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Research Article

Treatment Approach of Different Hormonal Therapy for Repeat Breeding Dairy Animals in Nepal

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Abstract

Repeat breeders are the animals with more than 3 to 4 inseminations after calving having regular cyclicity yet failed to conceive. Therefore, it is considered as one of the most emerging and frustrated reproductive disorders among dairy herds in Nepal that hinders favorable productivity and causes heavy economic losses to the livelihood of the farmers. We performed a systemic review to be acquainted with hormonal therapy as a treatment of repeat breeding. The aim of the present review is to summarize the current information about the ongoing methods in the treatment of repeat breeders. Based on the review of articles, it was found that gonadotropin-releasing hormone (GnRH) administration improved the conception rate in repeat breeder buffaloes and cows bearing dominant follicle in the ovary. In repeat breeder buffaloes and heifers with predominant corpus luteum and adequate body condition score (BCS), administration of PGF2α increased the pregnancy rates. Conception rate with Ovsynch protocol (GnRH-PGF2α -GnRH-TAI) usually was improved when initiation of program done during mid-diestrus i.e. days 5-12 of estrus cycle. Exogenous progesterone administration resulted in increased serum progesterone level during PGF2α injection that usually improves fertility of lactating dairy cows. In conclusion, hormonal treatment is considered to be most effective treatment to mitigate the problems of repeat breeding syndrome in Nepal. However, the first step of treatment involves good care, nutrition, better oestrus detection, timely insemination, periodic deworming to control parasite load and management of mating with bulls.

Keywords: Conception rate; GnRH; PGF2α; Progesterone; Repeat Breeder

1. Introduction

Eighty-three percent of the total population of Nepal lives in rural areas and their main source of income comes from agriculture and livestock. Livestock plays a significant role in agricultural development and the economy of the country contributing around 32% of agricultural Gross Domestic Product (GDP) and 11% to the national GDP of Nepal [1]. Cattle (*Bos tauras*) and water buffalo (*Bubalus bubalis*) has been considered as the important dairy animals in Nepal. About 25.68% of the peoples of the country are in animal husbandry practices in which cow (7.3 Million), and buffalo (5.17 Million) has a major contribution in GDP. The milk production from the cow and buffalo was 643806 and 121044 metric ton, respectively by fiscal year 2015-16 [2]. There was a target to reach 45% on the GDP contribution by livestock at the end of 20 years of Agricultural Perspective Plan (APP) program; which was by fiscal year 2014/15 but achievement was only 24.6%. However, the conditions of dairy animal in terms of health, nutrition, and management still is not in good condition to contribute to the livelihood and economies of people. Some of the major reproductive disorder recorded in many farms include abortion, infertility, long inter calving intervals, dystocia, uterine torsion, repeat breeding. Repeat breeding is regarded as one of the most important reproductive problems in buffaloes [3].

Repeat breeding is one of the most emerging and frustrated reproductive problems among dairy animals in Nepal. Animals are said to be repeated breeder if they have normal oestrus, oestrus cycle, as well as reproductive tract and, has been bred three or more times by a fertile bull or semen yet, failed to conceive [4]. The cause of repeat breeding is unclear and multifactorial. Hormonal insufficiency and dysfunction contribute about 40.1% causes of repeat breeding [5]. Prolonged duration of estrus, extended follicular phase, delayed luteinizing hormone (LH) surge and thus delayed ovulation, late postovulatory rise in plasma progesterone considered to be most prominent factors responsible for repeat breeding [6]. Failure of fertilization is mostly associated with poor heat detection by farmers, improper estimation of fixed-time artificial insemination. It is also due to the abnormalities related to poor semen quality. Either failure of fertilization or early embryonic death is considered to be major pathogenesis of repeat breeding animals [7]. Other risk factors include tubal obstructions, early or latent embryonic abnormalities, poor breeding and management techniques including genetic, nutritional and infectious factors.

