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Twining in Farm Animals with Special Reference to Cattle

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Abstract: Multiple births are uncommon in farm animals; cattle, buffaloes, she-camels, mares, sheep and goats. Occasionally, the reproductive process in cattle results in the birth of twins. But twinning has advantages and disadvantages on cows bearing twins and calves born as twins. This review throws light on the mechanism of twinning, the risk factors associated with twinning, the proper induction protocols, consequences of twinning and the different strategies for managing twinning. It is concluded that twinning can be a good approach to increase reproductive capacity, and consequently the production volume in beef and dairy production systems.

Key words: Cattle % Twining % Mechanisms % Risk-induction

INTRODUCTION

Multiple births are uncommon in buffaloes since most of animal species are uniparous. The occurrence of twin births was 0.14% in a population of Murrah buffaloes with 13,000 births from 1988-2016 without the use of reproductive protocols [1]. Cattle are monotocous species meaning that, under most circumstances, a successful pregnancy results in the birth of one calf. Occasionally, the reproductive process in cattle results in the birth of twins. In some beef cattle production systems,

twinning is considered a desirable trait that can enhance the overall profitability of the production by increasing weaned calf weight produced per cow [2, 3]. On the contrast, twinning in dairy cattle is an undesirable trait that reduces the overall profitability of dairy operation through negative effects on cows calving twins as well as on calves born as twins. Twinning in the dairy cattle population appears to be increasing over time [4]. If this trend continues, the dairy industry must be prepared to reduce the negative effects associated with twinning. Understanding the risk factors associated with twinning mechanism either physiologic or genetic basis may allow to develop management systems that minimize or eliminate the negative effects of twinning in dairy production systems. At present, dairy farmers and consultants are ill prepared to make sound management decisions to mitigate the negative effects of twinning on their operations due to a lack of basic and applied scientific data on twinning in dairy cattle.

This article aims to investigate the risk factors associated with twinning, the proper induction protocols as well as the different strategies for managing twinning in farm animals to avoid the adverse effects of twinning especially in dairy production systems.

1. Mechanisms of Twinning: Twinning in cattle can be classified into two types; monozygous twinning and dizygous twinning. Monozygous twinning rates in dairy cattle range from 7.4-13.6 % [5, 6] of all twin births or less than 0.3% of all births. These estimates seem high considering the frequency of double ovulation which would result in dizygous twins in the dairy cattle population [6, 7]. Thus, monozygous twinning occurs infrequently in cattle and likely accounts for relatively few twin births in the dairy cattle population. Dizygous twinning accounts for most twin births in dairy cattle [5-7]. Likewise, twinning and ovulation rate in cattle are strongly associated traits [8, 9] with the incidence of double ovulation in the dairy cattle population is reported to be 14% [7, 10].

Follicular growth during the estrous cycle in cattle occurs in regular periods of follicular growth, dominance and atresia, termed follicular waves. Normally, only one follicle within each wave is selected to become dominant and acquire ovulatory capacity through a process termed

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deviation [11]. Deviation, therefore, provides an intrinsic mechanism that restricts the number of ovulatory follicles present on the ovaries at any given time during the estrous cycle to one follicle. Unless monozygous twinning occurs, fertilization of the oocyte from one follicle normally results in the birth of one offspring. Occasionally, the follicular selection mechanism is abrogated and two follicles within the same follicular wave undergo deviation resulting in codominance. Cattle normally exhibit either two or three follicular waves during a cycle, and the relationship between the number of follicular waves and the incidence of codominance and/or twinning in cattle has not been established. Because both codominant follicles have undergone deviation and thereby have acquired dominance and ovulatory capacity, an endogenous or exogenous ovulatory stimulus that occurs when codominant follicles are present will result in a double ovulation and the release of two oocytes. Although double ovulation must precede the occurrence of dizygous twins, double ovulation does not consistently result in twinning because cows carrying twins experience high rates of embryonic loss and abortion during gestation [12].

