3 (early blastocyst or blastocyst) using International Embryo Transfer Society criteria (IETS, 2010). When embryo was identified, it was picked up by a 0.5 ml glass pack syringe connected to a capillary plastic hose and then transferred into a small dish (holding capacity of 35 x 10 mm) containing filtered culture media. Then embryo recovery rate (ERR) was calculated according to the following equation:

ERR(%)= (Total number of recovered embryos/number of CLs on day of flushing) x 100 by a single specialist.

All recovered embryos were measured including thickness (µm) of zona pellucid, diameter (µm) of intrazonal and diameter of the whole embryo (total diameter) with its coverings with microscope micrometer lens.

AMH and P4 blood analysis:

For measurements of AMH concentrations, one blood sample was collected at the beginning of second follicular wave (when antral follicle (2 to 5 mm) and follicular pool persist on the ovary) of a synchronized estrous cycle for each cow according to ultrasonography. For progesterone (P4) concentration measurements, blood samples were collected at days of superovulation treatment (days of oestrus, PMSG injection, PGF2α injection, AI and embryo collection). Blood samples were collected into evacuated collection tubes without EDTA by puncture of the coccygeal vein or artery. Blood samples were immediately centrifuged after collection at 3,000 × *g* for 20 min. Serum samples were then stored at −20°C until assayed for AMH and P4. AMH was analyzed by (MofA Global) using a bovine AMH ELISA. The AMH assay had an analytical sensitivity of 0.011ng/ml. Analysis of P4 was done by (RIA PROGESTERONE, IM1188, BECKMAN COULTER).

Statistical analysis:

Data were statistically analyzed by one way ANOVA using computer programme of SAS (2002) to test the difference between high and low AMH level. Pearson's correlation coefficient was used to determine the relationships between AMH level and different parameters studied.

RESULTS

AMH circulation levels:

In serum samples of 10 donor cows, Anti-Müllerian Hormone (AMH) ranged from 0.05 to 0.35 ng/ml, and averaged 0.174 ng/ml as general scale, when antral follicle and follicular pool appeared on the ovary at the beginning of the second follicular wave of the oestrus cycle. The distribution of AMH concentrations was divided into two levels, low AMH level (0.05-0.12 ng/ml, with average of 0.07±0.01 ng/ml, and high AMH level (0.20-0.35 ng/ml, with average of 0.27±0.02 ng/ml (Table 1).

NOTICE to the author: if blood sample are collected with anticoagulant in the collection tube, then you're working with PLASMA, NOT Serum

Table 1. Average and range of AMH levels in superovulated cows with high and low AMH category

Item	AMH (ng/ml)			
	High AMH Category (n=5)	Low AMH category (n=5)	P-value	Significant
		AMH profile (ng/ml):		
Average	0.27±0.02	0.07±0.01	0.0004	***
Range	0.20- 0.35	0.05 - 0.12	-	-

*** Significant differences at P<0.001.

AMH and superovulatory response:

Numbers of antral follicles (2- 5 mm in diameter) at the start of superovulation were greater (P<0.001) in high AMH level than low AMH level. Numbers of total follicles were insignificantly higher in high than in low AMH level during treatment days (oestrus, PMSG, PGF2α, and AI). Number of total follicles on flushing day showed an opposite trend but the differences were not significant. Mean numbers of total follicles on treatment days were significantly (P<0.001) higher in high than in low AMH level (Table 2).

The differences in average diameter of total follicles on day of oestrus, PGF2α, AI and flushing

were not significant. Only on day of PMSG injection, average diameter of total follicles was significantly (P<0.05) higher in low than in high AMH level. Large follicles diameter didn't differ significantly between the two levels during superovulation treatment (Table 2).

