**The Relationship between the Concentration of Anti-Mullerian Hormone (AMH) and Fertility in Replacement Females**

A.K. Redheada, C.D. Paula, A.K. Adebiyia, S.N. Carra, A.N. Nabersa, M. Knightsa\*

aDivision of Animal and Nutritional Sciences, West Virginia University, Morgantown, WV, 26506

\*Corresponding author: marlon.knights@mail.wvu.edu; 304-293-1946; Marlon Knights, West Virginia University, Division of Animal and Nutritional Sciences, P.O. Box 6108, Morgantown, WV 26506

**Abstract**

The effect of breed and age on the concentration of anti-mullerian hormone (AMH) and the relationship between AMH and fertility in replacement ewes were evaluated. In Experiment 1, a single blood sample was used to compare concentration of AMH in Dorset/Texel (DT; n= 238; age 8.7 ± 0.1 months), Suffolk (n= 44) and Katahdin (n= 77; age 6.9 ± 0.04 months) replacement females and to determine changes in systemic AMH with age in DT and Katahdin females. In Experiment 2, Katahdin and DT females were placed into LOW, MEDIUM and HIGH groups based on their systemic AMH determined from a blood sample collected 2 months prior to breeding. Females were treated with CIDR inserts (0.3g progesterone) for 5 days and were exposed to rams at insert removal for 30–35 days. Ewes were observed for estrus after 4 days of ram exposure, and pregnancy diagnosis was conducted via transrectal ultrasonography at the time of ram removal and again 20–25 days. In Experiment 1, Katahdin females had a higher AMH than DT and Suffolk females (P < 0.001: 566 ± 37 vs. 337 ± 14 vs. 237 ± 22 pg/ml, respectively). AMH decreased linearly with age in DT females (P = 0.03). In Experiment 2, Females with high AMH conceived and lambed to the first service in Katahdin but not the DT breed (Breed x AMH; P < 0. 05). Replacement females that conceived (Breed X Conception; P < 0.001) and lambed to the first service (Breed X lambing to 1st service; P < 0.001) had a higher AMH in Katahdin but not DT breed. In conclusion, AMH varies among different breeds of sheep and a single measure may be useful to select females with high reproductive performance in some breeds.

Key words: Anti-Mullerian Hormone, Reproduction, Ewe lambs, Age, Ovarian Reserve

1. **Introduction**

The profitability of sheep operations is highly correlated with reproductive performance of the flock. Replacement females can make up 25% of the breeding flock and their reproductive performance is generally lower than that of their flock mates (Quirke et al., 1977; Edwards et al., 2016). Additionally, replacement ewes that are successfully bred within their first year of age are more profitable and show greater lifetime reproductive performance than females bred to lamb at two years for the first time (Young et al., 2011; Kenyon et al., 2011; Kenyon et al., 2014).

Approaches to increase fertility in replacement females have been the subject of significant research efforts over the last five decades (Nieto et al., 2013; Kenyon et al., 2014; Knights et al., 2015). Recently, it was reported that the number of growing antral follicles in young adult cattle may be related to their fertility (Ireland et al., 2008; 2011) and low numbers of antral growing follicles have been related to suboptimal fertility in beef cattle (Cushman et al., 2009; 2010). Anti-Müllerian hormone (AMH) is a member of the transforming growth factor-β (TGF-β) family (Cate et al., 1986; Knight et al., 2006) and can be used as a marker of the ovarian follicular reserve in humans (Visser et al., 2005), mice (Kevenaar et al., 2006), cattle (Ireland et al., 2008; Monniaux et al., 2010; Rico et al., 2009; Batista et al., 2014) and bitches (Hollinshead et al., 2016).  In cattle, the concentration of AMH is positively and highly correlated with AFC (Ireland et al., 2008; 2011).

In dairy cattle, concentration of AMH showed a quadratic relationship with lactation number and females with low concentration of AMH had a lower pregnancy rate following first service, and a greater incidence of pregnancy loss between day 30 and 65 of gestation (Ribeiro et al., 2014). In addition, it was suggested that the concentration of AMH may be used as a diagnostic tool in young heifers to predict herd longevity (Jimenez-Krassel et al., 2015). Lahoz et al. (2012) reported that concentration of AMH determined at an early age in Rasa Aragonesa sheep, can be used to reliably predict fertility at first mating and suggested the cut off value of 97 pg/ml to distinguish between females with low and high fertility.

Concentrations of AMH vary among breeds of cattle (Baldrighi et al., 2014; Batista et al., 2014; Guerreiro et al., 2014; Stojsin – Carter et al., 2016) and with age (Cushman et al., 2010; Lahoz et al., 2014). The concentration of AMH and the relationship between concentration of AMH and fertility in replacement females might not be consistent across breeds.

Therefore, the objectives of this study were to evaluate the concentration of AMH in replacement females of different breeds and ages and to determine if the relationship between the concentration of AMH and fertility in replacement females varies with breed.