2. Risk Factors Associated with Twin Pregnancies

2.1 Genetic Risk Factors: Twinning in dairy cattle is caused by many different factors, both genetic (i.e., inherited) and non- genetic (i.e., animal management). In general, the breed of animal has an effect on the twinning rate as for most beef breeds of cattle, it is less than 1% [13] compared to twinning in dairy cattle population which ranges from 2.5-5.8%. In addition, the winning rate also varies widely among individual herds as indicated in some studies on Dutch Friesians and Dutch Friesian-Holstein crosses in Holland exhibited an overall twinning rate of 3.2% with a range among herds of 0-7.1% [14]. Similarly, the average twinning rate in a herd of North American Holsteins was 2.4% with a Range among herds of 0-9.6% [15]. In studies comparing the five major dairy breeds of cattle,Holsteins consistently exhibit the highest incidence of twinning [16, 17].

There is an established and ongoing effort by research groups across the globe to quantify the genetic component of twinning in cattle. Quantitative trait loci (QTLs, i.e., regions in the genome) previously identified as being associated with twinning in cattle further substantiates the existence of genomic component to twinning, with QTLs identified on Bos taurus autosome numbers 1, 5, 6,7, 8,10, 14, 15, 19, 23, and 24 [18, 19], Predictions for a subset of cows were compared to their on farm twin records. The heritability for twin pregnancies was 0.088, and genomic predicted transmitting abilities ((g)PTAs) ranged from 7.45-20.79. Effective inclusion of the prediction in a multitrait selection index offers producers a comprehensive tool to inform selection and management decisions [20].

2.2 Environmental or Management Risk Factors for Twinning

2.2.1. Parity: Twinning in dairy cattle increases with parity, ranging from 1% for first parity to nearly 10% during later parities. The greatest increase in twinning occurs between parity 1 (e.g., virgin heifers) and parity 2; twinning continues to increase during later parities but at a lesser rate. The effect of parity on twinning rate is not clearly understood but it may be explained by an increased ability of older cows to support twins throughout gestation, an increase in the incidence of double ovulation, or an interaction between these factors. Although increased uterine capacity of cows calving twins has been reported [13], several studies support increased ovulation rate as the primary factor explaining the effect of parity on twinning. Because monozygous twinning appears to be independent of parity, the effect of parity on twinning rate likely results from an increased frequency of dizygous twinning [21] and hence the incidence of double ovulation. Indeed, the incidence of double ovulation increases with parity in lactating dairy cows [22, 23]. Furthermore, other risk factors for twinning such as milk production are confounded with parity and may explain changes due to parity.

2.2.2. Ovulation Rate: There is a high correlation (0.8-0.9) between ovulation rate and twinning rate [8]. Heritability described as the proportion of genetic factor over the phenotypic variation observed. Low heritability and postpartum reproduction difficulties can cause slow response to selection. It appears to be challenging and time consuming due to the composite factor (genetic and non-genetic) effect, the development of a population with high lower twinning frequency would likely be required for consideration of a twinning technology by the beef cattle industry [24].

The heritability of multiple ovulation (0.028±0.003) and twin birth rate (0.017±0.004) was recently reported, for herds of cattle in Ireland from year 2002-2012 [25]. More recently, heritability estimates for twinning rate were 0.0192±0.0009 and 0.1420±0.0069 for linear and threshold models, respectively for US Holstein cattle [26]. Most sheep breeds have heritability of ovulation rate range about 0.15-0.25 [27].

