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Anti-Müllerian Hormone Related to Reproductive and Productive Longevity in Egyptian Buffaloes

Ghada H. Abdel- Rahman Hassan[1] and Jehan, A. Gafer[2*]

[1]Biology Department, Animal Reproduction Research Institute (ARRI), Agriculture Research Center (ARC), El-Giza, Egypt, ghada_hassan1971@yahoo.com

[2]Biotechnology Unit, Animal Reproduction Research Institute (ARRI), Agriculture Research Center (ARC), Giza, Egypt.

[*]Corresponding Author, Jehan, A. Gafer, E-mail: jehan.gafer@gmail.com

ABSTRACT

In Egypt, few measures were introduced to improve the reproductive performance rin buffaloes and up to quite recently little efforts were made to improve their genetic potential. Although anti-mullerian hormone (AMH) is the most reliable endocrine marker in assessing the potential for fertility over the ages, there is no age-specific reference range for peripheral AMH levels in buffaloes. The present field study aimed to establish age-specific serum AMH concentrations in buffaloes and their relation to reproductive and productive longevity. The conceivable relationships between Anti-Mullerian Hormone (AMH) concentrations with reproductive longevity and improve the buffalo's productivity were investigated by examining pregnancy rates and early pregnancy loss in three different age groups of buffaloes. Group 1 (heifer 18-24 months, *n* =15), Group 2 (buffaloes 3-6 years, *n* = 15), and Group 3 (old buffaloes 6-10 years, *n* = 15) were synchronized and time fixed inseminated. A single blood sample per animal was taken during oestrus just before artificial insemination (AI), (Day 0) for the AMH analysis. The result revealed that highest serum AMH concentrations were in the heifer group (154.1 7 ± 12.62) pg/ml, $P < 0.05$) Moreover, AMH concentrations and conception rates decreased with age. The AMH concentrations were higher in the pregnant animals at day 30 than in the non-pregnant and pregnancy loss animals between day 30 and day 60 after AI in each group. To the best of our knowledge this study provides first-hand information on age-specific serum AMH levels in Egyptian buffaloes. In conclusion, the AMH concentration analysis could be consider a remarkable biomarker for reproductive and productive longevity in buffaloes.

Keywords: AMH, ovarian reserve, Reproductive longevity, Productive longevity.

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INTRODUCTION

Water buffalo products regarded as an emerging industry Which depends on quality and diversity lead to improve abd expand the economy of many different countires which depends on quality and diversity lead to improve and expand the economy of many different countires on their quality and diversity, contribute to strengthening and expanding the economies of many countries. Via technical developments, numerous phenotypes that could be applied to boost productivity can be studied. **(FAO, 2016).**

However, there is a great heterogeneity in the development of milk and meat within the buffalo population, within the herd or under the same environmental conditions and management. **(Kamel 2018)** in Egypt, few steps to develop the production system of buffaloes have been implemented and no attempt has been made to improve their genetic potential. Anti-mullerian hormone (AMH) is a dimeric glycoprotein secreted from the beginning of life to the end of reproductive period by granulosa cells from developing follicles within the ovary (**Berdugo *et al.*, 2020).** AMH was used in women as a tool for diagnosing the condition of the ovarian fallback, especially the transition to menopause **(Broer *et al.*,**

2014). AMH used as a predictor of the response of follicular development to stimulation protocols in infertility treatments and pregnancy outcome. In the last decade, the subject of wide discussion has been whether this reserve is reversible or not in postnatal life and little is known about the renewal or lack of the ovarian reserve in livestock species **(Woods *et al.*, 2013).**

The loss of quantity and efficiency of the oocyte/follicle pool and hence the decline of reproductive capacity is the general definition of female reproductive aging. The ovaries are much more deeply affected by aging **(Amanvermez and Tosun, 2016).** Both the number and quality of the oocytes in the ovaries decline during the aging process and reach a point after that, all cyclic endocrinological activities stop, entering the menopause in women. However, menopause like stage has not been coined in farm animal species. There is a classical view of a finite primordial follicle reservoir in the ovaries called the ovarian reserve **(Broekmans *et al*., 2009).** This ovarian reserve drops progressively with increasing chronological age within predictable ranges.