1. **Materials and Methods**
	1. *Farm and Animals*

This study was conducted during the fall of 2013, 2014 and 2015 on three farms located in West Virginia and southwestern Pennsylvania. The animals used in this study consisted of Dorset x Texel (8.7 ± 0.1 months [range 4 – 10.4 months]; 38.9 ± 0.58 kg [range 23.1- 70.3 kg]), Katahdin (6.9 ± 0.04 months [range 3.6 – 5.6 months]; 23.6 ± 0.57 kg [range 13.1 – 35.8 kg]), and Suffolk (40.5 ± 0.75 kg [range 28.2 – 53.1 kg]) replacement females. Two months prior to beginning of the breeding season, replacement females were provided with a grain supplement (15% crude protein, 65% total digestible nutrients concentrate) ranging in amounts of 0.23 to 0.68 kg per head per day. All animals were managed on mixed grass legume pastures and were allowed ad libitum access to water and shade.

*2.2 Treatments*

The procedures used in these studies were approved by the West Virginia University Animal Care and Use Committee (IACUC # 13-1201).

*2.3 Experiment 1*

To determine the effect of breed of sheep on the concentration of AMH, a single blood sample was collected from Dorset/Texel (n = 238), Suffolk (n = 44) and Katahdin (n = 77) replacement females (Table 1) and assayed for AMH. To determine the relationship between age and concentration of AMH, blood samples were collected from Dorset/Texel and Katahdin replacement females ranging in age from 6 to 12 and 6 to 8 months, respectively.

*2.4 Experiment 2*

To determine the relationship between the concentration of AMH and fertility, the concentration of AMH was determined from a single blood sample collected 2 months prior to breeding. Females within the Katahdin breed and Dorset/Texel crosses, were placed into LOW, MEDIUM and HIGH groups respectively, equivalent to < mean - ½ standard deviation, ≥ mean - ½ standard deviation < mean + ½ standard deviation and ≥ mean + ½ standard deviation, respectively. Females within the Suffolk breed was not used as the producer experienced significant loss of animals due to sickness. All females were separated from rams prior to the beginning of the experiment and received progesterone via a CIDR device (containing 0.3g of progesterone; InterAg; Hamilton, New Zealand) for 5 days prior to ram introduction. At CIDR removal, replacement females were exposed to a group of sexually mature rams at a ratio not less than one ram per 15 replacement females.

.

*2.5 Estrous detection*

Rams fitted with marking harnesses were exposed to replacement females and managed as a single breeding group for approximately 60 days beginning at CIDR removal. To detect estrus at first service period, ewes were observed for the presence of raddle marks between 24 and 96 hours after ram introduction.

*2.6 Pregnancy diagnosis and lambing data*

Pregnancy diagnosis was conducted using transrectal ultrasonography (Aloka 500 Corometrics Medical Systems Wallingford, CT, USA) with a 7.5-mHZ linear trans-rectal probe between 30-35 and 50-55 days after ram introduction to detect pregnancy conceived at first and second service periods, respectively. Lambing records were collected and analyzed.

*2.7 Blood Collection and AMH assay*

Blood samples were collected into 10 ml tubes containing EDTA by jugular venipuncture. The samples were immediately placed on ice and later centrifuged at 3000 x g for 15 minutes for separation of plasma. The plasma samples were frozen at – 20 oC for later analysis. Plasma AMH was determined using an enzyme-linked immunosorbent assay ELISA kit (ANSH labs, Webster, Texas, USA). The sensitivity of the AMH assay was 0.009ng/ml and intra-assay CV was < 5 %.

*2.8 Statistical analysis*

To assess the effect of breed on the concentration of AMH, a one way analysis of variance (ANOVA) was conducted using the PROC MIX procedure of SAS (Statistical Analysis System version 9.4 for Windows; SAS Institute, Cary, NC, USA) and means were separated using Tukey’s HSD.

A polynomial regression was used to assess the relationship between concentration of AMH and age in replacement females, the regression between the concentration of AMH and age was determined using the PROC REG procedures of SAS.

A two-way Analysis of Covariance (ANCOVA) was conducted using the PROC MIX procedure of SAS to determine the effect of concentration of AMH (LOW, MEDIUM and HIGH groups), breed and breed x AMH interaction on reproductive responses controlling for age. Means were separated using Tukey’s HSD. Response variables included estrous response, conception rate, pregnancy to first and second services (ewe that lambed that were pregnant to first and second services, respectively), prolificacy (number of lambs born per ewe lambing), prolificacy to first service (number of lambs born per ewe lambing to first service only), proportion of ewes (ewes lambing by day 13 of the lambing period) lambing to first service, percentage lambed, lambing rate (lambs born per ewe exposed), ram introduction to lambing, lambing day (day that ewe lambed during the lambing period) and age to first lambing and the effect of conception, pregnancy to first service, percentage lambed and lambing to first service.

ANCOVA was used to investigate whether the concentration of AMH differed from replacement females experiencing a binary reproductive response or not controlling for age. These variables were used in a multivariable model that included the binary reproductive response as an independent variable to explain the variability of concentration of AMH (Ribiero et al., 2014). Reproductive responses investigated were estrous response, conception, pregnancy to first service, lambed and lambing to first service. Results were considered significant at a confidence interval of P ≤ 0.05 and a tendency when 0.05 < P ≤ 0.1.