There is a failure of conception even in normal cyclic repeat breeding buffaloes and this has led to an increase in calving interval and hence reduces the annual birth rate of calves [8]. When the calving interval increases beyond the end of lactation, there is a sharp decrease in the milk yield but the investment in health, management, and feeding of such repeat breeding still persists. The costs of herd management and rearing are increased due to the increment expenses on repeated unsuccessful artificial inseminations, culling costs and replacement of those animals that can't conceive [9]. Hence this causes heavy economic losses to the livelihood of farmers. Failure of conception even after repeated artificial insemination or natural mating compelled farmers to sell their dairy animals at cheap price. Thus, genetically potential buffaloes are slaughtered. Therefore, more and more animals are being imported from the neighboring country India for dairy herds. There is a danger in the near future that all the indigenous breed with higher genetic potential to get extinct from Nepal. The alternative approach of different hormonal therapy could be applied in dairy herds to increase the reproductive efficiency and minimize the reproductive problem related to

anestrus and failure of fertilization and conception [10]. The pregnancy rate with different hormonal protocols usually gets increased when synchronization is done at active breeding season which is August-September month in repeat breeder Murrah buffaloes [11]. Provision of mineral along with vitamin supplementation is very essential for repeat breeder animals to increase conception rate. Minerals such as Cu and Fe have played a significant role in the ovulatory process in crossbred cattle [12].

1. Methodology

A scientific literature review was done from different articles, published authenticate reports, conference proceedings and post-graduate dissertations and reliable information on the treatment approach of different hormonal therapy for repeat breeding dairy animals. We have summarized the relevant information in systemic way and also summarized in tabulated as well as figure form.

2. Hormonal treatment for the repeat breeders

3.1. Hormone Gonadotrophin Releasing Hormone (GnRH) administration

Conception rate in anestrous buffaloes and heifers having dominant follicle was found to be 100% within two months when treated with GnRH. Also, the conception rate in anestrous buffaloes with ovarian cysts when treated with GnRH injection found to be 100% within 2 months [13]. Different hormonal protocols in repeat breeder animals and their effect in conception rate is mentioned in **Table 1.** The conception rate in Nilli-Ravi buffaloes was effectively improved with being 42.8% with GnRH administration at the time of insemination, 28.6% after 12 h of post insemination and only 14.3% in control group which was under treatment of Normal Saline administration intramuscularly [14].

Repeat Breeder animal	**Treatment protocol**	**Conception rate**	**Citation**
Holstein (n= 10) and 3 Jersey cows	Single GnRH administration at the time of AI	46% within 50days	[15]
Murrah Buffaloes (n= 4) and heifers with BCS 2.5 and more (n= 4)	Single GnRH administration and insemination at estrus	100% within two months	[16]
Murrah Buffaloes (n=15)	Estrus detection 8-18hrs after GnRH administration and inseminate females	53.34% within 4 months	[17]
Murrah Buffaloes (n= 12)	GnRH + mineral mixture and AI on estrus	75.00% within 4 months	[17]
Jersey (n= 49) and Cross breed (n= 53) cows [Jersey x Red Sindhi]	GnRH along with first AI. Single and double inseminations (24hrs after 1st AI)	In Jersey 54.5% and 47% on single and double AI	[18]

		In Crossbred 50% and 42.8% on single and double AI	
Buffaloes (n= 10)	AI and GnRH on clear standing estrus	Overall 50% in 1st,2nd and 3rd repeated estrus cycle	[19]