At embryo collection time, the total CLs number of cows in high AMH level were greater (P<0.05) than in low AMH level. Ovulation rate in the two AMH levels of donor cows didn't differ significantly. (Table 2)

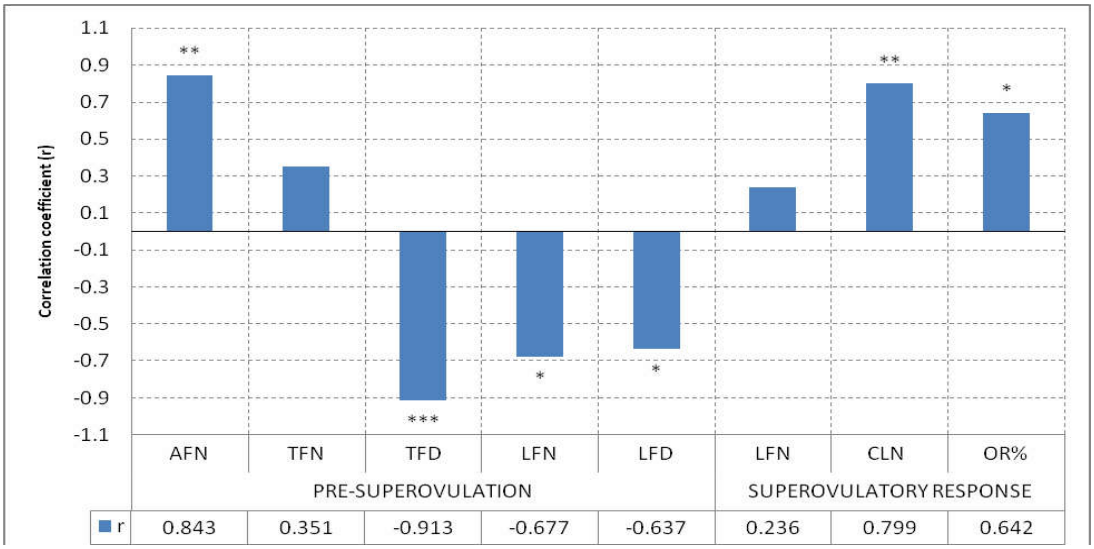
Table 2. Follicular and corpora lutea characteristics in superovulated cows with high and low AMH category during superovulation treatment days

Item	High AMH category	Low AMH category	P-value	Significant
Antral Follicles Number (AFN; n/cow; 8-10 d)	7.8±0.66	3.6±0.60	0.0015	**
Total follicular number/animal:				
Oestrus	3.4±0.92	2.6±0.24	0.4284	NS
PMSG	11.6±1.28	8.6±1.28	0.1383	NS
PGF2α	10.4±1.36	7.6±1.28	0.1817	NS
AI	9.6±1.40	7.2±1.49	0.2753	NS
Flushing	3.8±0.96	5.0±1.76	0.5670	NS
Mean	7.8±0.66	3.6±0.60	0.0015	***
Average diameter of total follicles (cm):				
Oestrus	0.92±0.08	0.79±0.07	0.2662	NS
PMSG	0.46±0.03	0.58±0.01	0.0238	*
PGF2α	0.80±0.06	0.77±0.04	0.6716	NS
AI	1.12±0.05	1.09±0.08	0.8023	NS
Flushing	1.49±0.04	1.58±0.13	0.5115	NS
Number of large follicles/animal:				
Oestrus	1.2±0.20	1.0±0.00	0.3466	NS
PMSG	0.4±0.24	0.8±0.20	0.2415	NS
PGF2α	2.0±0.89	0.8±0.37	0.2509	NS
AI	5.8±0.73	5.2±0.96	0.6351	NS
Flushing	3.4±0.81	3.8±1.62	0.8312	NS
Average diameter of large follicles (cm):				
Oestrus	1.4±0.13	1.2±0.04	0.3119	NS
PMSG	0.55±0.34	0.97±0.25	0.3557	NS
PGF2α	1.2±0.38	0.69±0.28	0.2857	NS
AI	1.38±0.11	1.19±0.04	0.1561	NS
Flushing	1.5±0.05	1.6±0.12	0.4732	NS
Total number of CL/animal:				
Oestrus	0.00±0.00	0.00±0.00	.	NS
PMSG	1.0±0.00	1.0±0.00	.	NS
PGF2α	1.0±0.00	1.0±0.00	.	NS
AI	0.00±0.00	0.00±0.00	.	NS
Flushing	5.0±0.89	2.2±0.58	0.0305	*
Ovulation rate	83.82±7.46	51.22±16.62	0.1115	NS

NS: Not significant differences. * Significant differences at P<0.05. ** Significant differences at P<0.01.