1. **Results**

*3.1 Relationship between concentration of AMH, breed and age*

The mean breeding age of the replacement females were 8.3 ± 0.01 months. The mean systemic concentration of AMH for Dorset, Katahdin and Suffolk replacement females were 337 ± 14, 566 ± 37 and 237 ± 22 pg/ml, respectively. AMH was higher in Katahdin replacement females than Dorset and Suffolk replacement females (P < 0.001). AMH decreased linearly with age (Figure 1a; P = 0.03) in Dorset/Texel replacements with age but there was no relationship between age and AMH in Katahdin replacement females (Figure 1b).

*3.2 Relationship between concentration of AMH and reproductive outcomes in Dorset and Katahdin replacement females*

There was a significant interaction of breed x AMH on conception rate (Table 2: P = 0.02) and lambing to the first service (Table 2; P = 0.01). More Katahdin replacement females with HIGH AMH conceived (P = 0.005; 90.0 ± 13.0 vs. 41.6 ± 11.9 %) and lambed to the first service period (P = 0.004; 90.6 ± 15.4 vs. 31.5 ± 13.6 %) compared to Katahdin females with LOW AMH. However, conception rate and proportion of females lambing to the first service did not differ among Dorset replacement females with LOW, MEDIUM and HIGH AMH.

Dorset replacement females (Table 3) had a higher estrous response (P = 0.01), pregnancy rate to first service (P = 0.002), pregnancy rate to second service (P = 0.002), prolificacy to first service (P = 0.03), percentage lambed (P < 0.001) and lambing rate (P < 0.001) than Katahdin replacement females. In addition, Dorset females tended to have a higher conception rate (P = 0.08) and overall prolificacy (P = 0.09) than Katahdin females.

More replacement females with HIGH AMH lambed to the first service period compared to females with LOW and MEDIUM AMH (Table 3; P = 0.04). Replacement females with HIGH AMH tended to have a higher conception rate (P = 0.07) lower number of days from ram introduction to lambing (P = 0.07), lambed earlier within the lambing period (P = 0.08) and lower age to first lambing (P = 0.08).

*3.3 AMH Concentrations in plasma according to binary reproductive responses*

AMH was higher in replacement females that conceived (P = 0.01; 471 ± 25 vs. 360 ± 37 pg/ml) compared to females that did not conceive. There was a significant interaction (Table 4; P = 0.0007) of breed x conception. Katahdin replacement females that conceived had a higher (P = 0.0008) concentration of AMH compared to Katahdin females that did not conceive. However, AMH did not differ between Dorset replacement females that conceived and Dorset females that did not conceive.

There was a significant interaction (Table 4; P = 0.009) of breed x pregnancy to the first service on AMH. Dorset replacement females that were pregnant to the first service had a lower (P = 0.03) AMH compared to Dorset females with HIGH that did not become pregnant to the first service.

AMH was higher in replacement females that lambed to the first service (P = 0.005; 507 ± 29 vs. 382 ± 34 pg/ml) compared to replacement females that did not lamb to the first service. There was a significant interaction (Table 4; P = 0.0009) of breed x lambing to first service. Katahdin replacement females that lambed to the first service had a higher (P = 0.0007) concentration of AMH compared to Katahdin females that did not lamb to the first service. However, AMH did not differ between Dorset replacement females that lambed to the first service and Dorset females that did not lamb to the first service.

1. **Discussion**

There are limited reports on the relationship between circulating concentration of AMH and reproductive variables in replacement females. The present study demonstrated that in replacement females (1) the concentration of AMH differs among breeds of sheep (2) a linear relationship exist between the concentration of AMH and age in Dorset replacement females (3) in some breeds the concentration of AMH may be a predictor of some reproductive performance variables.

In the present study, concentration of AMH varied among Suffolk, Dorset and Katahdin breeds of sheep. AMH also has been shown to vary with breeds of cattle (Baldrighi et al., 2014; Stojsin-Carter et al., 2016). AMH is produced by pre-antral and early antral follicles and there is a strong correlation between AFP and AMH (Batista el al., 2014; Ribeiro et al., 2014; Stojsin-Carter et al., 2016). AFP also varies across different breeds in cattle (Gimenes et al., 2009; Sartori et al., 2010) and sheep (Draincourt et al., 1986; Avdi et al., 1997; Webb et al. 1989). Therefore, intra and inter breed variations in concentration of AMH observed in sheep in this study, might be reflective of variations in the AFP. Different threshold values are used for different breeds of cattle to classify animals with low and high AFP populations (Batista et al., 2014; Guerreiro et al., 2014). Therefore it is suggested that because of the wide inter-breed variation observed in this study, a single value to predict reproductive outcome across breeds would not be sufficient.

The concentration of AMH decreased linearly with increasing age in Dorset/Texel replacement females. However the R2 value was 0.0178. A decline in AMH has been reported in the peripubertal period in other species. Monnaiux et al. (2013) reported that the concentration of AMH decreased between 6 months and 12 months of age in beef heifers. Lahoz et al. (2014) reported that the concentration of AMH declined between 6 and 19 months of age in Ras Aragonesa ewes, and Fanchin et al. (2003) reported a negative linear relationship between the concentration of AMH and age. In contrast, Hudson et al. (1990) reported that in humans, AMH changed quadratically with age from barely detectable at birth, increases at 11 to 19 years and decreased after 20 years of age. Visser et al. (2013) reported that AMH increased during the postnatal period up to 12 years and decreased at an older age. AMH is correlated with AFC (Ireland et al., 2009; Rico et al., 2009), the decrease in AMH observed in Dorset/Texel females may be due to the decline in AFC with increasing age. However, no relationship between age and AMH was observed in Katahdin females. Age range of the Katahdin females was small which might have precluded the determination of any relationship between age and concentration of AMH in this study.