A significant determinant of reproductive aging is the age-related depletion of ovarian follicular reserve. **(Lahoz *et al.*, 2014).** It is therefore a challenge to determine the reproductive capacity of an individual female in the herd for economic reasons. Currently, anti-mullerian hormone (AMH) appears to be the best endocrine marker in assessing the age-related decline in the ovarian pool in woman. **(van Rooij *et al*., 2005).** The measurement of serum AMH levels has been added to the panel of markers for ovarian aging **(de Vet *et al*., 2002; Fanchin *et al*., 2003),** ovarian follicular reserve **(van Rooij *et al*., 2002)** and ovarian responsiveness in assisted reproductive technology **(Elgindy *et al*., 2007).** The use of anti-Mtillerian Hormone (AMH) to predict field fertility in cattle has been investigated in study of **Jimenez-Krassel *et al.*, (2015)**.

There are several factors that have a negative effect on the reproductive performance of buffalo and cause severe economic losses **(Suthar 2010)**. Nevertheless, the reproductive efficiency of buffalo can be improved directly by implementing efficient management systems. Therefore, the present field study aimed to abode the question whether ovarian secretion of AMH would be affected by synchronization with CIDR and PGF2α hormones challenge and, to establish age-specific serum AMH concentrations in buffaloes as a biomarker for reproductive and productive longevity in buffaloes.

MATERIALS AND METHODS

Animals

The present study was performed on the experimental farm of Animal production Research Institute (APRI). A total of 45 Egyptian bufaloes, 15 buffaloes heifer 18-24 months and 250-300 kg; 15 Egyptian buffalo 3-6 years of age and 350-400 kg body weight and 15 Egyptian buffalo (6-10 years old and 400-500 kg live body weight) were used in this study selected randomly from the flock. All buffaloes were healthy and clinically free from external and internal parasites. By rectal palpation all experimental buffalo and heifers used in this study normally cyclic with normal genital tract free from any diseases and disorders. Animals housed in semi open pens. According to standard farming practice of ARRI, the animals were fed twice a day and had free access to drinking water and mineral blocks. They were fed with good-quality Egyptian clover, berseem (alfa alfa) every day and were offered a standard total mixed ration according to **NRC (2007).**

Experimental design

Animlas were grouped into two groups according to the body weight and age. All groups were subjected to oestrus synchronization protocol after the determination of the completion of the uterine involution ultrasonographically following the 60th postpartum day. According to this protocol the CIDR, an intra vaginal P4 device, CIDR, (1.9 g progesterone Canada) was inserted for 9 days, regardless reproductive status, and then animals were intramuscularly injected with 2.5 ml PGF2α/animal (Estrumate, Essex Animal Health Fresoythe Sedelsberger Strasse 2-4. 26169 Friesoythe, Germany) 24 h prior to CIDR removal. Animals in heat within 24-72 h after CIDR withdrawal and a fixed-time by artificial insemination were carried out at the same time from fertile buffalo bulls **(Abo-Farw *et al.*, 2019)**.

Blood samples and AMH assay

Blood samples were collected from the jugular vein when the animals were in the oestrus just before the artificial insemination (Day 0), then in the 30th and 60th days after artificial insemination). The oestrus was detected using the clinical techniques mentioned by **Roelofs *et al.*, (2010).** The collected blood samples were centrifuged at 3000 g for 15 min to obtain the serum. The serum samples were stored at −20 °C until analysis. Bovine anti- Müllerian Hormone was assayed by ELISA technique using commercial diagnostic kit (Nova Tec, Immudiagnostica GmbH Waldstraβe 23 A6, D-63128 Dietzenbach, Germany). The assay had a sensitivity of 0.04pg/ml and coefficient of variation of 2.2٪. This assay had high sensitivity and excellent specificity for detection of bovine AMH. No significant cross-reactivity or interference between

bovine AMH and analogues was observed. Intra-assay Precision (Precision within an assay): CV%<15% Three samples of known concentration were tested twenty times on one plate to assess. Inter-assay Precision (Precision between assays): CV%<15% Three samples of known concentration were tested in twenty assays to assess.

Pregnancy diagnosis and monitoring

The pregnancies were assessed by identifying the asymmetry in the uterine horns and the presence of an embryonic vesicle with a tra 0 =kjuhs rectal ultrasonographic examination (real-time B-mode ultrasonography device with a 6.5-mHz linear probe, BCF Easy-Scan, BCF Technology Ltd., UK) on the 30th day (Day 30) after insemination. The animals were noted as pregnant and non-pregnant according to this first pregnancy examination. Then, the animals were Re-checked for the pregnancy continuity by rectal ultrasonography on the 60th day (Day 60) after insemination, and the pregnancy losses were recorded.