Table 1: Hormone GnRH effect on conception rate

Treatment with GnRH at the time of insemination improves the conception rate of repeat breeding dairy cows [20] and the treatment protocol is shown in **Figure 1**. GnRH from the hypothalamus stimulates increased secretion of follicle-stimulating hormone (FSH) and LH from anterior pituitary during proestrus and estrus. The combined action of both of these hormones is associated with follicular development, ovulation, and corpus luteum (CL) function. LH is believed to rupture the follicular cyst present within the ovaries and bring back the noncyclic cows into normal cyclicity [15]. GnRH injection probably led the LH surge that hastens the ovulation and conception may take place. Heavy fertility losses in repeat breeder heifer are primarily due to fertilization failure and reduced embryo survival [21], [22]. The effects of GnRH treatment at artificial insemination (AI) on rates of fertilization and embryonic mortality are unknown but may be related to the timing of ovulation and progesterone secretion by the corpus luteum [18]. GnRH when given either at the time of insemination or between 11 and 14 days after Timed Artificial Insemination (TAI) regarded to increase the pregnancy rates in cattle [23]. GnRH therapy along with mineral-vitamin mineral mixtures results the highest conception rate in repeat breeder buffaloes [17]. This relies on the fact that repeat breeding being multi-factorial causes including hormonal aberration and other factors which respond better on both hormonal and mineral mixture supplement. By maintaining and synchronizing the hormonal status through GnRH treatment, the conception rate in repeat breeder buffaloes can be improved.

Figure 1: Artificial insemination along with GnRH hormone administration intramuscularly after the detection of heat.

3.2. Hormone Prostaglandin PGF2α Administration

Conception rate in anestrus heifer and buffaloes with predominant corpus luteum and adequate BCS was found to be 100% within two months after treatment with PGF2α [13]. Repeat breeder with the luteal stage when subjected to prostaglandin administration shows improved pregnancy rate as shown in **Table 2**.

Repeat Breeder animal	Status of animal	Treatment protocol	Conception rate	Citation
Murrah buffalo(n=3) and heifers(n=3)	Functional CL and heifer with BCS 2.5 or more	Single PGF2α and natural insemination	100% within 1month in buffalo and within 2 months in heifer	[16]
Cross-bred cows (n=11)	No detectable reproductive tract infections	2 PGF2α doses 11 days apart and AI on estrus with mineral supplementation for 20days	45.45% within 3 months	[24]
Cross-bred cows(n=10)	No detectable tract infections	2 doses of PGF2α 11days apart and AI on detected estrus within a week	30% within 3months	[24]
Holstein dairy cows(n=10)	BCS 2.25-3, no detectable tract infections	Saline lavage, intrauterine infusion of Cepharin, 2 doses of PGF2α 13days apart and AI on detected heat	70% within 45days	[9]
Buffaloes(n=10)	Persistent corpus luteum, no detectable genital abnormalities	Mid-cycle PGF2α injection after palpation of CL on Day0 and 1st TAI 72hours and 2nd TAI 96 hours later	Overall 70% in 1st, 2nd and 3rd repeated estrus cycle	[19]

Table 2: Hormone PGF2α effect on conception rate

This means PGF2α responds more in repeat breeder which are in the luteal phase with normal CL after silent ovulation. Treatment with PGF2α along with mineral mixture improves the conception rate in cross-breed dairy cows irrespective of examination of CL and BCS [24]. Randomly cyclic cows on their late luteal stage (cycle day11 to 14) when subjected to the double regiment of 14-day prostaglandin protocol, maximum repeat breeder cows show positive response towards pregnancy [25]. Conception rate reached 70% with saline lavage, intrauterine infusion of cephapirin , then 2 doses of PGF2α 13 days apart and AI on detectable heat [9].

Prostaglandin is a naturally occurring hormone that causes regression of the corpus luteum (luteolysis) and decreases progesterone secretion which results in a return to estrus. Estrus can be expected to return within two to four/five days following injection of prostaglandin. Prostaglandin causes lysosomes within the granulosa cells to rupture, thus releasing their enzymes. These lysosomal enzymes cause further deterioration of tissue at the apex of follicle and cause the release of ova. Endometritis is one of the possible cause of repeat breeding in buffaloes [16]. PGF2α stimulates myometrial contraction that removes debris and microorganisms from the contaminated uterine lumen. As a result of its luteolytic action, PGF2α considered as treatment of choice for endometritis in cows with functional corpus luteum [9]. Protocols include PGF2α one-shot method and two-shot method as shown in **Figure 2 and Figure 3**. Animals should be on luteal stage of estrus cycle bearing CL for the treatment with prostaglandin.