More females with a high concentration of AMH tended to conceive and lamb to the first service. Previous reports observed a positive association between concentration of AMH and fertility. In sheep, Lahoz et al. (2012) reported that ewes with higher prepubertal concentrations of AMH showed a higher probability of becoming pregnant at first mating than those with a lower concentration of AMH. In addition, ewes which failed to conceive had a lower concentration of AMH (Lahoz et al., 2012). Dairy cows with low AMH concentrations had lower pregnancy following first service and greater incidence of pregnancy loss between day 30 and 65 of gestation (Ribeiro et al., 2014) and a reduced survival rate after birth of first calf (Jimenez-Krassel et al., 2015).

AMH is positively correlated with AFP (Ireland et al., 2009; Rico et al., 2009; Batista el al., 2014; Ribeiro et al., 2014) and oocyte yield (Majumder et al., 2010). Wiweko et al. (2016) reported that serum and follicular fluid AMH concentrations were also positively correlated with the total number of oocytes and number of mature oocytes. Takahashi et al. (2008) reported the concentration of AMH was higher in oocytes that were successfully fertilized compared to those that were not successfully fertilized. Therefore, AMH could be a reliable endocrine marker of both oocyte yield and quality (Takahashi et al. 2008; Lehman et al., 2014; Zhu et al., 2016). Further, it is tempting to suggest that replacement ewe lambs with a high concentration of AMH and a higher number of follicles (Ireland et al. 2009; Rico et al. 2009) will ovulate oocytes of better quality resulting in improved reproductive outcome.

In contrast, low concentration of AMH was not correlated with negative reproductive outcomes in humans aged 23 to 41 years (Streuli et al., 2014; Fraisse et al., 2008) and hamsters aged 9 months (Roosa et al., 2016). Smeenk et al. (2007) reported that basal AMH is not related to embryo quality or to the probability of achieving pregnancy in humans. This indicates that a single factor is not sufficient to predict pregnancy outcome across all species and physiological states.

**5.0 Conclusion**

In conclusion, a single measure of concentration of AMH of replacement females may be a useful tool to select replacement females with a high reproductive performance. However, it is important to consider breed when developing threshold values to delineate the potential for high and low reproductive outcome as the concentration of AMH varies among breeds of sheep,

Table 1. Concentration of AMH used to classify animals as low, medium and high and total number of animals for experiment 1 and experiment 2.

|  |  |  |
| --- | --- | --- |
| Breed | Concentration of AMH (pg/ml) | N |
| **Low** | **Medium** | **High** |  |
| Dorset | < 227 (n = 89) | 227 – 447 (n = 89) | > 447 (n = 60) | 238 |
| Katahdin | < 403 (n = 24) | 403 – 728 (n = 33) | > 728 (n = 20) | 77 |
| Suffolk | < 164 (n = 17) | 164- 311 (n=18) | > 311 (n= 9) | 44 |

Table 2. Effect of breed (Dorset/Texel Crosses, n = 238 and Katahdin, n = 77) and concentration of AMH (Low, Medium and High) on reproductive responses of replacement females. Values are least square means ± SEM. Significant interaction P < 0.05.

|  |  |  |
| --- | --- | --- |
|  Reproductive Variable | Breed of Replacement female (B) | P-value |
| **Dorset/Texel Cross** | **Katahdin** |
| **Concentration of AMH (pg/ml)** |
| **Low** | **Medium** | **High** | **Low** | **Medium** | **High** | **Interaction****(B x AMH)** |
| Conception rate (%)a | 81.2 ± 5.0 | 84.1 ± 4.9 | 75.7 ± 6.7 | 41.6 ± 11.9 | 66.6 ± 10.7 | 90.0 ± 13.0 | **0.02** |
| Pregnancy rate (%) 1st serviceb | 61.8 ± 5.3 | 64.9 ± 5.1 | 46.4 ± 6.3 | 25.7 ± 10.8 | 34.2 ± 9.0 | 46.8 ± 11.0 | **0.07** |
| Prolificacy 1st servicec | 1.34 ± 0.08 | 1.23 ± 0.07 | 1.32 ± 0.1 | 1.03 ± 0.23 | 1.04 ± 0.17 | 1.03 ± 0.17 | **0.09** |
| Lambing to 1st service (%)d | 59.4 ± 6.7 | 68.1 ± 6.1 | 53.6 ± 7.8 | 31.5 ± 13.6 | 62.4 ± 13.7 | 90.6 ± 15.4 | **0.01** |

aNumber of replacement females diagnosed pregnant as a percentage of ewe lambs marked by rams

bNumber of replacement females diagnosed on day 30 to 35 as a percentage of all ewe lambs exposed to rams

cLambs born per replacement female lambing to the first service period (first 14 days of lambing season)