Statistical analysis: The data were subjected to statistical analysis of varience (ANOVA) according to **Sendecor and Cochran (1982).**

RESULTS

According to the single blood sample collected in estrus for the AMH measurement, the 30th and 60th day of pregnancy findings were evaluated. The highest serum AMH concentrations were detected in the heifer group, followed by the young and old buffaloes groups, respectively. Heifer group had highest concentration of AMH compared to other groups (p <0.05). The experimental groups of the serum AMH concentrations are shown in Table (1) and Fig.1.

Table 1: Concentrations of serum AMH in the heifer, young, and old buffaloes groups on Day 0.

Groups	Age	Mean AMH concentrations • ±S.E.M.(pg/ml)
Heifer	18-24 months (n=15)	154.1 7 ± 12.62[a]
Young buffaloes	3-6 years (n=15)	132.49 ±7.83[b]
Old buffaloes	6-10 years (n=15)	81.56 ± 5.96[c]

a, b and c The difference between the mean values with the different Between rows significant, P < 0.05.
S.E.M. = standard error of the mean (concentration).

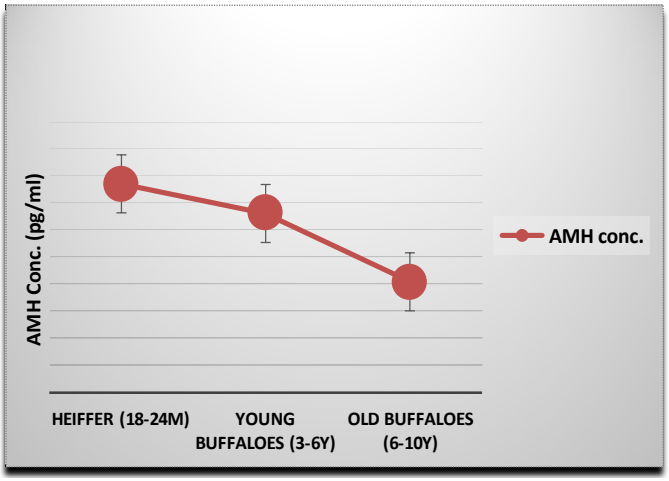

Fig. 1: Concentration of serum AMH in heifer, young and old buffaloes on day 0.

In the first pregnancy examination performed on Day 30 and 60 the numbers of pregnant animals were 12, 11, and 10 for the heifer, young and old buffalos' groups, respectively. In the first pregnancy test performed on Day 30 after the artificial insemination, the AMH concentrations were higher in the pregnant animals than in the non-pregnant animals ($P < 0.05$) in each group. Additionally, the AMH concentrations were higher in all the pregnant animals than in the no pregnant animals ($P < 0.05$). The serum AMH concentrations for the pregnant and non-pregnant animals in the first examination are shown in Table 2 and Fig.2.

Table 2: The serum AMH concentrations from the day of insemination for the animals becoming pregnant and nonpregnant in the examination on the 30th day after insemination

Groups	Pregnant	Non pregnant
Heifer 18-24 months	167.2 9 ± 2.62[a] (n=12)	94± 5.42[c] (n=3)
Young buffaloes 3-6 years	144.37 ±3.23[b] (n=11)	63±4.34[a] (n=4)
Old buffaloes 6-10 years	91.68 ± 4.32[c] (n=10)	48± 2.31[b] (n=5)

[a, b and c] The difference between the mean values with the different letters between columns and rows is significant, $P < 0.05$
S.E.M. = standard error of the mean (concentration).

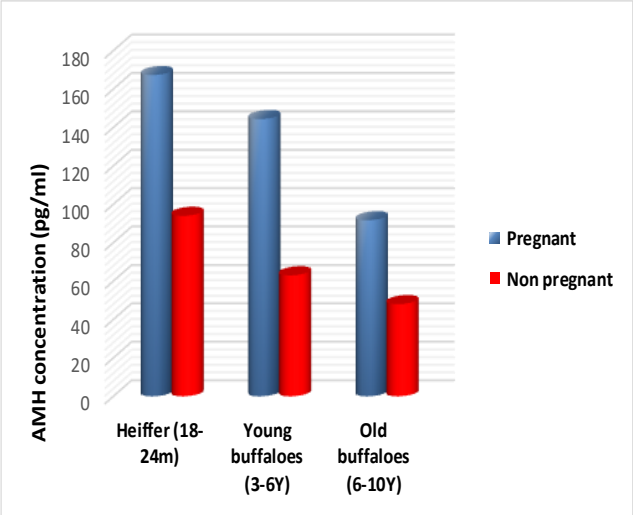

Fig. 2: Concentration of serum AMH in pregnant and non-pregnant buffaloes on 30th day after insemination

The AMH concentrations were significantly higher in all the pregnant animals than in the animals with a pregnancy loss ($P < 0.05$) in the last examination performed on Day 60 of the pregnancy. The serum AMH concentrations of the pregnant animals and the animals with a pregnancy loss on Day 60 are shown in Table 3 and Fig.3.