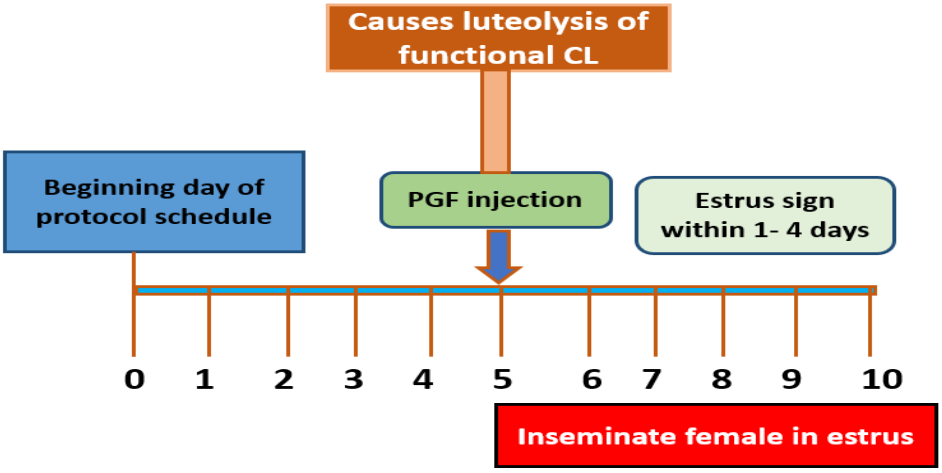

Figure 2: Protocol starting from day 0, prostaglandin hormone administration intramuscularly at day 5 and insemination of female on estrus.

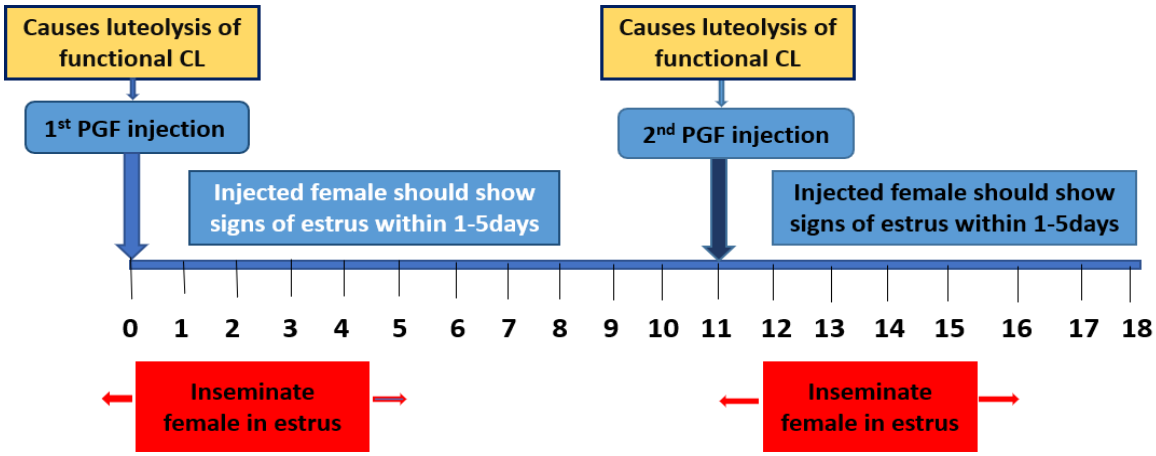

Figure 3: Prostaglandin hormone administration at day 0, animals showing estrus within in 1-4 days are inseminated and second shot prostaglandin administration at day 11 on those that do not came to estrus on first shot of prostaglandin.

