

Different Therapeutic Approaches for Treatment of Repeat Breeder Cows and its Effect on Maximization Conception Rate in Holstein Dairy Cows

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ABSTRACT

The objective of this study was to compare therapeutic approaches of repeat breeder cases in Holstein dairy cows and its effect on pregnancy rate/artificial insemination (PR/AI). The repeat breeder cows (n=62) which received intrauterine infusion of penicillin-streptomycin combination (Pentomycin) after A.I which yielded the highest pregnancy results (68.33%), while intrauterine benzyl penicillin to repeat breeder cows (n=32) lead to establishment of pregnancy at a rate of (31.25%) of treated cows. Insertion of CIDR on 5th day after A.I in repeat breeder cows (n=25) was accompanied by (52%) PR/AI, and, a twice used PRID2 in cows (n=12) gave satisfactory PR/AI (41.67%). Single administration of hCG 2500 IU (n=47) on 5th day after A.I was accompanied by an increased PR/AI to (89.36%). In spite of that a single dose of 3300 IU hCG (n=126) on day 5 after AI decreased PR/AI in treated cows to (48.41%) and the same trend was observed in repeat breeder cows which received 5000 IU hCG n=(17) single dose (47.05%). Moreover, repeated administration of a small dose of hCG 1000 IU on days 4, 5 and 6 after AI (n=104) gave (60.58%) PR/AI.

Keywords: *Pregnancy rate, Repeat breeder, Holstein dairy cows.*

INTRODUCTION

Repeat breeding is the main infertility complication in dairy herd associated with great effect on fertility and dairy farm economics (Zemjanis, 1980) with consequent results on number of parities and milk yielding (Gustafsson and Emanuelsson, 2000). A repeat breeder cow or heifer is one that fails to conceive to three or more fertile services without any clinical affections of the reproductive tract and usually returns to estrus at regular normal-length intervals (Sood *et al.*, 2015). Abnormal extension of the estrus period or absence of external signs as silent estrus reach to 50% of repeat breeding cows (Cummins *et al.*, 2012). The reproductive loss in heifers and cows after breeding can reach 40-50% and including failure of

fertilization (10%) and embryonic deaths (25-30%), and abortion (5-10%) (Roche, 1981 and Sreenan *et al.* 2001).

Pregnancy rate (PR) in repeat breeder cow is influenced by individual animal factor, intrinsic factors and endocrine events between the maternal environment and conceptus, resulting in early embryonic death (Albihn *et al.*, 1991; Bage *et al.*, 2002).

The repeated disorders during estrus of repeat breeder animal affect the preovulatory LH peak (Albihn *et al.*, 1991). In normal heifers, the induced supra basal P4 levels during estrus substantiated the occurrence of spontaneous repeat breeders (Duchens *et al.*, 1995). The short proestrus phase and LH concentration in repeat breeding cows may affect the oocyte developmental competence (Sood *et al.*, 2015). Delayed ovulation and ovulatory failure have

been reported as the underlying etiology of repeat breeding among cattle in several research papers (Bage *et al.*, 2002; Lopez-Gaitus *et al.*, 2005; Saumande and Humblot, 2005; Bloch *et al.*, 2006; Demetrio *et al.*, 2007; Cummins *et al.*, 2012). Repeat breeder cows usually ovulate smaller follicles less than 11.53 ± 0.32 mm (Kapse *et al.*, 2017). Other factors have been recommended as actual causes of reduced conception rate and repeat breeding including feeding, physiological disturbances, management, early embryonic death, genetic diseases, anatomical defects and errors of estrus detection (Roussel *et al.*, 1988; Jainudeen and Hafez, 1993; Heuwieser *et al.*, 1997) and stress of heat (Badinga *et al.*, 1985). Treatment of repeat breeding animals using GnRH improve conception rates (Sankhi, 1996; Sah and Nakao, 2006; Sah *et al.*, 2012; Savalia *et al.*, 2014).