2.2.3. Milk Production: A positive association between milk production and twinning in dairy cattle has been observed in some studies [28] but, not in others [29]. However, effects of milk production have been reported when comparing dams carrying or calving twins with their non twinning herd mates. Cows calving twins produce less milk during the subsequent lactation compared with cows calving singletons [30]. This reduction in milk production may result from increased incidences of metabolic disorders experienced by cows calving twins during the early stages of lactation. By contrast, milk production at 100 d was greater during the lactation when cows were carrying twin fetuses compared with cows carrying singletons; however, no difference in milk production was detected at later points in lactation [30]. Similarly, cows calving twins produced 2.7 kg more milk at peak production than cows calving singletons, although total production for the lactation did not differ between groups. The single largest contributor to the increase in twinning over a 10-yr period (1983 to 1993) was the concurrent increase in peak milk production during that time. Although the direct relationship between the incidence of double ovulation and twinning is unclear, cows with greater than average milk production near the time of artificial insemination after a synchronized ovulation exhibited a 3-fold greater incidence of double ovulation than cows with less than average milk production [31]. Future studies should continue to investigate the relationships between milk production, ovulation rate, and twinning in dairy cattle.

2.2.4. The Effect of Season on Twin Pregnancies: The effect of sire, live body weight (LBW) and age of dam and calving season on twinning rate and its consequent effects on post-partum reproductive performance of dairy Friesian cows was studied. Twinning rate increased significantly ($P<0.05$)with increasing live body weight and age of dams. Winter season significantly showed the lowest twinning rate (3.76%) compared to the highest rate (6.20%) in autumn season [32].

3. The Protocols of Twinning

3.1. Recombinant Human (rh-FSH): Induction of twinning in single-bearing Noemi ewes is recently performed after the insertion of CIDR pessary for 10 days in the ewe vagina and intramuscular administration of six descending daily twice doses of 60, 60; 40, 40 and 30, 30 IU of recombinant human FSH given at days 8, 9, and 10, respectively in addition to an equivalent dose (260 IU) of human Chorionic Gonadotrophin (hCG) is given at D 10 before CIDR is removed [33]. Also, in another trial, induction of twinning in single- bearing Noemi ewes using recombinant human (rh-FSH) at six descending doses of a total 180IU in Noemi ewes produced two viable neonates. The percent of pregnant ewes within a group giving birth to multiple (>1 lamb) progeny was 0, 72.7, and 80.0%, in the control, porcine FSH, and Human FSH-treated groups, respectively [34].

3.2. Immunization Against Inhibin: The immunization against inhibin-based peptide immunogens in Norduz sheep was Investigated through administration of two doses of inhibin "-subunit 1-32 porcine vaccine with 3 weeks interval. ultrasonographic inspection showed that significantly high number of follicles with 5 mm and larger diameter has developed in immunized ewes. Moreover, 3 of eight immunized ewes have lambed twins without observation of twin birth was in control group [35]. Active immunization against different concentrations of inhibin DNA vaccine of 0.5, 1.0, 1.5, or 2.0 mg and boosted with half of the primary immunization dose improved the behavioral of estrous and ovulation, eventually leading to an augment in the conception rates and twinning rate of beef cattle [36].

3.3. Equine Chorionic Gonadotropins (eCG): Bos taurus beef heifers administered two different doses of eCG either 200 or 300 IU at the end of a fixed-time artificial insemination (FTAI) treatment had greater double ovulation rate and a higher twinning rate on day 30 of gestation and parturition. Those beef heifers carrying two fetuses suffered greater pregnancy losses than cows with singletons[37[zebu beef cows treated with different doses of eCG; 300 (300 IU of eCG), 600 (600 IU of eCG), and 900 (900 IU of eCG displayed estrus within 48 h after removal of the PD (42.3% vs. 11.6%) and ovulated more than one follicle (42%, 58/138 vs. 1.8%, 1/54).Twin pregnancy was higher in cows treated with eCG (42%, 58/138) than controls (0%, 0/54). However, few cows (33.3%) were able to keep both fetuses intact until birth [38]. Group 2 Barki ewes treated with an intravaginal progesterone impregnated sponge for 12 days with an intramuscular injection of prostaglandin F2" at Day 9 and Group 3 ewes synchronized as G2 were received an intramuscular injection of PMSG (750IU / ewe) in descending doses for three days from days 10-12 with sponges' removal at the 12th day and intramuscular injection of hCG hormone (500 IU/ewe) on the 14th day. The twining rates significantly increased in group G3, 32.00% compared to 13.04 and 10.00% in G2 and G1(control group), respectively. The triple rate increased significantly in G3 as compared to other groups, with values of 4.0, 0.0 and 0.0%, respectively[39].