3.3. Hormone GnRH and PGF2α administration

Ovsynch, GnRH and PGF2α protocol was first developed by [26] as shown in **Figure 4.** and found to be the most efficient TAI protocol for cows. The conception rate for the repeat breeder cattle having cystic ovary when treated with the combination of two reproductive hormones GnRH and PGF2α was improved [27]. For this therapy to be responsive, repeat breeders must come in cyclic heat. Following treatment with PGF2α, when AI is performed after observed signs of estrus, the conception rate is usually maximized [28]. Stage of the cycle should be taken into consideration when initial GnRH is to be administered [29]. Conception rate in lactating dairy cows is usually improved when the initiation of program done during mid diestrus i.e. days 5-12 of cycle. This is because there will be increase in ovulation of first wave (initiation on days 5-9) and number of cows with high progesterone at the moment of PGF2α. There is increased in pregnancy rate with administration of 2nd GnRH injection after 48 h of injection of PGF2α [30]. The reason for increasing pregnancy rate is due to prevention of delayed ovulation as well as anovulatory defects.

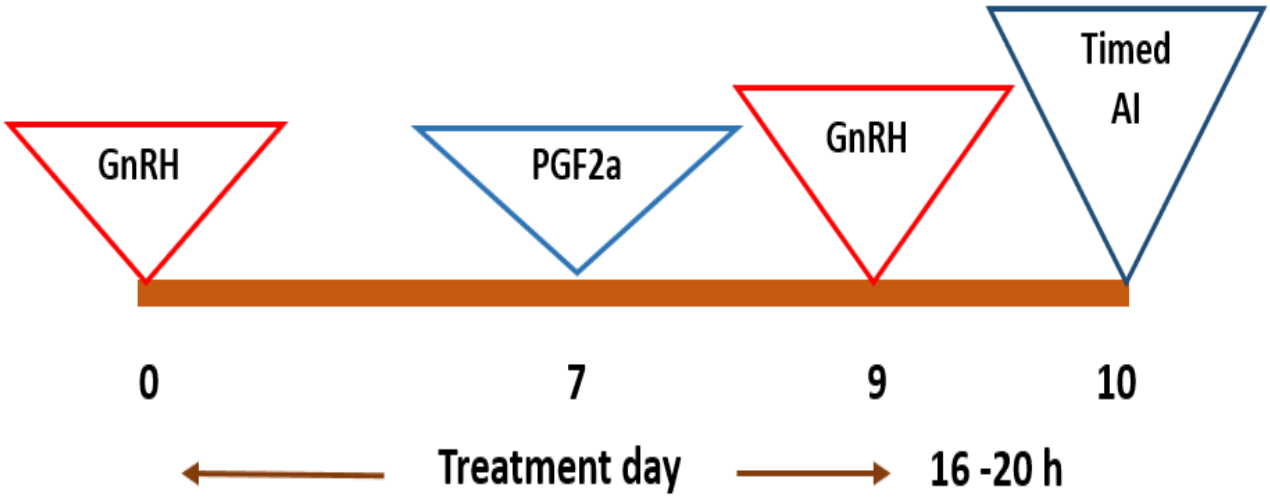

Figure 4: Ovysynch Protocol (Hormone GnRH administration at day 0, PGF2α hormone administration at day 7, another similar dose of GnRH administration intramuscularly at day 9 and timed artificial insemination 16-20 h later).

Fertility in primiparous Holstein cows was increased with double Ovsynch protocol resulting induction of ovulation in non-cyclic cows [31]. Conception rate varies with status of repeat breeder animal when subjected to Ovsynch protocol that is mentioned in **Table 3**. Administration of GnRH is associated with the release of FSH and LH, thus causing follicular development, ovulation and CL function. Administration of PGF2α is involved in the lysis of CL, hence reducing progesterone level and return to estrus cycle. Both ovulatory response and pregnancy rate in repeat breeder cows were found to be greater when GnRH dose increased (N & K, 2010). Treatment with 2nd GnRH injection prior to insemination is responsible for timely ovulation and increase in progesterone that necessarily supports the development of an embryo, hence resulting in better conception in repeat breeder buffaloes [19].