Correlation between AMH and super-ovulatory response parameters:

Results of correlation coefficients between serum AMH circulation levels and different follicular and CL characteristics are illustrated in Fig. 1. These results revealed that AMH concentration had a significant positive correlation with antral follicles number (r=0.842, P<0.01), total CL number (r=0.799, P<0.01), and ovulation rate (r=0.641, P<0.05). However, AMH level had significant high negative-correlation with total follicle diameter, number and diameter of large follicles during pre-superovulation period. On the other hand, AMH had insignificant and weak correlation with each of total follicles number during pre-superovulation period and large follicle number on AI day.



AFN: Antral Follicle Number at start of superovulation, TFN: Total Follicles Number, TFD: Average Diameter of Total Follicles, LFN: Large Follicles Number, LFD: Large Follicles Diameter, CLN: Corpora Lutea Number, OR: Ovulation Rate.

Fig. 1: Correlation between AMH level, follicular and corpus luteum characteristics in superovulated cows.

Progesterone profile on superovulation days:

The differences in serum P4 concentrations between high and low level AMH were not significant on different days of superovulation treatment. Although P4 level on flushing day was higher in high than low AMH, the differences were not significant and indicating wide variation in P4 concentrations within high AMH level category (Table 3).

Table 3. Progesterone profile (ng/ml) in blood serum of superovulated cows with high and low AMH category on different superovulation day

Time	High AMH category	Low AMH category	P-value	Significant
Oestrus	0.43±0.07	1.02±0.38	0.1768	NS
PMSG	3.1±0.76	2.7±0.60	0.6925	NS
PGF2α	3.38±1.57	4.36±0.50	0.5711	NS
AI	1.02±0.47	0.41±0.10	0.2490	NS
Flushing	16.38±6.34	4.28±1.24	0.0983	NS

NS: Not significant differences.

Correlation between AMH and P4 concentrations

Results of correlation coefficients between AMH circulation levels and concentration of serum P4 on different superovulation days are illustrated in Fig. 2. These results revealed that AMH level highly correlated with P4 level only on the day of embryo collection (r=0.625, P<0.05). However, AMH level had weak correlation with P4 level, being positive on days of PMSG and AI, while was negatively on day of oestrus and PGF2α (P≥0.05).

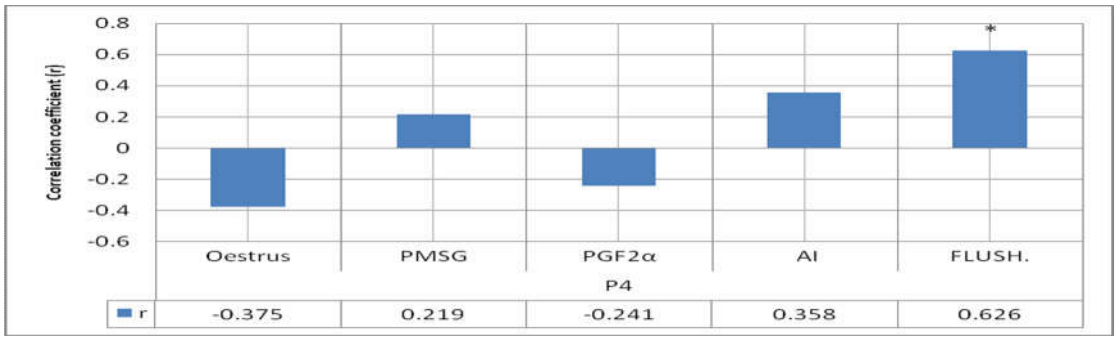


Fig. 2: Correlation coefficient (r) between AMH level and progesterone level in blood serum of cows on different superovulation days.

Embryo production:

Results in Table 4 showed that average number and recovery rate of recovered embryos from donor cows with high AMH level was greater (P<0.01; P<0.001) than those recovered from cows in low AMH level. The yield and percentage of good and acceptable (excellent and good) embryos were significantly higher in high than in low AMH. However, embryos in excellent, fair and poor grades were not affected by both AMH levels. The yield and percentage of embryos at blastocyst stage were found in high AMH level, but non embryos at blatocyst and morula stages were recovered from cow with low AMH level. Meanwhile, embryos at compact morula and 4-cell stages were not affected in cows with both AMH levels (Table 4).