3.4. Embryo Transfer and Artificial Insemination: Angus-cross Cows were assigned either for only artificially inseminated (AI) or received an embryo transfer following artificial insemination (ET + AI) after estrous-synchronization with CO-Synch + CIDR; GnRH (86 mcg/2 mL IM, CIDR®; 1.38 g of progesterone and25 mg intramuscular injection of prostaglandin F2" [40]. Ultrasound examination detected 56% pregnancy risk for both groups, with sensitivity, specificity, and accuracy of 75, 100, and 90.5%, respectively, for bilateral twin detection. Using ET + AI proved to increase twinning rate, and growth when raising both twins with their dams. Monozygotic multiple pregnancies after transfer of single *in vitro* produced or *in vivo* equine embryos were 62% (254/410) and 83% (413/496) respectively. The incidence of multiple pregnancy was 1.6% (4/254) and 0% (0/413) for IVP and *in vivo* blastocysts, respectively. More specifically, three IVP blastocysts yielded twin embryo proper fetuses, and one IVP conceptus developed three distinct embryonic bodies. Two twin pregnancies aborted spontaneously at 3 and 9 months, respectively, while the heartbeat was lost from all three embryos in the triplet pregnancy before day 35 of gestation[41].

3.5. Immunization Against Steroid Hormones: Heifers immunized against androstenedione conjugated to immunogenic carrier protein called keyhole limpet hemocyanin (KLH) had higher twinning rate (21%) and double ovulations (29%) compared to heifers immunized with Estradiol or KLH or saline [42].

3.6. Pregnant Mare Serum / Gonadotropin (PMSG + GnRH) Protocol: The twining rate of does was 18.8 % in both does groups either treated with double doses of PGF2" in 12 days apart or impregnated only with vaginal sponge for 12 days. While twinning rates were 23.52 and 33.33 %, respectively in does groups impregnated with vaginal sponges for 12 days, either with only 300 IU of PMSG or 300 IU of PMSG+ GnRH 10µg at the time o sponge removal and the time of breeding [43].

4. Consequences of Twinning

4.1. Negative Impacts of Twinning in Dairy Cattle

4.1.1. Impact of Twinning on Cows: Twinning reduces reproductive performance by increasing average days open and services per conception during the subsequent lactation Cows calving twins are at greater risk for many reproductive disorders including retained placenta, dystocia, and metritis as well as metabolic disorders including displaced abomasum, and ketosis [28,40]. Not surprisingly, cows calving twins are culled at a greater rate than non-twinning herd mates.

4.1.2. Impact of Twinning on Calves: Incidence of abortion, stillbirth, neonatal calf Mortality and reduced birth weight are greater among twin compared with singleton calves, probably due to reduced gestation length and increased incidence of dystocia among cows calving twins [30, 42].

4.1.3. Freemartinism: Freemartinism in heifers occurs when embryonic membranes of a male and a female conceptus occupying the same uterus fuse during gestation resulting in exchange of blood between the fetuses. Endocrine factors or cells from the male fetus causes abnormal development of the reproductive organs of the female fetus resulting in infertility [44].

5. Strategies for Managing Twinning

5.1. Early Identification of Twins: Ultrasonography can be used to identify cows carrying, twin fetuses at 40-55 d post-AI. Palpation per rectum between 50-70 d post-AI also, results in an acceptable degree of accuracy among experienced veterinarians [45].

Systematic identification of cows carrying twin fetuses allows for differential management of these cows later during gestation, especially during dry and transition periods.

5.2. Elective Abortion and Culling: Continued management of the cow carrying twins could be avoided either by culling the cow or by aborting the pregnancy during the first trimester of gestation, usually through administration of an ecbolic agent such as prostaglandin F2". The following reasons are considered. The first reason is that Cows carrying twins experience greater rates of early embryonic loss than cows carrying singletons and, on occasion, loose one fetus while maintaining the other [46]. Second, the risk for a twin pregnancy during the subsequent gestation is increased because cows calving twins are at greater risk for subsequent twinning [28, 45].