dProportion of replacement females lambing by day 14 of the lambing period

|  |  |  |  |
| --- | --- | --- | --- |
| Reproductive Response | Breed of Replacement Female | Concentration of AMH (pg/ml) | P-Value |
| **Dorset/Texel Cross** | **Katahdin** | **Low****(L)** | **Medium (M)** | **High****(H)** | **Breed** | **AMH****H vs. L** |
| Estrous Response (%)a | 71.5 ± 3.0 | 54.4 ± 5.9 | 67.8 ± 5.5 | 64.1 ± 4.8 | 56.9 ± 5.9 | 0.01 | NS |
| Conception rate (%)b | 80.3 ± 3.2 | 66.1 ± 7.2 | 61.4 ± 4.4 | 75.3 ± 5.9 | 82.8 ± 7.3 | 0.08 | 0.07 |
| Pregnancy rate (%) 1st servicec | 57.7 ± 3.3 | 35.6 ± 6.2 | 43.8 ± 5.9 | 49.6 ± 6.3 | 46.6 ± 6.3 | 0.002 | NS |
| Pregnancy rate (%) 2nd serviced | 82.3 ± 2.7 | 63.0 ± 5.3 | 74.2 ± 5.0 | 72.2 ± 4.5 | 71.5 ± 5.3 | 0.002 | NS |
| Prolificacye | 1.18 ± 0.04 | 1.03 ± 0.08 | 1.11 ± 0.07 | 1.10 ± 0.07 | 1.09 ± 0.07 | 0.09 | NS |
| Prolificacy 1st servicef | 1.30 ± 0.05 | 1.03 ± 0.11 | 1.19 ± 0.12 | 1.13 ± 0.09 | 1.17 ± 0.09 | 0.03 | NS |
| Lambing to 1st service (%)g | 60.4 ± 4.0 | 61.5 ± 8.4 | 45.5 ± 7.5 | 65.2 ± 7.4 | 72.1 ± 8.6 | NS | 0.04 |
| Lambed (%) | 68.7 ± 3.1 | 41.0 ± 5.9 | 60.5 ± 5.5 | 51.9 ± 5.0 | 52.1 ± 6.0 | < 0.001 | NS |
| Lambing rate (%)h | 83.9 ± 4.2 | 39.9 ± 8.1 | 68.2 ± 7.6 | 58.7 ± 6.8 | 58.7 ± 8.1 | < 0.001 | NS |
| Ram introduction to lambing (d)i | 155.9 ± 0.9 | 159.04 ± 2.0 | 160.7 ± 1.7 | 155.4 ± 1.7 | 156.3 ± 2.0 | NS | 0.08 |
| Lambing day (d)j | 14.6 ± 0.9 | 13.6 ± 2.0 | 17.4 ± 1.8 | 12.1 ± 1.8 | 12.8 ± 2.0 | NS | 0.08 |
| Age to first lambing (d) | 398.61 ± 1.71 | 401.60 ± 1.9 | 403.3 ± 1.7 | 398.2 ± 1.7 | 398.8 ± 2.0 | NS | 0.08 |

Table 3. Main effects of breed (Dorset/Texel crosses, n = 238 and Katahdin, n = 77) and concentration of AMH (Low, Medium and High) on reproductive responses of replacement females. Significant difference P < 0.05.

fLambs born per replacement female lambing to the first service period (first 14 days of lambing season)

gProportion of replacement females lambing by day 14 of the lambing period

hLambs born per replacement female exposed

IMean number of days from ram introduction to lambing

JMean day replacement female gave birth within the lambing period (day 1 = day the first ewe lambed).

aNumber of replacement females marked by rams of all ewe lambs exposed

bNumber of replacement females diagnosed pregnant as a percentage of ewe lambs marked by rams

cNumber of replacement females diagnosed on day 30 to 35 as a percentage of all ewe lambs exposed to rams

dNumber of replacement females diagnosed on day 50 to 55 as a percentage of ewe lambs not pregnant on day 30 -35

eLambs born per replacement female lambing

Table 4. The relationship between concentration of AMH and reproductive responses in Dorset/Texel crosses (n = 238) and Katahdin breed (n = 77) ewe lambs. Values are least square means ± SEM. Significant difference and interaction P < 0.05.

|  |  |  |
| --- | --- | --- |
| Reproductive Response(RR) | Breed of Replacement Female | P – Value |
| **Dorset/Texel Cross** | **Katahdin** |
| **Incidence of Event** **AMH Concentration (pg/ml)** | **Reproductive Response****(RR)** | **Breed****(B)** | **Interaction****(B X RR)** |
|  | **Yes** | **No** | **Yes** | **No** |  |  |  |
| Estrous Response | 316.30 ± 18.7 | 395.8 ± 31.3 | 559.2 ± 40.8 | 571.70 ± 39.2 | NS | < 0.001 | NS |
| Conceptiona | 313.0 ± 19.0 | 355.5 ± 39 | 629.8 ± 47 | 365.5 ± 64 | 0.01 | 0.0006 | 0.0007 |
| Pregnancy to 1st serviceb | 315.9 ± 21.1 | 384.7 ± 25.3 | 632.8 ± 51 | 519.0 ± 37 | NS | < 0.001 | 0.009 |
| Lambing to 1st servicec | 330.4 ± 24.3 | 354.7 ± 30.7 | 683.3 ± 52.7 | 408.7 ± 62.0 | 0.005 | < 0.001 | 0.0009 |
| Lambed | 335.3 ± 20.1 | 362.5 ± 30.4 | 534.8 ± 42.8 | 573.2 ± 45.5 | NS | < 0.001 | NS |

aNumber of replacement females diagnosed pregnant and marked by rams

bNumber of replacement females diagnosed pregnant on day 30 to 35 of all ewes exposed to rams

cNumber of replacement females lambing by day 14 of the lambing period

1. Dorset Replacement Females
2. Katahdin Replacement Females

Figure 1. Effect of age on the concentration of AMH in (a) Dorset (n = 238) and (b) Katahdin (n = 77) replacement females. Each circle represents data from one ewe lamb.