Table 3: The serum AMH concentrations from the day of insemination of the pregnant animals and the animals with a pregnancy loss detected on the 60th day after insemination (lost between D30 and D60).

Groups	Pregnant	Pregnancy loss
Heifer 18-20 months	167.2 9 ± 6.12^{a} (n=9)	112.17± 4.42^{a} (n=3)
Young buffaloes 2-5 years	144.37 ±5.03^{b} (n=7)	88.50±3.34^{b} (n=4)
Old buffaloes 6-10 years	91.68 ± 2.22^{c} (n=5)	83.60± 2.31^{c} (n=5)

a, b and c The difference between the mean values with the different letters in the same line is significant, $P < 0.05$ S.E.M. = standard error of the mean (concentration).

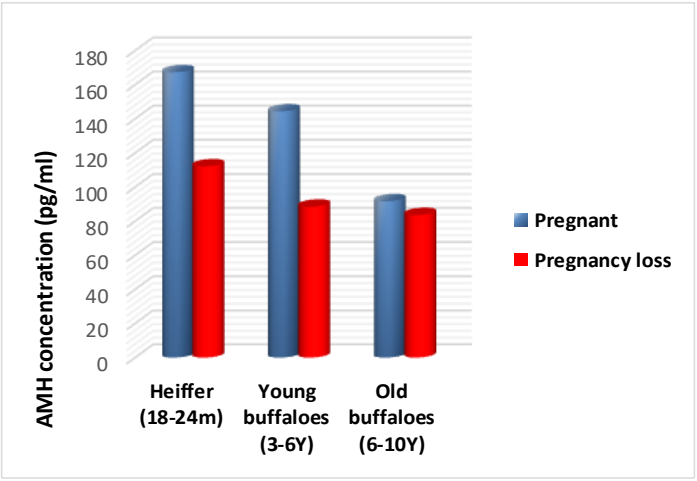

Fig. 3: Concentration of serum AMH in pregnant and buffaloes with pregnancy loss (loss between day 30- day 60) on 60th day after insemination

DISCUSSION

Results revealed that under Egyptian conditions. Ovarian dynamics, which are considered to be an indicator of fertility, reproductive and productive longevity have also gained attention in buffaloes. previous study have tried to obtain information about the quantity and quality of the ovarian reserve by the AMH measurements and success to recognised AMH concentration as a marker of an ovarian reserve in women **(Arouche *et al.*, 2015).**

It is much easier to determine the ovarian reserve and potential fertility by evaluating the AMH concentrations in a blood sample rather than performing an ultrasonographic examination **(Pfeiffer *et al.*, 2014).** The present study aimed to explore the relationship of fertility, productivity and reproductive longevity to the AMH measurements in different age groups of buffaloes under oestrus synchronization, which is substantially well practical compared with the ultrasonographic antral follicle count (AFC) in veterinary practice. Previous study demonstrated that plasma AMH concentrations in cattle was not sufficient to make a significant difference **(Vernunft *et al.*, 2015).** These concentrations displayed a minimal change throughout the oestrous cycle unless a super-stimulation protocol was performed **(Baruselli *et al.*, 2015).**

The AMH measurement has been recently characterized as an independent marker of ovarian activity in cattle due to its relatively stable concentrations throughout the oestrus cycle **(Baruselli *et al.*, 2015; Vernunft *et al.*, 2015).** A synchronisation protocol was used in the present study to provide sexual uniformity during the evaluation of the relationship between the pregnancy rates and the

continuity with the AMH concentrations of the buffaloes with the different age groups. However, the feasibility of the AMH measurement at any time during the sexual cycle exhibits a great advantage from a practical point of view and makes it valuable especially in the health of the herd and reproductive management.