It is of interest to note that four cows (produced >1 embryo/cow) responded to superovulation treatment (80%) versus one non-responded cow (20%, produced one embryo) were recorded out of five cows with high AMH level. The yield and percentage of yield, quality and stage of embryos in responded and non-responded cows within high AMH level are presented in Table 5. Results revealed greater yield, better quality and higher developmental competence of embryos recovered from responded than no-responded cows. However, there were no responded cows (cow which produce >1 embryos) in low AMH level of donor cows.

Correlation between AMH and embryonic characteristics:

Results of correlation coefficients between serum AMH circulation levels and embryo characteristics (yield, grade and stage) are presented in Fig. 3. The results indicated highly, significant and positive correlation of AMH level with total number, recovery rate, excellent, good grade of embryos, blastocysts, compact morulae and morulae. In negative pattern, AMH level showed significant-negative correlation with each of poor (P<0.01) and 4-cell (P<0.001) embryos.

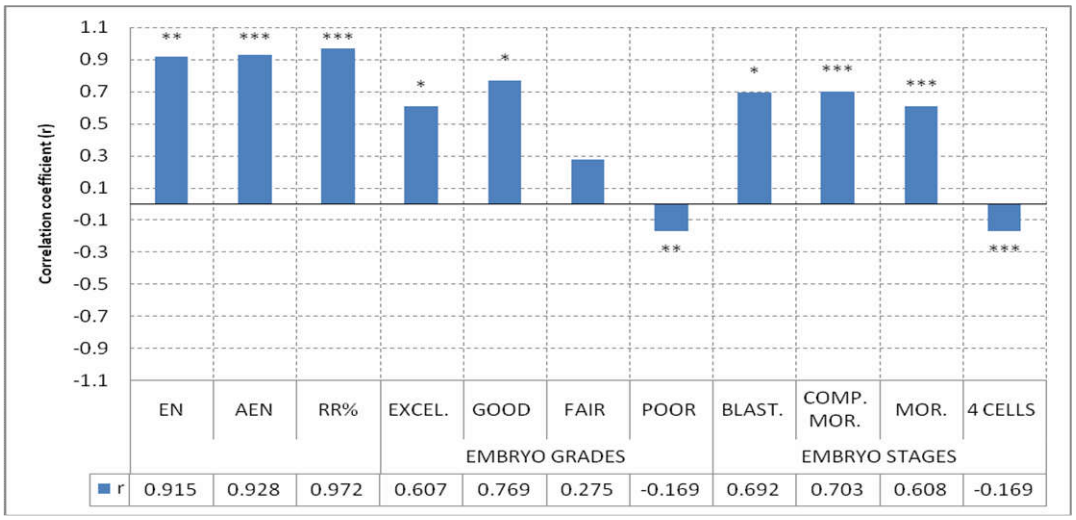
Table 4. Yield, quality and stage of embryos recovered from superovulated cows with high and low AMH category

Item	High AMH Category (n=5)	Low AMH category (n=5)	P-value	Significant
Total embryo number/cow	3.8±0.86	0.4±0.24	0.0052	**
Embryo recovery rate	72.62±8.07	11.66±7.25	0.0005	***
Embryo quality:				
Excellent (n/cow)	1.2±0.96	0.2±0.20	0.3420	NS
Excellent (%)	20.66±16.13	20.0±20.00	0.9801	NS
Good (n/cow)	2.0±0.44	0.00±0.00	0.0021	**
Good (%)	56.34±15.86	0.00±0.00	0.0033	**
Acceptable (n/cow)	3.2±0.86	0.2±0.20	0.0094	**
Acceptable (%)	86±9.79	20±20.00	0.0180	*
Fair (n/ow)	0.6±0.40	0.00±0.00	0.1720	NS
Fair (%)	14.0±9.79	0.00±0.00	0.1909	NS
Poor (n/cow)	0.00±0.00	0.2±0.20	0.3466	NS
Poor (%)	0.00±0.00	20.0±20.00	0.3466	NS
Embryonic stage:				
Blastocyst (n/cow)	0.6±0.24	0.00±0.00	0.0400	*
Blastocyst (%)	12.34±5.20	0.00±0.00	0.0453	*
Compact morula (n/cow)	1.6±0.87	0.2±0.20	0.1562	NS
Compact morula (%)	32.32±13.87	20.00±20.00	0.6264	NS
Morula (n/cow)	1.6±0.50	0.00±0.00	0.0138	**
Morula	55.34±16.18	0.00±0.00	0.0091	**
4-cell (n/cow)	0.00±0.00	0.2±0.20	0.3466	NS
4-cell (%)	0.00±0.00	20.00±20.00	0.3466	NS

NS: Not significant differences. * Significant differences at P<0.05. ** Significant differences at P<0.01.
*** Significant differences at P<0.001.