Repeat breeder cows show typically lower circulating progesterone and delayed low post A.I progesterone increase, when compared to normal cows (Tiwari *et al.*, 2019). The early rise in progesterone after insemination and endometrial secretions are important for development of embryo during early stages of pregnancy (Geisert *et al.* 1992). The pattern of post-ovulation progesterone rise is very critical for future embryonic development (Maurer and Echtenkamp, 1982; Bage *et al.*, 2002).

In spite of that, treatment of repeat breeder cows with single intramuscular injection of progesterone did not improve fertility (Prakish *et al.*, 2018). Injection of repeat breeder cows with 500 mg hydroxyl progesterone caproate IM on day four after insemination with a conception rate of 66.67% (Sharma *et al.*, 2003). While, the conception rates after using P4 after insemination were 56 and 29.6% in CIDR-treated and control repeat breeding cows, respectively (Khoramian *et al.*, 2011). Positive effects on conception rates and growth of embryo after using or modifying CIDR in repeat breeder cows (Mann *et al.*, 2001; Mann *et al.*, 2006; Larson *et al.*, 2007).

Repeat breeding heifers have a lower P4 by day 7 of the cycle and absence of maternal recognition at days 16 and 17, with subsequent early embryonic death (Bage *et al.*, 2002). Improving pregnancy rate in low fertility cows can be induced by supplementation of P4 before day 6 (Mann and Lamming, 1999), confirming the importance of sufficient P4 production in the early luteal phase. Therefore,

the aim of the present study was to comparing between different therapeutic approaches for the treatment of repeat breeding Holstein dairy cows.

MATERIALS AND METHODS

Animals

The current study was carried out on a total of 440 mixed parity Holstein dairy cows (lactation number ranged between 1 and 7) in a well-managed private Holstein herd (Umer dairy farm, Sheikhpura, Pakistan) during the period from (January 2018 to March 2019). The average daily temperature ranged between 16 and 25 °C while monthly fluctuations of relative humidity ranged from 20 to 40%. All animals were proven free from any infectious and contagious diseases and were routinely vaccinated according to a scheduled vaccination program. Animals were fed a totally mixed ration (TMR) according to nutrition research council recommendations (NRC, 2001).

Reproductive examination

Cows (n= 440) diagnosed as repeat breeders with a previous history of infertility (loss in the conception rate) with apparently healthy reproductive organs (no any inflammation, no any abnormal discharge. In addition, normal appearance of uterus with ultrasound and no any pathological affections. All cows in this experiment were checked rectally, by ultrasonography and visual examination of their mucous to determine if any cow has abnormalities or endometritis (Kasimanickam *et al.*, 2004; Hussein *et al.*, 2017). These cows were classified to two experiments based on therapeutic diagnosis only without selection or sorting of these cows.

First experiment

Cows (n=131) were classified to the following groups based on treatment options. The 1st group was consisted of 62 repeat breeder cows which received intrauterine administration of 20 ml penicillin + streptomycin (Pentomycin) (PNS-SM), containing 200 mg/ml Procaine benzyl penicillin and 200 mg/ml Dihydrostreptomycin". 12 hour after insemination. The 2nd group consisted of 32 repeat breeder cows which was administration with Benzil pencilline sodium 1.000.000 IU 5 vials 12 hour after insemination (Schlegl *et al.*, 2020). The 3rd group consisted of 25 repeat breeder cows which was treated with intravaginal administration of CIDR

(Controlled internal drug releasing device, each one contains 1.38 g progesterone, Zoetis USA) and have a silicone-coated nylon core. It was inserted intra-vaginally by specialized applicator device) on 5th day after insemination and removed on 18th day after A.I (Larson *et al.*, 2007). The 4th group consisted of 12 repeat breeder cows treated intravaginal administration of (" PRID DELTA2 is (PRID[®] DELTA) which is an intravaginal progesterone releasing device which contains 1.55 g progesterone, (Ceva Santé Animale, France) but used 2 times before and applied on 5th day after insemination and removed on 18th day after A.I.