AMH concentrations remained constant during pregnancy and during ovarian cycle **(Rico *et al*., 2011)**, explaining why a single AMH measurement has been usually sufficient. Hence, a single AMH measurement in the plasma of animals at any age or physiological stage could be useful for the assessment of reproductive status in cattle. Administration of exogenous hormones during estrus synchronization could not affect plasma AMH concentrations in cattle **(Pfeiffer *et al*., 2014).** This could be due to the fact that AMH was not involved in feedback mechanisms of the hypothalamus-pituitary-gonadal axis **(Visser *et al*., 2012).**

Jimenez-Krassel *et al*., (2015) found a shorter reproductive herd life, a reduced survival rate of the first calf, and lower pregnancy rates with low AMH concentrations in Holstein heifers. They concluded that a single AMH measurement is a predictive tool for the longevity of the future herd. In the present study, the mean AMH concentrations of the entire pregnant animals were found to be significantly higher than in the non-pregnant animals ($P < 0.05$).

Additionally, the AMH concentrations in the pregnant heifers were higher than the non-pregnant heifers ($P < 0.05$). Similar data was obtained for the young and old buffaloes groups, exhibiting the relationship between the AMH measurement and the pregnancy rates ($P < 0.05$). These results were inconsistent with the findings of the study in which heifers with low AMH concentrations were reported to have poor fertility **(Jimenez-Krassel *et al*., 2015).** Although our results, with a limited animal number, reflect the outcome that the higher AMH concentrations corresponded to higher fertility, productivity and reproductive longevity further studies are needed with expanded populations.

The highest serum AMH concentrations were determined in the heifer group ($P < 0.05$) in the present study. Additionally, the AMH concentrations and pregnancy rates decreased with the age. Although the subgroups with high and low AMH concentrations per group could not be established due to the sample size in the present study, the highest concentrations found in the youngest group and the lowest concentrations found in the oldest group supported the association of the AMH concentrations with the ovarian reserve, which decreased with the age. In agreement with the present study, (**Ahmet Sabuncu1 *et al*., 2019)** could prove the fact that younger cows have a higher plasma AMH concentration may indicate that AMH plays a role in reproductive efficiency. Then added that AMH levels are closely linked to ovarian follicular reserve, and plasma AMH levels could be a candidate endocrine marker for assessing individual reproductive status in cattle. Due to the rarity of the literature on serum AMH in buffaloes it is difficult to narrate our discussion. To the pest of our knowledge this study provides first-hand information on age-specific serum AMH levels in Egyptian Buffaloes.

CONCLUSION

The following conclusions were obtained from the present study: The relation between the AMH and age, the AMH levels decrease with the increasing age; AMH measurement can give sufficient information about the reproductive efficiencies. The scientific data emerging from the present study might lead to new investigating probe on AMH, a valuable parameter for veterinary reproduction. The AMH concentration analysis could be consider a remarkable biomarker for reproductive and productive longevity in buffaloes. Further studies are needed to evaluate the AMH measurement, especially for buffaloes livestock management.

Declaration of competing interest

There is no conflict of interest to declare

REFERENCES

ABO-FARW, M. A.1; SH. A. GABR; W. M. NAGY1; M. EL. FATEH HAMMAD AND E. A. A. EL-EMARY, 2019. Synchronization of Estrus and Ovulation Using CIDR nd Prostaglandin for Improving Pregnancy Rate of Repeat Breeder Egyptian Buffaloes. J. of Animal and Poultry Production, Mansoura Univ.,Vol 10 (9):305 – 312.

AHMET S., GAMZE E. DAL1, SINEM O. E., OMUR K. AND RAMAZAN A. 2019. Association of Anti-Müllerian Hormone concentrations between the pregnancy rates and pregnancy continuity of cows in different age groups. Veterinarni Medicina, 64, (07): 302–308.

AMANVERMEZ, R. AND TOSUN, M. 2016. An update on ovarian aging and ovarian reserve tests. *Int. J. Fertil. Steril.*, **9**: 411- 415.

BARUSELLI PS, BATISTA EOS, VIEIRA L.M AND SOUZA A.H. 2015. Relationship between follicle population, AMH concentration and fertility in cattle. Animal Reproduction 12, 487–497.

BERDUGO, J. A. A. M. TARAZONA, J. J. ECHEVERRI, W. D. CARDONA-MAYA AND A. LÓPEZ-HERRERA 2020. Differences in plasma Anti Müllerian hormone levels and reproductive parameters between two bovine species: *Bos indicus*

and *Bubalus bubalis* VETERINARSKA STANICA 51 (5), x-x, 2020.

BROEKMANS, F.J., SOULES, M.R. AND FAUSER, B.J. 2009. Ovarian aging: mechanisms and clinical consequences. *Endocr. Rev*., **30**: 465-493.