Table 5. Yield, quality and stage of embryos recovered from responded and non-responded cows with high AMH category

Item	High AMH category	
	Responded	**Non-responded**
Cow (%)	4/5 (80)	1/5 (20)
Total follicles number /cow:	12.00±1.58	10.0±0.00
Antral follicles number/cow	8.25±0.63	6.0±0.00
P4 at PGF2α (ng/ml)	4.02±1.86	0.80±0.00
Total embryo number/cow	4.5±0.65	1.0±0.00
Embryo recovery rate	78.27±7.44	50.0±0.00
Concentration of AMH (ng/ml)		
Average	0.28±0.03	0.22±0.00
Range	0.2-0.35	-
Embryo quality:		
Excellent (%)	25.82±19.73	0.00±0.00
Good (%)	56.67±17.15	100.0±0.00
Acceptable embryos (%)	82.49±11.82	100.0±0.00
Fair (%)	17.51±11.81	0.00±0.00
Poor (%)	0.00	0.00
Embryonic stage:		
Blastocyst (%)	15.6±5.42	0.00±0.00
Compact morula (%)	40.4±14.56	0.00±0.00
Morula (%)	44.0±15.12	100.0±0.00



EN: Embryos Number, AEN: Acceptable Embryo Number, RR: Recovery Rate

Fig. 3: Correlation between AMH level and embryo characteristics in superovulated cows.

DISCUSSION

Results from the limited number of previous studies investigating the relationship between circulating AMH and in vivo embryo production following superovulation of dairy cattle have been encouraging. These studies are consistent in showing that the circulating AMH is a good predictor of superstimulation, superovulation, and embryo production. In addition, the largest studies were done in nonlactating (Rico *et al*., 2012), crossbred (Monniaux *et al*., 2010), or beef (Hirayama *et al*., 2012) cattle. Given the potential importance of AMH, as a predictor of reproductive capacity. The present study aimed to evaluate the relationship between the AMH profile and the follicular dynamics, yield, quality and stage of embryos in superovulated Friesian cows.

In the present study, AMH in serum of 10 superovulated Friesian cows ranged from 0.05 to 0.35 ng/ml, and averaged 0.174 ng/ml as general scale, when antral follicle and follicular pool persisted on the ovary at the beginning of the second follicular wave (8-10 day) of the oestrus cycle. These

results indicated a wide variation of AMH in blood serum of Friesian cows. According to the limited number of cows used in this study, superovulated animals were divided into two AMH categories with low (0.05-0.12 ng/ml) and high (0.2-0.35 ng/ml)). By the same pattern, AMH level was classified into high (0.4±0.02 ng/ml) and low (0.2±0.01 ng/ml) categories (Guerreiro *et al*., 2014), or into low ranged (0.01 to 0.14 ng/ml, intermediate (0.141 to 0.45 ng/ml), and high (0.451 to 3.19800 ng/ml) (Ribeiro *et al*., 2014). In addition, Hirayama *et al*. (2017) used the 25th (0.118 ng/ml) and 75th (0.488 ng/ml) percentile values of plasma AMH concentration for classification of cows with low and high superovulatory responses. Ghanem *et al*. (2016) sorted the cows into 3 groups as low (<0.1 ng/ml), medium (0.1≥ to <0.25 ng/ml) and high (≥0.25 ng/ml) in terms of their AMH levels. In other ways, Souza *et al*. (2015) reported an AMH range from 0.00001 to 0.37430 ng/ml, while Monniaux *et al*. (2010) reported AMH concentrations to range from 0.0011 to 0.5312 ng/ml. In addition, Rico *et al*. (2009) reported AMH concentrations ranges of 0.025-0.228, 0.049-0.359and 0.026-0.212 ng/ml. In another study, Rico *et al*. (2012) reported AMH concentrations ranging from 0.005 to 0.244 ng/ml where cattle below 0.087 ng/ml identified as poor responders to superovulation and having less than 15 large follicles near the time of estrus. Differences in cut-off points between studies and the present levels of AMH may be related to several factors, such as differences in measure of superovulatory response parameters (Rico *et al*., 2012) versus CL counts on the day of embryo collection; (Souza *et al*., 2015), or/and different AMH assays, and different methods used to collect plasma (Rico *et al*., 2012).