Repeat Breeder	Status of animal	Treatment protocol	Conception rate	Citation
Cows(n=20) out of which 6 has cystic ovaries	Normal anatomical condition of reproductive organ, 2nd or more parity, <10yrs age, >3 BCS	GnRH (d0) + PGF2α(d7) +GnRH (9) and FTAI 16-20hrs of 2nd GnRH	Overall conception rate 55% within 2 months and 66.7% in cystic ovaries cows	[27]
Jersey crossbred cattle (n=17)	No detectable reproductive problems, no cystic ovary	Deworming and mineral supplementation 20days prior to treatment, GnRH(d0) +PGF2α(d7) GnRH(d9) and TAI at 12hrs and 24hrs of 2nd GnRH	58.82% within 3months	[29]
Cross breed cows (n=13)	No detectable tract infections	Deworming, Mineral supplementation 20days prior to treatment, GnRH(d0), PGF2α(d7) GnRH(d9), FTAI 15hrs later	61.53% within 3months	[24]
Cross breed cows (n=12)	No detectable tract infections	Deworming and GnRH(d0), PGF2α(d7) GnRH(d9), FTAI 15hrs later	50% within 3months	[24]

Table 3: Ovsynch protocol and its effect in conception rate

3.4. Hormone Progesterone Administration

Regardless of the causative factor, treatment with a combination of progesterone based Ovsynch protocol and GnRH after AI was found to be effective for increasing the conception rate in repeat breeder cows [32]. Progesterone level in repeat breeder cows rises slower than the normal rise and has lower progesterone level [33]. Serum progesterone level in that of repeat breeder animal ranges from 1.44 ± 0.39 as compared to 3.66 ± 0.84 in that of normal animals [34]. An attempt to increase progesterone level after TAI by using exogenous progesterone in dairy cows has been increased [35]. Greater ovulatory response to second GnRH administration in progesterone based Ovsynch protocol (GnRH–progesterone– 7d–PGF2α–2d–GnRH–16h–AI–7d–GnRH) is due to high level of progesterone during PGF2α injection [32]. Exogenous progesterone administration resulted increased serum progesterone level during PGF2α injection that usually improves fertility of lactating dairy cows [36]. Exogenous administration of progesterone and its effect on conception rate of repeat breeder cattle is shown in **Table 4**.

Repeat breeder animal	Status of animal	Treatment protocol	Conception rate	Citation
Cows (n=175)	No abnormalities in tract	GnRH-Progestagen-7D-PGF2α-2dGnRH-16hr FTAI-7dGnRH	40.6% within 62 days	[32]
Holstein dairy cows (n=143)	1st-5th lactating cows producing >2 kg milk/day	Day0-AI-day5-PRID-day19-removal of PRID	36.36% within 40-47 days (effective conception rate in young 1st and 2nd late lactating cows)	[35]

Table 4: Hormone Progesterone effect on conception rate

Treatment with Controlled internal drug release device (CIDR) based Timed AI protocol as shown in **Figure 5,** improved conception rate up to 70% in true anestrous buffaloes [19]. This increase in pregnancy rate using CIDR-based TAI protocol is due to new follicular wave emergence, follicular growth, ovulation and increased conception rate in repeat breeder buffaloes. However, CIDR based TAI protocol (CIDR+GnRH-PGF2α-GnRH-FTAI) resulted lower pregnancy rate than the previous one [37]. This is because of presence of part of supra-basal P4 concentration during estrus and periovulatory periods. Embryonic mortality is another most common cause of repeat breeding and this is due to luteal insufficiency. Young 1st and 2nd parity late lactating Holstein dairy cows usually were benefited in terms of maintaining pregnancy rates after post-insemination Progesterone releasing intravaginal device (PRID) supplementation for 14 days with protocol (AI-day5-PRID-day19-removal of PRID) [35]. Continuous release of progesterone from CIDR and PRID helps to suppress ovulation and estrus and thus helps in maintaining pregnancy and reduce early embryonic death in repeat breeder cows. Early rise in serum P4 concentration due to exogenous progesterone supplementation is essential for the production of protein growth factors which is most for supporting the growth of embryo. Also P4 is associated with sufficient release of Interferon-gamma that blocks the release of endogenous PGF2α [33]. The commercially available hormones in Nepal are as follows: Gonadotrophins (Fertigyl, Buserelin, Recepetal, Cystorelin), Prostaglandin (Lutalyse, Estrumate, Prostamate,) and Progestins (PRID, CIDR).