BROER, S. L., F. J. BROEKMANS, J. S. LAVEN and B. C. FAUSER 2014. Anti-Mullerian hormone: ovarian reserve testing and its potential clinical implications. Hum. Reprod. Update 20, 688-701.

DE VET, A., LAVEN, J.S., DE JONG, F.H., THEMMEN, A.P.N. AND FAUSER, B.C. 2002. Antimullerian hormone serum levels: a putative marker for ovarian aging. *Fertil. Steril*., **77**: 357– 362.

ELGINDY, E.A., EI-HAIEG, D.O. AND EI-SEBAEY, A. 2007. Anti- Müllerian hormone: correlation of early follicular, ovulatory and midluteal levels with ovarian response and cycle outcome in intracytoplasmic sperm injection patients. *Fertil. Steril*., **89**: 1670-1676.

FANCHIN, R., SCHONAUER, L.M., RIGHINI, C., GUIBOURDENCHE, J., FRYDMAN, R. AND TAIEB, J. 2003. Serum anti-Mullerian hormone is more strongly related to ovarian follicular status than serum inhibin B, estradiol, FSH and LH on day 3. *Hum. Reprod*., **18**: 323–327.

JIMENEZ-KRASSEL, F., D. M. SCHEETZ, L . M. NEUDER, J. L . IRELAND, J. R. PURSLEY, G. W. SMITH, R. J. TEMPELMAN, T. FERRIS, W. E . ROUDEBUSH, F. MOSSA, P. LONERGAN, A. C . EVANS AND J. J. IRELAND 2015. Concentration of anti-Mullerian hormone in dairy heifers is positively associated with productive herd life. J. Dairy Sci. 98, 3036-3045.

Kamel M.E. AND Mohammed 2018. Application of advanced reproductive biotechnologies for buffalo improvement with focusing on Egyptian buffaloes. Asian Pacific Journal of Reproduction. All rights reserved. doi: 10.4103/2305-0500.241177.

LAHOZ, B., ALABART, J.L., COCERO, M.J., MONNIAUX, D., ECHEGOYEN, E., SÁNCHEZ, P. AND 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**: 347-357.

NRC (2007): Nutrient Requirement of Dairy Cattle. (7th Ed), National Academy Press.

PFEIFFER K.E, JURY L.J. AND LARSON J.E. 2014. Determination of anti-Mullerian hormone at estrus during a synchronized and a natural bovine estrous cycle. Domestic Animal Endocrinology 46, 58–64.

ROELOFS J, LOPEZ-GATIUS F, HUNTER RH, VAN EERDENBURG FJCM, HANZEN C. 2010. When is a cow in estrus? Clinical and practical aspects. Theriogenology 74, 327–344.

SENDECOR, G.W. AND COCHRAN, W.G. 1982. Statistical methods. 7th Edition, the Iowa State University Press, Iowa.

SUTHAR V.S. AND DHAMI A.J. 2010: Estrus detection methods in buffalo. Vet World ; 3(2): 94-96.

VAN ROOIJ, I.A.J., BROEKMANS, F.J.M., SCHEFFER, G.J., LOOMAN, C.W.N., HABBEMA, J.D.F., DE JONG, F.H., FAUSER, B.J.C.M., THEMMEN, A.P.N. AND TE VELDE, E.R. 2005. Serum anti- Mu¨llerian hormone levels best reflect the reproductive decline with age in normal women with proven fertility: a longitudinal study. *Fertil. Steril*., **83**: 979–987.

VAN ROOIJ, I.A., BROEKMANS, F.J., TE VELDE, E.R., FAUSER, B.C., BANCSI, L.F., DE JONG, F.H. AND THEMMEN, A.P.N. 2002. Serum anti-Mullerian hormone levels: a novel measure of ovarian reserve. *Hum. Reprod*., **17**: 3065–3071.

VERNUNFT A., SCHWERHOFF M., VIERGUTZ T., DIEDERICH M. AND KUWER A. 2015. Anti-Muellerian hormone levels in plasma of Holstein-Friesian heifers as a predictive parameter for ovum pick-up and embryo production outcomes. Journal of Reproduction and Development 61, 74–79.

WOODS, D. C., Y. A. R. WHITE, AND J. L. TILLY. 2013. Purification of oogonial stem cells from adult mouse and human ovaries: An assessment of the literature and a view toward the future. Reprod. Sci. 20:7–15.

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