Second experiment

The second choice for treatment of repeat breeder cows include treatment with hCG as follow, Cows (n=309) were classified to 15 repeat breeder cows were injected with hCG at dose level of 1500 IU, 47 repeat breeder cows received hCG at 2500 IU, 126 repeat breeder cows received 3300 IU of hCG and 17 received the hormone at 5000 IU, all of the cows received hormone on day five after AI by IM route. The last group consisted of 104 repeat breeder cows, which received hCG in three shots on day 4, 5 and 6 after insemination (Santos *et al.*, 2001; Zolini *et al.*, 2019).

Pregnancy diagnosis

Pregnancy diagnosis in all cows was carried out 35 days using ultrasonographic portable device equipped with a 7.5 MHz linear rectal probe SonoScape A5. Confirmatory findings of pregnancy were the embryo and the

surrounding amniotic membrane; embryonic heartbeats and clear hypo-echoic fluids surrounding the embryo (Kasimanickam *et al.*, 2018; Kasimanickam *et al.*, 2020). All data were recorded in dairy management program (dairy comp 305) for calculate and judging the conception rate of all group.

RESULTS

Treatment of repeat breeder cows using intrauterine penicillin-streptomycin combination (Pentomycin) yielded the highest pregnancy results (68.33%), while intrauterine Benzyl penicillin treatment led to establishment of pregnancy at a rate of 31.25% of treated cows. Insertion of CIDR was accompanied by 52% PR/AI, and, a twice used PRID gave satisfactory PR/AI (41.67%). Pregnancy rate per artificial insemination in repeat breeder cows as shown in table (1) and fig. (1).

Single administration of 1500 IU hCG on day 5 after insemination achieved pregnancy in 86.67% of treated cows as shown in table (2) and fig. (2). Increasing the dose of hCG to 2500 IU was accompanied by an increased PR/AI to 89.36. Despite, a single dose of 3300 IU hCG on the 5th day after AI decreased PR/AI in treated cows to 48.41%, and the same trend was observed in repeat breeder cows which received 5000 IU hCG single dose (47.05%). Moreover, repeated administration of a small dose of hCG (1000 IU) on days 4, 5, and 6 after AI gave 60.58% PR/AI.

Table 1: Effects of different local treatment protocols on pregnancy rate per artificial insemination in repeat breeder cows

Item	Inseminated	Pregnant	PR/AI
IU Penicillin-streptomycin	62	41	68.33 ^b
IU benzyl penicillin	32	10	31.25 ^a
CIDR	25	13	52.00 ^{ab}
PRID2	12	5	41.67 ^{ab}

Pregnancy rate per artificial insemination differed significantly (Chi square = 10.98; P value = 0.012) among different treatment protocols. PR/AI: Pregnancy rate per artificial insemination; IU: Intrauterine; CIDR: Controlled internal drug release; PRID2: Progesterone releasing intra-vaginal device previously used twice.

Table 2: Effects of hCG treatment on pregnancy rate per artificial insemination in repeat breeder cows

Dose of hCG	Inseminated	Pregnant	PR/AI
1500 IU	15	13	86.67 ^b
2500 IU	47	42	89.36 ^b
3300 IU	126	61	48.41 ^a
5000 IU	17	8	47.05 ^a
1000 IU three shots	104	63	60.58 ^{ab}

Pregnancy rate per artificial insemination differed significantly (Chi square = 29.67; P value = 0.0001) among different HCG dosages protocols. HCG: Human chorionic gonadotropin; IU: International unit; PR/AI: Pregnancy rate per artificial insemination