5.3. Management of Cows Carrying Twins: If a cow carrying twins is to be maintained in the herd until parturition, several management strategies should be considered. Feeding dairy cows carrying twin fetuses a higher plane of nutrition, especially during the last trimester of gestation may be beneficial [28, 46]. Earlier dry off and feeding of a transition diet may reduce the incidence of physiologic and metabolic disorders associated with cows calving twins [6]. Providing assistance at calving for cows carrying twins may reduce complications associated with dystocia and the incidence of neonatal calf mortality.

CONCLUSION

It is concluded that twinning can be a good approach to increase reproductive capacity, and consequently the production volume in beef and dairy production systems. Strategies for managing twinning such as early identification of twins by ultrasonography, feeding a higher plane of nutrition especially during the last trimester of gestation, earlier dry off and feeding of a transition diet may reduce the incidence of physiologic and metabolic disorders associated with cows calving twins. Providing assistance at calving for cows carrying twins may reduce complications associated with dystocia and the incidence of neonatal calf mortality.

REFERENCES

1. Santana, R.L.G., D.J. Abreu dos Santos, H. Tonhati, R.B. Costa and G.M. Ferreira de Camargo, 2019. Twinning rate in buffaloes: A case report, Reproduction in Domestic Animals, 54: 808-811.
2. De Rose, E.P. and J.W. Wilton, 1991. Productivity and profitability of twin births in beef cattle. J. Anim. Sci., 69: 3085-3093.
3. Hemken, R.W., R.J. Harmon, W.J. Silvia, W.B. Tucker, G. Heersche and R.G. Eggert, 1991. Effect of dietary energy and previous bovine somatotropin on milk yield, mastitis, and reproduction in dairy cows. J. Dairy Sci., 74: 4265-4272.
4. Koong, L.J., G.B. Anderson and W.N. Garrett, 1982. Maternal energy status of beef cattle during single and twin pregnancy. J. Anim. Sci., 54: 480-484.
5. Erb, R.E. and R.A. Morrison, 1959. Effects of twinning on reproductive efficiency in a Holstein-Friesian herd. J. Dairy Sci., 42: 512-519.
6. Ryan, D.P. and M.P. Boland, 1991. Frequency of twin births among Holstein-Friesian cows in a warm dry climate. Theriogenology, 36: 1-10.
7. Fricke, P.M. and M.C. Wiltbank, 1999. Effect of milk production on the incidence of double ovulation in dairy cows. Theriogenology, 52: 1133-1143.
8. Morris, C.A. and A.M. Day, 1986. Ovulation results from cattle herds with high twinning frequency. Proc. 3rdWorld Congress on Genetics Applied to Livestock Production, pp: 96-100. Held July 16-22, 1986 in Lincoln, Nebraska, USA.
9. Van Vleck, L.D., K.E. Gregory and S.E. Echternkamp, 1991. Ovulation rate and twinning rate in cattle: heritabilities and genetic correlation. J. Anim. Sci., 69: 3213-3219.
10. Ginther, O.J., M.C. Wiltbank, P.M. Fricke, J.R. Gibbons and K. Kot, 1996. Minireview. Selection of the dominant follicle in cattle. Biol. Reprod., 55: 1187-1194.
11. Van Vleck, L.D., K.E. Gregory and S.E. Echternkamp, 1991. Ovulation rate and twinning rate in cattle: Heritabilities and genetic correlation. J. Anim. Sci., 69: 3213. Kirkpatrick, B.W.; Morris, C.A. A Major Gene for Bovine Ovulation Rate. PLOS ONE 2015, 10, e0129025.
12. Pfau, K.O., J.W. Bartlett and C.E. Shuart, 1948. A study of multiple births in a Holstein-Friesian herd. J. Dairy Sci., 31: 241-254.
13. Labhsetwar, A.P., W.J. Tyler and L.E. Casida, 1963. Analysis of variation in some factors affecting multiple ovulations in Holstein cattle. J. Dairy Sci., 46: 840-842.
14. Cady, R.A. and L.D. Van Vleck, 1978. Factors affecting twinning and effects of twinning in Holstein dairy cattle. J. Anim. Sci., 46: 950-956.
15. Foote, R.H., 1981. Factors affecting gestation length in dairy cattle. Theriogenology, 6: 553-559.
16. Moioli, B., R. Steri, C. Marchitelli, G. Catillo and L. Buttazzoni, 2017. Genetic parameters and genome-wide associations of twinning rate in a local breed, the Maremmana cattle. Animals, 11: 1660-1666.
17. Kim, E.-S., X. Shi, O. Cobanoglu, K. Weigel, P.J. Berger and B.W. Kirkpatrick, 2009. Refined mapping of twinning-rate quantitative trait loci on bovine chromosome 5 and analysis of insulin-like growth factor-1 as a positional candidate gene1. J. Anim. Sci., 87: 835-843.
18. McGovern, S.P., D.J. Weigel, B.C. Fessenden, D. Gonzalez-Peña, N. Vukasinovic, A.K. McNeel and F.A. D i Croce, 2021. Genomic Prediction for Twin Pregnancies. Animals, 11: 843-864.
19. Koong, L.J., G.B. Anderson and W.N. Garrett, 1982. Maternal energy status of beef cattle during single and twin pregnancy. J. Anim. Sci., 54: 480-484.