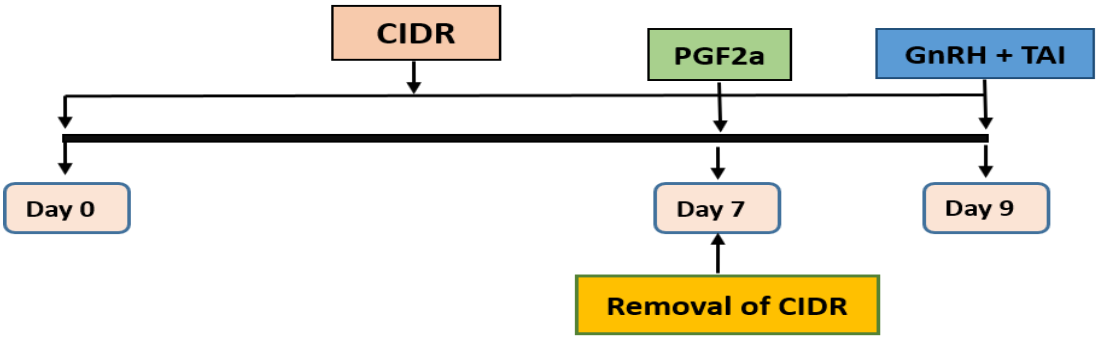

Figure 5: CIDR device placed intravaginally at day 0, removal of CIDR device at day 7, PGF2α hormone administration at day 7 and GnRH hormone administration along with Timed Artificial Insemination at day 9.

3. Other Remedial Measures to solve the problem

Washing the vagina of repeat breeder showing cervicitis with 1L of 1% Lugol's iodine solution. 100% success in pregnancy rate in repeat breeding buffaloes with this treatment approach within 6 months. Treating the repeat breeder heifer with oral administration of vitamin-mineral mixture (30 g/day) up to 3 weeks resulted in a 100% conception rate within 6 months [16]. Repeat breeders should be provided with deworming to reduce a load of internal parasite along with intrauterine infusion of nitrofurazone solution. Supplementation of sodium phosphate for 1 month with dose 40gram/head/day in the diet along with 500 ppm of zinc acetate in the drinking water to the repeat breeder buffaloes improved conception rate by 80% [38]. Feeding repeat breeders with Beta-carotene improved the conception rate by 33.3% as compared to 27.2 % in control group [39]. Other assisted reproductive techniques which have provided new therapeutic options for resolving this syndrome includes *in vitro* production or embryo transfer, intraperitoneal insemination [7]. Special care should be given to feed additives to control this syndrome.

4. Conclusion

On the basis of this study, it could be concluded that repeat breeding cattle and buffaloes respond quickly and excellently to hormone GnRH along with mineral and vitamin supplementation. Better conception rate could be achieved if repeat breeders are in luteal stage. Ovsynch protocol gives better conception in cystic ovaries repeat breeders. This protocol gives better conception rate when initiation of program done at 5-9 days of estrus cycle. Hormone progesterone administration could be a treatment of choice for endometritis repeat breeders and to minimize the early and latent embryonic mortality in repeat breeders. Hence different treatment strategies as studied in this review article could be adopted as guideline to improve conception rate in repeat breeding cattle and buffaloes in Nepal. However, the first step of treatment involves good care, nutrition, better oestrus detection, timely insemination and management of mating with bulls.

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Conflict of interest

The authors have no conflict of interest.

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