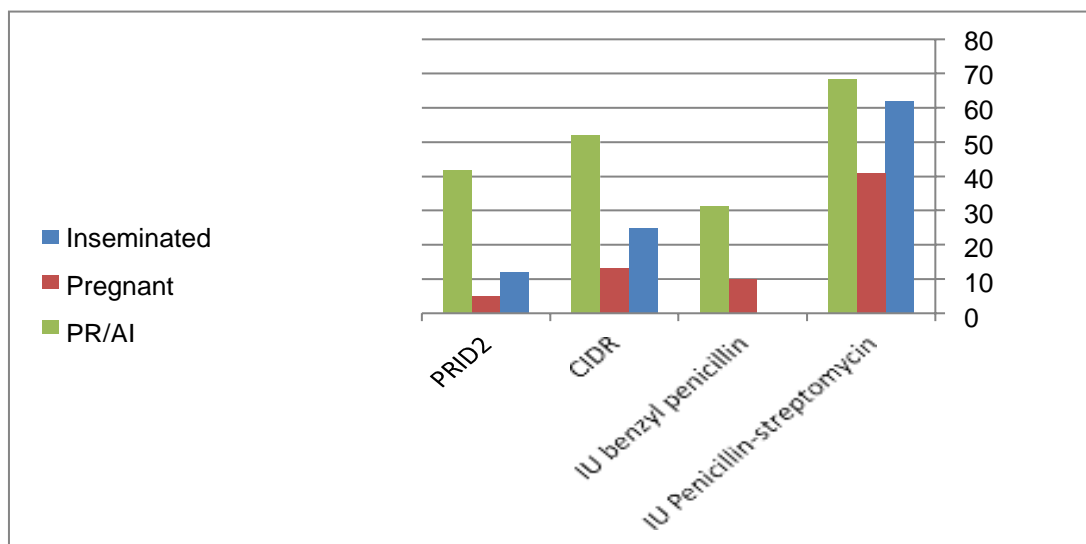


Fig.1: Effects of different local treatment protocols on pregnancy rate per artificial insemination in repeat breeder cows

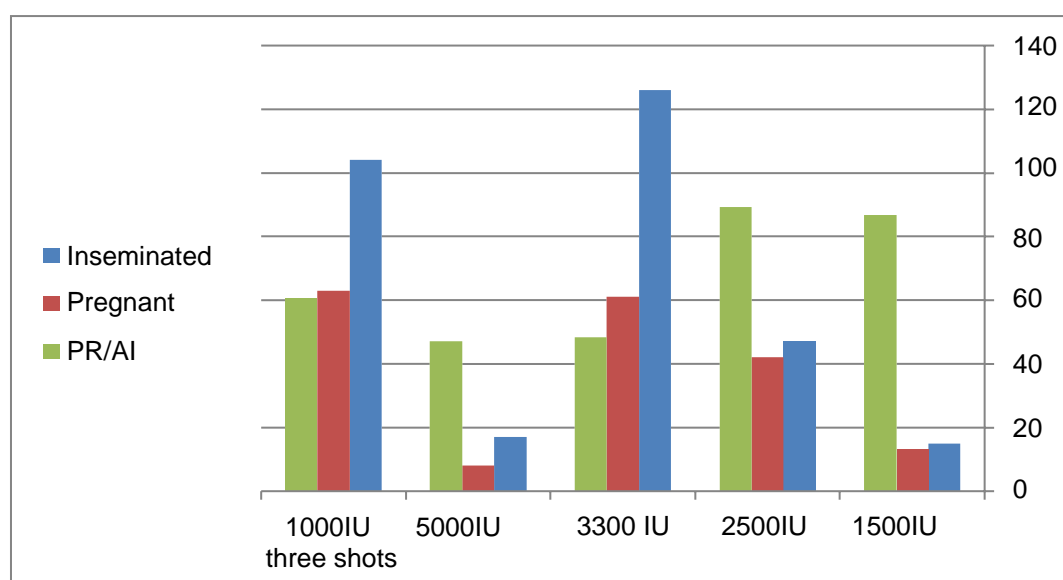


Fig.2: Effects of HCG treatment on pregnancy rate per artificial insemination in repeat breeder cows.