**Acknowledgements**

The authors would also like to thank the staff from Reymann Memorial Farms; George and Lisa Wherry, and James Dean for the use of their flocks and for their assistance in data collection.

**Conflict of Interest**

This work was supported by Hatch 476 (NE–1027) of the West Virginia Agricultural and Forestry Experiment Station, and the USDA-Northeast Sustainable Agriculture Research and Education program (LNE14-333-29001). The authors of this study do not hold equity, consult with or advise this funding agency.

**References**

Avdi,M. Chemineau, P. Draincourt, M.A. (1997). Alterations in follicular maturation associated with within-breed variation in ovulation rate in Chios sheep. *Animal Reproduction Science*, 46, 223-235.

Baldrighi, J.M., Sá Filho, M.F., Batista, E.O.S., Lopes, R.N.V.R., Visintin, J.A., Baruselli, P.S., Assumpção, M.E.O.A. (2014). Anti-Mullerian hormone concentration and antral ovarian follicle population in Murrah heifers Compared to Holstein and Gyr kept under the same management. *Reproduction in Domestic Animals*, 49, 1015-1020.

Batista, E. O S, Macedo, G. G., Sala, R. V., Ortolan M.V., Sá Filho, M. F., Del Valle, T. A., Jesus, E. F., Lopes, R. N. V. R., Rennó, F. P., Baruselli, P.S.(2014). Plasma Anti-Mullerian hormone as a predictor of ovarian antral follicular population in Bos Indicus (Nelore) and Bos Taurus (Holstein) Heifers. *Reproduction in Domestic Animals*, 49, 448–52.

Carter, A.S., Mahboubi, K., Costa N.N., Gillis D.J, Carter T.F., Neal M.S., Miranda M.S, Ohashi O.M., Favetta L.A., King W.A. (2016). Systemic and local anti-Mullerian hormone reflects differences in the reproduction potential of Zebu and European type cattle. *Animal Reproduction Science*, 167, 51–58.

Cate R.L., Mattaliano R.I, Hession C., Tizard R., Farber N.M., Cheung A., Ninfa E.G, Frey, A.Z, Gash, D.I., Chow, E.P, Fisher, R.A., Bertonis, J.M., Torres, G., Wallner, B.P., Ramachandran, K.L., Ragin, R.C., Manganaro, T.F., Maclaughlin, D.T., Donahoe P.K. (1986). Isolation of the bovine and human genes for Mullierian-inhibiting substance and expression of the human gene in animal cells*. Cell* 45, 685-698.

Cushman, R. A., Allan, M. F., Kuehn, L. A., Snelling, W. M., Cupp, A. S., Freetly, H. C. (2009). Evaluation of antral follicle count and ovarian morphology in crossbred beef cows: investigation of influence of stage of the estrous cycle, age, and birth weight. *Journal of Animal Science*, 87, 1971–80.

Cushman, R.A., Wood J.R., Slattery R.G., Clopton D.T. (2010). Reproductive aging influences ovarian function in beef cows. *Nebraska Beef Cattle Reports*, Paper 558.

Draincourt, M.A., Gauld, I.K., Terqui, M., Webb, R. (1986). Variations in patterns of follicle development in prolific breeds of sheep. *Journal of Reproduction and Fertility*, 78, 565-575.

Edwards, S.J., B. Smaill, A.R. O’Connell, P.D. Johnstone, D.R. Stevens, L.D. Quirke, P.A. Farquhar, J.L. Juengel. (2016). Reduced ovulation rate, failure to be mated and fertilization failure/embryo loss are the underlying causes of poor reproductive performance in juvenile ewes. *Animal Reproduction Science*, 167, 125-132.

Fanchin, R., L.M. Schonäuer, C. Righini, J. Guibourdenche, R. Frydman, J. Taieb. (2003). Serum Anti-Müllerian hormone is more strongly related to ovarian follicular status than serum inhibin b, estradiol, FSH and LH on day 3. *Human Reproduction*, 18, 323–27.

Fraisse, T., Ibecheole,V., Streuli, I. bischof, P. de Ziedler,D. (2008). Undetectable serum anti-Mullerian hormone levels and occurrence of ongoing pregnancy. *Fertility Sterility*, 89, 723.e9–723.e11.

Gimenes, L.U., Sa Filho, M.F., Carvalho, N.A.T., Torres Junior, J.R.S., Souza, A.H., Madureira, E.H., Trinca, L.A., Sartorelli, E.S., Barros, C.M., Carvalho, J.B.P. Mapletoft, R.J., Baruselli, P.S. (2009). Follicular dynamics of Bos indicus, Bos Taurus and Bubalus bubalis heifers treated with norgestomet ear implant associated or not to injectable progesterone. *Animal Reproduction*, 6, 256.