20. Fricke, P.M. and M.C. Wiltbank, 1999. Effect of milk production on the incidence of double ovulation in dairy cows. Theriogenology, 52: 1133-1143.
21. Marcusfeld, O., 1987. Periparturient traits in seven high dairy herds. Incidence rates, association with parity, and interrelationships among traits. J. Dairy Sci., 70: 158-166.
22. Gregory, K.E., G.L. Bennett, L.D. Van Vleck, S.E. Echternkamp and L.V. Cundiff, 1997. Genetic and environmental parameters for ovulation rate, twinning rate, and weight traits in a cattle population selected for twinning. Journal of Animal Science, 75: 1213-1222.
23. Fitzgerald, A.M., D.P. Berry, T. Carthy, A.R. Cromie and D.P. Ryan, 2014. Risk factors associated with multiple ovulation and twin birth rate in Irish dairy and beef cattle. Journal of Animal Science, 92: 966-973.
24. Lett, B.M. and B.W. Kirkpatrick, 2018. Heritability of twinning rate in Holstein cattle. Journal of Dairy Science, 101: 4307-4311.
25. Vinet, A., L. Drouilhet, L. Bodin, P. Mulsant, S. Fabre and F. Phocas, 2012. Genetic control of multiple births in low ovulating mammalian species. Mammalian Genome, 23: 727-740.
26. Kay, R.M., 1984. Changes in milk production, fertility and calf mortality associated with retained placentae or the birth of twins. Vet. Rec., 102: 477-479.
27. Deluyker, H.A., J.M. Gay, L.D. Weaver and A.S. Azairi, 1991. Change of milk yield with clinical diseases for a high producing dairy herd. J. Dairy Sci., 74: 436-445.
28. Nielen, M., Y.H. Schukken, D.T. Scholl, H.J. Wilbrink, and A. Brand, 1989. Twinning in dairy cattle: a study of risk factors and effects. Theriogenology, 32: 845-862.
29. Kinsel, M.L., W.E. Marsh, P.L. Ruegg and W.G. Etherington, 1998. Risk factors for twinning. J. Dairy Sci., 81: 989-993.
30. Shamiah, S.M., A.A. Shitta and H.M.A. Gaafar, 2007. Some factors affecting on postpartum reproductive performance of Friesian cows. Egyptian J. Anim. Prod., 44: 111-119.
31. Zeitoun, M.M., 2021. Production of Live Twins of Single-Bearing Ewes: A Way for Increasing the Sheep Smallholder's Income in the Arabian Gulf Region. Animal Science, 1: 14-20.
32. Zeitoun, M.M., M.A. Ali and A.O. El-Dawas, 2020. Induction of twinning Noemi ewes using two protocols of recombinant human follicle stimulating hormone versus porcine pituitary derived FSH and their subsequent impacts on maternal hormones. Mac. Vet. Rev., 43: 111-123.
33. Bingol, M., I. Daskiran and F. Cedden, 2012. Inhibin immunization in Norduz sheep. Archiv Tierzucht, 55: 179-183.