DISCUSSION

Repeat breeding in lactating cows have many factors, which can affect the pathogenesis such as negative energy balance, hormonal imbalance, and postpartum affections (**Bage et al., 2002**).

In this experiment, repeat breeder dairy cows were submitted to several therapies to improve conception figures. Treatment options were intrauterine administration of PNS-SM combination, intrauterine administration of Benzyl PNS, intravaginal insertion of twice used PRID or insertion of new CIDR after AI. Significant variations were observed in pregnancy rates where intrauterine PNS- SM preparation was accompanied by higher PR/AI. Meanwhile, intrauterine administration of Benzyl PNS yielded the poorest pregnancy. In

addition, PR/AI in repeat breeders did not vary between CIDR group and twice-used PRID group.

As regards to post-insemination local application, both twice-used PRID and CIDR gave comparable pregnancy rates in treated cows. This could be an indication for future research testing the efficiency of PRID for supporting progesterone in early pregnancy in cattle after repeated applications. The fact that PRID has greater surface area of contact with vaginal wall, compared to CIDR and that his greater surface area is correlated with its progesterone secretory potential might be the underlying etiology behind its greater efficacy in maintaining pregnancy in repeat breeder cattle (Van Werven *et al.*, 2013).

In the second experiment we tried to find the

most suitable dose regimen of HCG which when administered five days after the best pregnancy rates in repeat breeder cows. Very good pregnancy rates were obtained from different doses ranging from 47.05% for 5000 IU single dose to 89.36% for 2500 IU single dose. The obtained results are greater than those reported by Shams-Esfandabadi *et al.*, (2007), Nascimento *et al.*, (2013). However, these two studies were carried out on different animal categories. The first one was a conclusive meta-analytic study and the second was carried out using a single 3000 IU injection on day 5 after AI in normal cows not suffering from repeat breeding. Shams-Esfandabadi *et al.*, (2007), recorded that, there is no improvements were observed in treated cows' pregnancy rates when compared to control cows. Alnimer and Shamoun, (2015) reported that single 1500 IU hCG injection on day 6 after AI improved pregnancy rates in repeat breeder cows by reducing pregnancy losses and increasing post AI concentrations of progesterone. In that study, however, the pregnancy rates were far lower from ours regarding the 1500 IU hCG dose (38.09 versus 86.67%). Meanwhile, in the current study, pregnancy diagnosis was carried out on day 35 after insemination, thus, it is likely that late embryonic deaths might have been responsible for this variation. Other studies report reduced pregnancy rates in cows treated by hCG after AI on day 5 (Schmitt *et al.*, 1996; Hanlon *et al.*, 2005). Discrepancies might be due to different doses, parities and sources of the drug.

As regards to the 3300IU single dose, the pregnancy rate observed during work was (48.41%) was similar to that reported by (Zolini *et al.*, 2019). Similarly, Santos *et al.*, (2001) found pregnancy rate on day 28 after AI, 45.8%. Santos *et al.*, (2001) confirmed also that the beneficial effects of hCG on pregnancy were brought by its ability to increase luteal secretion of progesterone, not via increased luteal tissue mass. In the present trial, administration of 5000 IU of hCG on day 5 after estrus in cows yielded lower pregnancy results, when compared to cows injected by 1500 or 2500 IU.

Partially agreeing, Helmer and Britt (1986) found that pregnancy rates in cows receiving a single 5000 IU HCG after estrus was not accompanied by any improvements in pregnancy rates.

CONCLUSION

The highest pregnancy rates were achieved in cows received penicillin-streptomycin (Pentomycin) than intrauterine Benzyl penicillin. Insertion of CIDR on 5th day after AI give satisfactory results than PRID. Single administration of 2500 IU hCG on 5th day after A.I was increased PR/AI than 5000 IU and 1000 IU of hCG.