Guerreiro, B.M., E.O.S. Batista, L.M. Vieira, M.F. Sá Filho, C.A. Rodrigues, A. Castro Netto, C.R.A. Silveira, B.M. Bayeux, E.A. Dias, F.M. Monteiro, M. Accorsi, R.N. Lopes, P.S. Baruselli. (2014). Plasma Anti-Mullerian Hormone: An endocrine marker for in vitro embryo production from Bos taurus and Bos indicus donors. *Domestic Animal Endocrinology*, 49, 96–104.

Hollinshead, F. K., C. Walker, D. W. Hanlon. (2016). Determination of the normal reference interval for anti-Mullerian hormone (AMH) in bitches and use of AMH as a potential predictor of litter size. *Reproduction in Domestic Animals*, 5, 1–6.

Hudson,P.L., Douglas, I., Donahoe, P.K., Cate, R.L., Epstein, J., Pepinsky, R.B., MacLaughlin, D.T. (1990). An immunoassay to detect human Mullerian inhibiting substance in males and females during normal development. *Journal of Clinical Endocrinology and Metabolism*, 7091, 16-22.

Ireland, J. J. A., G. W. B. Smith, D. A. Scheetz, J. K. A. Folger. (2011). Does Size Matter in Females ? An Overview of the Impact of the High Variation in the Ovarian Reserve on Ovarian Function and Fertility, Utility of Anti-Mullerian Hormone as a Diagnostic Marker for Fertility and Causes of Variation in the Ovarian Reserve in Cattle. *Reproduction Fertility and Development*, 23, 1–14.

Ireland, J. L. H., Scheetz, D., Jimenez-Krassel, F., Themmen, A.P.N, Ward, F., Lonergan, P., Smith, G.W., Perez, G.I., Evans, A.C.O, Ireland, J.J. (2008). Antral Follicle Count Reliably Predicts Number of Morphologically Healthy Oocytes and Follicles in Ovaries of Young Adult Cattle. *Biology of Reproduction*, 79, 1219–25

Ireland, J.J., Zielak-Steciwko, A.E., Jimenez-Krassel, F., Folger, J., Bettegowda, A., Scheetz, D., Walsh, S., Mossa, F., Knight, P.G., Smith, G.W., Lonergan, P., Evans, A.C. (2009). Variation in the ovarian reserve is linked to alterations in intrafollicular estradiol production and ovarian bio- markers of follicular differentiation and oocyte quality in cattle. *Biology of Reproduction*, 80, 954-64.

Jimenez-Krassel, F., Scheetz, D.M., Neuder, L.M., Ireland, J.L.H., Pursley, J.R., Smith, G.W., Tempelman, R.J., Ferris, T., Roudebush, W.E., Mossa, F., Lonergan, P., Evans, A.C., Ireland, J.J. (2015). Concentration of anti-Müllerian hormone in dairy heifers is positively associated with productive herd life. *Journal of Dairy Science,* 98, 1–10.

Kenyon, P. R., A.N. Thompson, S.T. Morris. (2014). Breeding ewe lambs successfully to improve lifetime performance. *Small Ruminant Research*, 118, 2–15.

Kenyon, P., van der Linden, D., West, D., Morris, S. (2011). The effect of breeding hoggets on lifetime performance. *New Zealand Journal of Agricultural Research*, 54, 321–330.

Kevenaar, M.E, Mohamed, F., Meerasahib, P.K., van de Lang-Born, B.M.N., de Jong, F.H., Groome, N.P., Themmen, A.P.N., Visser, J.A. (2006). Serum anti-Mullerian hormone levels reflect the size of the primordial follicle pool in mice. *Endocrinology*, 147, 3228–34.

Knight, P.G., Glister, C. (2006). TGF-β Superfamily Members and Ovarian Follicle Development. *Reproduction*, 132, 191–206.

Knights, M., Redhead, A., D’Souza, K., Baptiste, Q. (2015). Effect of stimulation with a gonadotropin mixture on reproductive outcome in nulliparous ewes bred during seasonal anestrus and early breeding season. *Animal Reproduction Science*, 159, 198-204.

Lahoz, B., Alabart, J. L., Monniaux, D., Mermillod, P., Folch, J. (2012). Anti-Müllerian hormone plasma concentration in prepubertal ewe lambs as a predictor of their fertility at a young age. *BMC Veterinary Research*, 8, 118.

Lahoz, B., Alabart, J. L., Cocero, M. J., Monniaux, D., Echegoyen, E., Sánchez, P., Folch, J. (2014). Anti-Müllerian hormone concentration in sheep and its dependence of age and independence of bmp15 genotype: an endocrine predictor to select the best donors for embryo biotechnologies. *Theriogenology*, 81, 347–57.

Lehmann, P., Velez, M.P., Saumet, J., Lapensee, L., Jamal, W., Bissonnette, F., Phillips, S., Kadoch, I.J. (2014). Anti-mullerian hormone (AMH): a reliable biomarker of oocyte quality in IVF. *Journal of Assisted Reproduction Genetics*, 31, 493-498.

Majumder, K., Gelbaya, T.A., Laing, I., Nardo, L.G. (2010). The use of anti-mullerian hormone and antral follicle count to predict the potential of oocytes and embryos. *European Journal of Obstetrics, Gynecology and Reproductive Biology*, 150, 166-170.