34. Jinzhu Meng, Qiuye Li, Lilin Xiao, Weichen Liu, Zhengjie Gao, Lin Gong, Xianyong Lan and Shuilian Wang, 2024. Immunization against inhibin DNA vaccine as an alternative therapeutic for improving follicle development and reproductive performance in beef cattle. Endocrinol., 14: 1-9.
35. Cuadro, F., C. García Pintos, R. Núñez-Oliveram, C. Brochado, G.A Bó and A. Menchaca, 2024. Equine chorionic gonadotropin (eCG) treatment in heifers: Double ovulation, twinning rate, and pregnancy losses in twin pregnancies. Theriogenology, 226: 213-218.
36. Alvarez, Rafael Herrera; Pugliesi, Guilherme; Nogueira Natal, Fabio Luis; Rocha, Cecilia Constantino; Ataide Junior, Gilmar Arantes; Ferreira Melo, Alfredo Jose; Otzuk, Ivani Posar; de Oliveira, Claudio Alvarenga; Humblot, Patrice, 2018. Reproductive performance of Bos indicus beef cows treated with different doses of equine chorionic gonadotropin at the end of a progesterone-estrogen based protocol for fixed-time artificial insemination. Theriogenology, 118: 150-156.
37. Ashour, G/, M.F. El-Bassiony, S.M. Dessouki and M.A. El-Wakeel, 2018. Application of Different Hormonal Protocols for Improving Reproductive Performance of Barki Ewes. World Vet. J., 8: 55-64.
38. Lamb, G.C., 2010. Estrus Synchronization Protocols for Cows. In Proceedings of the Applied Reproductive Strategies in Beef Cattle, San Antonio, TX, USA, 28-29 January.
39. Wise, T.H. and B.D. Schanbacher, 1983. Reproductive effects of immunizing heifers against androstenedione and oestradiol-17$. J. Reprod. Fert., 69: 605-612.
40. Echternkamp, S.E. and K.E. Gregory, 1999. Effects of twinning on gestation length, retained placenta, and dystocia. J. Anim. Sci., 77: 39-47.
41. Dijkstra, A., J. Cuervo-Arango, T.A.E. Stout and A. Claes, 2019. Monozygotic multiple pregnancies after transfer of single *in vitro* produced equine embryos. Equine Veterinary Journal, 52: 258-261.
42. Muller, U., 1992. Y chromosome and its role in primary sex differentiation. J. Anim. Sci., 70: 1.

43. Anand Kumar, E.S., K.R. Reddy, A.G. Reddy, K.B.P. Raghavavender, D.A. Kumar and L. Ramsingh, 2018. Efficacy of estrus synchronization protocols on reproductive performance in goats. The Pharma Innovation Journal, 7: 03-06.
44. Davis, M.E. and G.K. Haibel, 1993. Use of real-time ultrasound to identify multiple fetuses in beef cattle. Theriogenology, 40: 373-82.
45. Day, J.D., L.D. Weaver and C.E. Franti, 1995. Twin pregnancy diagnosis in Holstein cows: Discriminatory powers and accuracy of diagnosis by transrectal palpation and outcome of twin pregnancies. Can. Vet. J., 36: 93-97.
46. Bendixen, P.H., P.A. Oltenacu and L. Andersson, 1989. Case-referent study of cystic ovaries as a risk indicator for twin calvings in dairy cows. Theriogenology, 31: 1059-1066.