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Conflict of interest

The authors declare they do not have any conflict of interest.

REFERENCES

- Albihn, A., Gustaffson. H., Hurst. M., Rodriguez-Martinez. H. (1991): Embryonic ability to prolong the inter-estrus interval in virgin and repeat breeding heifers. *Anim Reprod Sci.*, 26:193-210
- Alnimer, M.A., Shamoun. A.I. (2015): Treatment with human chorionic gonadotropin 4 or 6 days after TAI to improve pregnancy outcomes in repeat breeding dairy cows. *Anim Reprod Sci.*, 157: 63-70.
- Bage, R., Gustafsson, H., Larsson, B., Forsberg, M., Rodriguez-Martinez, H. (2002): Repeat breeding in dairy heifers: follicular dynamics and estrous cycle characteristics in relation to sexual hormone patterns. *Theriogenology*, 57: 2257-2269.
- Bloch, Y., Folman, M., Kaim, Z., Roth, R., Braw-Tal, D. Wolfenson (2006): Endocrine alterations associated with extended time interval between estrus and ovulation in high-yield dairy cows. *J. Dairy Sci.*, 89: 4694-4702
- Cummins, S.B., Lonergan, P., Evans, A.C., Butler, S.T. (2012): Genetic merit for fertility traits in Holstein cows: II. Ovarian follicular and corpus luteum dynamics, reproductive hormones and estrus behavior. *J Dairy Sci*, 95:3698-3710.
- Demetrio, R.M. Santos, C.G.B. Demetrio, J.L. M. Vasconcelos (2007): Factors affecting conception rates following artificial insemination or embryo transfer in lactation Holstein cows. *J. Dairy Sci.*, 90: 5073-5082