Monniaux, D, Drouilhet, L, Rico, C, Estienne, A., Jarrier, P., Touze, J.L., Sapa, J., Phocas, F., Dupont, J., Dalbies-Tran, R. (2013). Regulation of anti- Mullerian hormone production in domestic animals. *Reproduction, Fertility and Development*, 25, 1–16.

Monniaux, D., Barbey, S., Rico, C., Fabre, S., Gallard, Y., Larroque, H. (2010). Anti-Mullerian hormone: a predictive marker of embryo production in cattle? *Reproduction, Fertility and Development*, 22, 1083-1091.

Quirke, J.F, Hanrahan, J.P. (1977). Comparison of the survival in the uteri of adult ewes of cleaved ova from adult ewes and ewe lambs. *Journal of Reproduction and Fertility*, 51, 487–489.

Ribeiro, E.S., Bisinotto, R.S., Lima, F.S., Greco, L.F., Morrison, A., Kumar, A., Thatcher, W.W, Santos, J.E.P. (2014). Plasma Anti-Müllerian Hormone in Adult Dairy Cows and Associations with Fertility. *Journal of Dairy Science*, 97, 6888–6900.

Rico, C., Fabre, S., Médigue, C., di Clemente, N., Clément, F., Bontoux, M., Touzé, J. (2009). Anti-Mullerian hormone is an endocrine marker of ovarian gonadotropin-responsive follicles and can help to predict superovulatory responses in the cow. *Biology of Reproduction*, 80, 50–59.

Rosales Nieto, C.A., Ferguson, M.B., Macleay, C.A., Briegel, J.R., Martin, G.B., Thompson, A.N. (2013). Selection for superior growth advances the onset of puberty and increases reproductive performance in ewe lambs. *Animal*, 7, 990-997.

Roosa, K.A., Zysling, D.A., Place, N.J. (2016). An assessment of anti-Mullerian hormone in predicting mating outcomes in female hamsters that have undergone natural and chemically-accelerated reproductive aging. *General and Comparative Endocrinology*, 214, 56-61.

Sartori,R., Bastos, M.R., Baruselli, P.S., Gimenes, L.U., Ereno, R.L., Barros, C.M. (2009). Physiological differences and implications to reproductive management of Bos Taurus and Bos indicus cattle in a tropical environment. *Society of Reproduction and Fertility Supplement*, 67, 357-375.

Smeenk, J.M., Sweep, F.C., Zielhuis, G.A., Kremer, J.A., Thomas, C.M., Braat, D.D. 2007. Anti mullerian hormone predicts ovarian responsiveness, but not embryo quality or pregnancy, after in vitro fertilization or intra-cytoplasmic sperm injection. Fertility Sterility 87:223-226.

Streuli, I., de Mouzon, J., Paccolat, C., Chapron, C., Petignat, P., Irion, O.P., de Ziegler, D. (2014). AMH concentration is not related to effective time to pregnancy in women who conceive naturally. *Reprodution Biomedical*, 28, 216–224.

Takahasi, C., Fujito, A., Kazuka, M., Sugiyama, R., Ito, H., Isaka, K. (2008). Anti-Mullerian hormone substance from follicular fluid is positively associated with success in oocyte fertilization during in vitro fertilization. *Fertlity and Sterility*, 89, 586-591.

Visser, J.A, Themmen, A.P.N. (2005). Anti-Müllerian Hormone and folliculogenesis. Molecular and Cellular Endocrinology, 234, 81–86.

Visser, J.A., Hokken-Koelega, A.C., Zandwijken, G.R., Limacher, A., Ranke, M.B., Fluck, C.E. (2013). Anti-Mullerian Hormone levels in girls and adolescents with Turner syndrome are related to karyotype, pubertal development and growth hormone treatment. *Human Reproduction*, 28, 1899-1907.

Webb,R. Gauld, I.K., Draincourt, M.A. (1989). Morphological and functional characterization of large antral follicles in three breeds of sheep with different ovulation rates. Journal of *Reproduction and Fertility*, 87, 243-255.

Wiweko, U. Anggraheni, E. Mansyur, T. Yuningsih, A.K. Harzief, G. Pratama, K. Sumapraja, M. Natadisastra, A. Hestiantoro. (2016). Serum AMH level predicts oocytes quality better than follicular fluid AMH. *Asian Pacific Journal of Reproduction*, 5, 361-364.

Xu,J., C.V. Bishop, M.S. Lawson, B.S. Park, F. Xu. (2016). Anti-Mullerian hormone promotes pre-antral follicle growth, but inhibits antral follicle maturation and dominant follicle selection in primates. *Human Reproduction*, 31, 1522-1530.

Young, J. M., A.N. Thompson, M. Curnow, C.M. Oldham. (2011). Whole-farm pro fi t and the optimum maternal live weight pro file of Merino ewe flocks lambing in winter and spring are influenced by the effects of ewe nutrition on the progeny’ s survival and lifetime wool production. *Animal Production Science*, 51, 821–833.

Zhu, J. J. OU, W. Xing, W. Li, W. ZHU. 2016. Anti-Mullerian hormone, antral follicle count and follicle stimulating hormone for predicting the number of oocytes retrieved in IVF/ICSI cycles. *Journal of Reproduction and Contraception*, 27, 89-93.