A large variability of AFN is reported among different cows, however antral follicle population (AFP) is highly repeatable within animal (Burns *et al*., 2005; Ireland *et al*., 2007), and AMH can be considered a reliable endocrine marker of ovarian reserve (Ireland *et al*., 2007, 2008; Monniaux *et al*., 2012). As applied in our study, Ireland *et al*. (2008) used a single measurement of AMH concentration in serum to determine the count of the ovarian reserve of follicles. The obtained results show that among the follicular characteristics, number of antral follicles at the start of superovulation, total number of follicles on different superovulation days (P<0.001), and total follicle diameter pre-superovulation significantly increased in animals with high than low AMH level. In accordance with this trend, some authors found that cows with high serum AMH level had higher follicular counts and ovulatory responses to superovulatory treatment than those with low AMH level (Ireland *et al*., 2008, 2010; Rico *et al*., 2009; Center Keith, 2015).

A greater number follicle (3-7 mm) before the superovulatory treatment was associated with higher AMH concentrations and a higher ovarian response (Rico *et al*., 2009). Furthermore, Ireland *et al*. (2010) found that AMH concentrations were found to be 2 and 6 folds higher for cattle with the intermediate or great number than that with low number of follicles. In another study, Center Keith (2015) reported that donor cows within high AMH category had the best superovulatory response. In addition, Ireland *et al*. (2008) found a link between follicle number and fertility after measure of AMH from day 6 through day 2 of the subsequent estrous cycle. Generally, there are many studies stating the existence of a positive correlation between AMH level and follicle number, oocyte count and superovulation response (Fanchin *et al*., 2003; Guerreiro *et al*., 2014; Baldrighi *et al*., 2014). The AMH was correlated with AFP in cattle of different genetic groups, Bos indicus, Bos taurus and Bubalus bubalis (Baldrighi *et al*., 2014; Guerreiro *et al*., 2014; Batista *et al*., 2016).

There is a practical advantage in utilizing AMH to predict AFP instead of direct counting AFP with ultrasound equipment (Baruselli *et al*., 2016). The present results were indicated by the recorded higher positive correlation between AMH and each of number of antral follicles pre-superovulation (r=0.842, P<0.01) and CL number (r=0.799, P<0.01). Similarly, Rico *et al*. (2009) reported a strong and significant correlation between plasma AMH concentrations and numbers of large follicles after superstimulation (r = 0.83) and number of CL after superovulation (r = 0.64). Recent results of Rico *et al*. (2012) indicated a high correlation of circulating AMH concentrations to number of large follicles (r = 0.56)or CLs (r = 0.43) after superovulation in nonlactating Holstein cows. Also, Center Keith (2015) reported that donor cows within high AMH category had more CLs than donor cows in the lowest AMH level. The elevation in AMH level associated with increasing follicular count may be due to superovulatory treatment inducing the follicular growth of small-sized follicles (not detected by ultrasonography). In the present, antral follicular count was measured at the start of the 2nd follicular wave when the follicular pool and antral follicles (2-5 mm in diameter) were appeared on the ovarian surface. Also, superovulatory treatment with gonadotropin may have increased AMH secretion via granulosa cells (Rico *et al*., 2009, 2012).