- Duchens, M., Maciel, M., Gustafsson, H., Forsberg, M., Rodriguez-Martinez, H., Edqvist, L.E. (1995): Influence of perioestrous suprabaasal progesterone levels on cycle length, oestrous behaviour and ovulation in heifers. *Anim. Reprod. Sci.* 37: 95-108.
- Geisert, R.D., Short, E.C., Zavy, M.T. (1992): Maternal recognition of pregnancy. *Anim. Reprod. Sci.* 28: 287–298.
- Gustafsson, H. and Emanuelsson, U. (2000): Has a repeat breeder cow in the present lactation a higher risk to become a repeat breeder in the next lactation? *Proc 14th Inter Cong Anim Reprod* 2: 100.
- Hanlon, D. W., Jarratt, G. M., Davidson, P. J., Millar, A. J., Douglas, V. J. (2005): The effect of hCG administration five days after insemination on the first service conception rate of anestrous dairy cows. *Theriogenology*, 63:1938–1945
- Helmer, S.D., Britt, J.H. (1986): Fertility of dairy cattle treated with human chorionic gonadotropin to stimulate progesterone secretion. *Theriogenology*, 26(5): 683-695.
- Heuwieser, W., Oltenacu, P.A., Lednor, A.J., Foote, Rh. (1997): Evaluation of different protocols for prostaglandin synchronization to improve reproductive performance in dairy herds with low estrus detection efficiency. *J Dairy Sci.*, 80:2766–2774.
- Hussein, M.M., Khalil, A.A.Y., Al Agawany, A.A., Zyada, A. A. (2017): Efficacy of ultra-sonographic examination and combined use of PGF2 alpha plus cephaprin to identify and treat endometritis in dairy cows. *J. veterinary medical research*, 24(2): 322-336.
- Jainudeen, M.R., Hafez, E.S.E., (1993): Reproductive failure infemales. In: Hafez ESE (ed.), *Reproduction in Farm Animals*, 6th edn. Lea and Febiger, Philadelphia, pp. 261–311.
- Kapse, S., Singh, M., Sharma, A., Kumar, P. (2017): Dominant follicle diameter at insemination related to positive pregnancy outcome in dairy cattle. *Indian J. Anim. Reprod.*, 38 (1): 56-57.
- Kasimanickam, R., Duffield, T. F., Foster, R. A., Gartley, C. J., Leslie, K. E., Walton, J. S., Johnson, W. H.(2004): Endometrial cytology and ultrasonography for the detection of subclinical endometritis in postpartum dairy cows. *Theriogenology*, 62(1-2):9-23.
- Kasimanickam, R.R., Hall, J.B., Estill, C.T., Kastelic, J.P., Joseph, C., Abdel Aziz, R.L., Nak, D. (2018): Flunixin meglumine improves pregnancy rate in embryo recipient beef cows with an excitable temperament. *Theriogenology*, 107: 70-77.
- Kasimanickam, R., Kasimanickam, V., Kastelic, J.P., Ramsey, K. (2020): Metabolic biomarkers, body condition, uterine inflammation and response to superovulation in lactating Holstein cows. *Theriogenology*, 146: 71-79
- Khoramian, B., Farzaneh, N., Talebkhan, M., Mohri, M. (2011): Comparison of the effects of gonadotropin-releasing hormone, human chorionic gonadotropin or progesterone on pregnancy per artificial insemination in repeat-breeder dairy cows. *Res Vet Sci*, 90: 312-315.
- Larson, S.F., Butler, W.R., Currie, W.B. (2007): Pregnancy rates in lactating dairy cattle following supplementation of progesterone after artificial insemination. *Anim. Rep. Sci.* 102:172–179.
- López-Gatiús *et al.*, (2005): Ovulation failure and double ovulation in dairy cattle: Risk factors and effects. *Theriogenology*, 63: 1298-1307
- Mann, G.E., Lamming, G.E. (1999): The influence of progesterone during early pregnancy in cattle. *Reprod. Domest. Anim.*, 34: 269-274.
- Mann, G.E., Fray, M.D., Lamming, G.E. (2006): Effects of time of progesterone supplementation on embryo development and interferon γ production in the cow. *Vet. J.* 171: 500–503.
- Mann, G.E., Merson, P.M., Fray, M.D., Lamming, G.E. (2001): Conception rate following progesterone supplementation after second insemination in dairy cows. *Vet. J.* 162: 161–162.
- Maurer, R. R., Echtenkamp, S. E. (1982): Hormonal asynchrony and embryonic development. *Theriogenology*, 17: 11-22.
- Nascimento, A.B., Bender, R.W., Souza, A.H., Ayres, H., Araujo, R.R., Guenther, J.N., Sartori, R., Wiltbank, M.C. (2013): Effect of treatment with human chorionic gonadotropin on day five after timed artificial insemination on fertility of lactating dairy cows. *J Dairy Sci*, 96: 2873-2882.
- N.R.C (2001): *Nutrient Requirements of Dairy Cattle* (7th rev. ed.), Natl. Acad.

- Sci., Washington, DC.
- Prakish, S.S., Makwana, R.B., Savaliya, B.D., Patbandha, T.K., Murthy, K.S. (2018): Effect of hormonal therapy on fertility in repeat breeding Gir cows. *Int J Curr Microbiol Appl Sci*, 7: 4952-4958.
- Roche, J.F. (1981): Reproductive wastage following artificial insemination. *Vet Rec*. 109:401-404.
- Schlegl, R., Drillich, M., Ballas, P., Reinlander, U., Iwersen, M., Baumgatner, W., Ehling-Schulz, M., Wagener, K.(2020): Field trial on the post-insemination intrauterine treatment of dairy cows with mild endometritis with cephalixin. *Theriogenology*, 156(15):20-26.
- Roussel, J.D., Beatty, J.F., Koonce, K. (1988): Gonadotropin releasing hormone therapy in functional infertility of dairy cattle. *Theriogenology*, 30:1115-1119
- Sah, S.K., Nakao, T. (2006): Characteristics of repeat breeding buffaloes in Nepal. *J. Reprod. Dev.* 52:335-341.
- Sah, S.K.S., Yadav, J.L., Kaphle, K. (2012): Conception rate in repeat breeding buffaloes using hormone GnRH and mineral-vitamin mixtures under farmers managed condition in Chitwan, Nepal. *Proceedings of 10th National Veterinary Conference* 51-56.
- Sankhi, K.P. (1996): Effect of gonadorelin in cows and heifers associated with reproductive problems. *Proc 5th National Vet Conf*, 42-45.
- Santos, J.E.P., Thatcher, W.W., Pool.L.,Overton, M.W(2001):Effect of human chorionic gonadotropin on luteal function and reproductive performance of high producing lactating Holstein dairy cows. *J Anim Sci*, 79:2881-2894.
- Saumande and Humblot , (2005) :The variability in the interval between estrus and ovulation in cattle and its determinants *Animal Reproduction Science* 85(3-4):171-82
- Savalia,A. J., Dhama,K. K., Hadiya,K. R., Patel,Nitesh Sarvaiya(2014):Influence of controlled breeding techniques on fertility and plasma progesterone, protein and cholesterol profile in true anestrus and repeat breeding buffaloes. *Veterinary World* 7(9):727-732
- Schmitt, E.J., Diaz, T., Barros, C.M., de La Sota, R.M., Drost, M., Fredricksson,E.W., Staples, C.R., Thorner, R., Thatcher, W.W (1996): Differential response of the luteal phase and fertility in cattle following ovulation of the first wave follicle with human chorionic gonadotropin or an agonist of gonadotropin releasing hormone. *J Anim Sci*, 74: 1074- 1083.
- Shams-Esfandabadi, N., Shirazi, A., Mirshokrai, P., Bonyadian, M. (2007): Influence of hCG administration after AI on conception rates and serum progesterone concentration in cattle. *Pak J Biol Sci*, 10: 2709-2713.
- Sharma, A., Jindal, R., Singh, N., Singh, R.V. (2003): Effect of progesterone supplementation on conception rate and hormonal profile in repeat breeding buffaloes. *Indian J Anim Sci*, 73(7): 773-774.
- Sood, P., Zachut, M., Dube, H., Moallem, U. (2015): Behavioral and hormonal pattern of repeat breeder cows around estrus. *Reproduction* 149: 545-554.
- Sreenan, J.M., Diskin, M.G., Morris, D.G. (2001): Embryo survival rate in cattle: a major limitation to the achievement of high fertility. Fertility in the high-producing dairy cow. In Diskin, M.G. (Editor), *Animal Science Vol. 1, Occasional Publication No. 26, British Society of Animal Science*, 93-104
- Tiwari, I., Shah, R., Kaphle, K., Gautam, M. (2019): Treatment approach of different hormonal therapy for repeat breeding dairy animals in Nepal. *Arch Vet Sci Med*, 2(3): 28-40.36, 795-807.
- Van Werven,T.,Waldeck,A.,Souza,A.H.,FlochS andEnglebienne,M.(2013):Comparison of two intravaginal progesterone releasing devices (PRID-delta versus CIDR) in dairy cows: blood progesterone profiles and field fertility. *Anim Reprod Sci.*, 138:143-149.
- Zemjanis, R. (1980): Repeat-breeding or conception failure in cattle. In. *MORROW, D.A. Current therapy in Theriogenology. Philadelphia: Saunders, Cap.5, p.205-213.*
- Zolini, A.M., Ortiz, W.G., Estrada-Cortes, E., Ortega, M.S., Dikmen, S., Sosa, F., Giordano, J.O., Hansen, J.P. (2019): Interactions of human chorionic gonadotropin with genotype and parity on fertility responses of lactating dairy cows. *J Dairy Sci.*, 102: 846-856.