The present study investigated the differences in embryonic yield, quality and stage. The obtained results indicated significantly higher production of embryos with high quality (good) and acceptable embryos at blastocyst and morula stages, in high than in low AMH level. In this line, there are a strong correlation of AMH level with total (r=0.914, P<0.01), acceptable (r=0.928, P<0.01), excellent (r=0.607, P<0.05), good (r=0.769, P<0.05), blasocyst (r=0.691, P<0.05), compact morula (r=0.703, P<0.001), and morula (r=0.607, P<0.001) embryos. Souza *et al*. (2015) reported that total numbers of recovered embryos were greater for donors in the highest AMH quartile versus the lowest, but AMH quartile had no effect on the percentage of transferrable or fertilized embryos. In comparable

with the present correlations, Monniaux *et al*. (2010) reported that plasma AMH concentrations were correlated with average numbers of embryos (r = 0.49) and transferable (r = 0.320) embryos per donor crossbred Holstein × Normande dairy cows. However, Hirayama *et al*. (2012) reported a correlation between AMH concentrations and numbers of transferable embryos (r = 0.390).

The correlation observed between AMH and P4 level (r=0.625, P<0.05) may reflect the tendency of higher P4 concentration on day of flushing in high vs. low AMH levels, but the difference was not significant. This may be due to an indicator of CLs number on day of flushing.Low concentrations of progesterone in circulation are associated with higher embryonic mortality in cattle (Mann and Lamming, 1999). Both beef and dairy cows characterized as having low antral follicle counts also have progesterone concentrations that are 40 to 50% lower during the luteal phase of the estrous cycle than similar cows having high antral follicular counts (Jimenez-Krassel *et al*., 2009). Similar correlation (r = 0.79; P < 0.001) between AMH and number of antral follicles detected via ultrasonography before the start of superovulatory treatment (Rico *et al*., 2009).

CONCLUSION

Circulating AMH concentration, as endocrine marker, is highly associated individually with superovulatory response and embryo production potential in cows. The repeatability and utility of the AMH assay could make it a valuable practical method for improving the efficiency of multiple-ovulation embryo transfer programs in dairy herds.

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الأنتيموليريان كدلالة هرمونية لانتاج الأجنة من أبقار الفريزيان فائقة التبويض

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هدفت الدراسة الحالية الى تقييم ديناميكية التطور الحويصلى وانتاج وجودة ومراحل تطور الأجنة فى الأبقار الفريزيان الفائقة التبويض ذات مستوى AMHالمرتفع والمنخفض. أجريت التجربة على 10بقرات فريزيان نظم شياعهم بواسطة حقن ٣ مل من هرمون البروستاجلاندين لادخالهم فى شياع قبل بدء معاملة التبويض الفائق. حقنت الأبقار بـ ٢٥٠٠ وحدة دولية من هرمون PMSGفى اليوم العاشر من الشياع ، ثم حقنت الأبقار بعد ٤٨ ساعة بـ ٣ مل من هرمون البروستاجلاندين ، وفى اليوم ١٤ من الشياع حقنت الأبقار بـ ٥ مل من هرمون GnRHثم بعد ذلك تم تلقيح الأبقار اصطناعيا مرتين بعد 12و 24ساعة من حقن الـ GnRH. تم جمع الأجنة بعد ٧ ايام من التلقيح. أخذت عينة دم واحدة عند بداية الموجة الحويصلية الثانية والتى حددت وفقا لجهاز التشخيص بالموجات فوق الصوتية لتقدير هرمون AMHفى سيرم الدم. أظهرت النتائج أن عدد الحويصلات الأولية والكلية كان أعلى معنويا فى الأبقار ذات مستوى AMHالمرتفع عنها فى منخفضة مستوى AMH.عدد الأجسام الصفراء كان أعلى معنويا فى الأبقار ذات مستوى AMHالمرتفع عنها فى منخفضة مستوى AMH.وجد أن هناك ارتباط معنوى موجب بين تركيز هرمون الـ AMHو عدد الحويصلات الأولية قبل المعاملة ، عدد الأجسام الصفراء فى يوم جمع الاجنة ومعدل التبويض. الإختلافات فى عدد الأجنة المتحصل عليها و معدل استرداد الأجنة من الأبقار ذات مستوى AMHالمرتفع كانت أعلى معنويا عنه فى ذات المستوى المنخفض. وجد ارتباط موجب عالى المعنوية بين مستوى الـ AMHو عدد و جودة و مراحل الأجنة.

استنتج من هذه الدراسة أن تركيز هرمون AMH كدلالة هرمونية له ارتباط وثيق باستجابة الأبقار لمعاملة التبويض الفائق وانتاج الأجنة.