Efficacies of $PGF_{2\alpha}$, Ovsynch, and CIDR Protocols on Synchronization of Estrus in Buffaloes: A Comparative Study

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Abstract: The present study aimed to compare the efficacies of $PGF_{2\alpha}$, GnRH, and CIDR on estrus synchronization of native buffaloes in Bangladesh. A total of 93 buffaloes of second to fifth parity were treated either with $PGF_{2\alpha}$, Ovsynch, or CIDR protocol. In the $PGF_{2\alpha}$ experiment, buffaloes were treated with either 500 µg (n=20) or 875 µg (n=20) of cloprostenol, and artificial inseminations (AI) were done at 72 hrs after $PGF_{2\alpha}$ injection. In the Ovsynch protocol, buffaloes were treated with 200 µg (n=11) or 350 µg (n=16) of GnRH at day 0, followed by 875 µg of $PGF_{2\alpha}$ at day 7, and again 200 µg or 350 µg of GnRH at day 9, and AI was performed at 16 hrs after the second GnRH administration. In the CIDR protocol, a CIDR implant was placed intravaginally for 12 days with either 500 µg (n=13) or 875 µg (n=13) of cloprostenol at 24 hrs before the removal of the CIDR, and AI was performed at 72 hrs after the removal of the CIDR. The results showed that estrus response and conception rates did not differ significantly between $PGF_{2\alpha}$ and GnRH protocols. Higher doses of $PGF_{2\alpha}$ and GnRH did not result in any significant increase in estrus response in buffaloes. CIDR induced estrus in all buffaloes in both doses of $PGF_{2\alpha}$. Estrus rate was significantly higher (P=0.035) in buffaloes of the CIDR protocol than in the $PGF_{2\alpha}$ and Ovsynch groups. Conception rates of buffaloes did not differ significantly (P=0.823) among the protocols. The calving rates were higher (P=0.278) in buffaloes synchronized with CIDR than in $PGF_{2\alpha}$ and Ovsynch groups. The costs of materials per buffalo synchronized, conceived, and calved were higher in the CIDR protocol and lower in the $PGF_{2\alpha}$ than in other protocols. However, considering all the expenses and calving rates, costs per buffalo calves were cheaper in the CIDR group with a higher dose of $PGF_{2\alpha}$. In conclusion, CIDR can be applied to increase the reproductive efficiency of buffaloes.

Keywords: Calving, CIDR, conception, estrus synchronization, GnRH, $PGF_{2\alpha}$.

INTRODUCTION

The buffalo (*Bubalus bubalis*) is an important livestock resource in many countries of the world, particularly in Asia, Latin America, and the Mediterranean region. They are important components of livestock agriculture in developing countries for producing draft power, milk, meat, and hides. High feed conversion efficiency, high butter fat content in milk, low maintenance requirement, high draught output, and high disease resistance make buffaloes popular [1]. The productivity of female buffalo is affected by poor reproductive efficiency, such as late maturity, poor expression of the estrus signs, irregular estrus cycle, silent heat, seasonality in breeding, poor conception rate/early embryonic mortality, prolonged inter-calving interval, and reduced ovarian activity during the hot season [2-4]. In addition, due to poor estrus detection, a variable duration of estrus, and the difficulty encountered in predicting the time of ovulation, success in artificial insemination (AI) in buffaloes is also low [5]. High reproductive efficiency is important for achieving the maximum economic benefits from buffalo farming. Assisted reproductive technologies (ARTs) should be applied to improve the reproductive efficiency in buffaloes.

Estrus synchronization, an important tool of ARTs, is usually applied to optimize the time of estrus and to improve the reproductive efficiency in buffaloes. It is particularly beneficial while estrus detection is difficult due to silent heat symptoms in buffaloes [6]. Synchronization of estrus helps in fixing the breeding time within a short, predefined period and thereby scheduling the parturition time at the most favorable season, when the newborns can be reared in a suitable environment with ample feed for enhancing their survivability. Estrus synchronization provides more economic returns by improving the production efficiency in animals [6]. The current and future direction of estrus synchronization is to focus on combining traditional methods of controlling cycle length with the manipulation of follicular development. Regulation of corpus luteum (CL) function and follicular

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development is essential for estrus synchronization more precisely and to control the time of ovulation more exactly to allow timed insemination without the need for detection of behavioral estrus [6].

Numerous protocols to synchronize estrus in buffaloes have been described in recent years. Estrus synchronization protocols differ in hormones used, method of hormone administration, number of injections, timing of injections, and heat detection requirements. Three basic approaches are being used for synchronization of estrus in domestic animals: pharmacological methods using prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), gonadotropin-releasing hormone (GnRH), and controlled internal drug release (CIDR). $PGF_{2\alpha}$ is used to bring females into heat and shorten the estrous cycle, whereas GnRH induces ovulation or starts the development of a new follicular wave, and CIDR embedded with progesterone is applied to keep animals out of heat and extend the estrous cycle [7].

Prostaglandins are widely used to synchronize estrus in different species. Naturally, it is produced in the uterus. There are several prostaglandin' analogues (cloprostenol, luprostiol, dinoprost, etc.) that are utilized in cattle and buffalo [8]. The efficacy of these analogues depends on their luteolytic action on the CL, which causes a rapid decline in progesterone level and finally results in ovulation [8]. $PGF_{2\alpha}$ is widely used for synchronization of estrus in buffaloes [9]. It regresses the CL and ultimately decreases the levels of progesterone, leading to the development of follicles [10]. It has been reported that when $PGF_{2\alpha}$ is administered in the presence of functional CL, about 60-70% of treated animals showed estrus [11].

There are two applicable methods developed for estrus synchronization using $PGF_{2\alpha}$ or its analogues [12]. The first $PGF_{2\alpha}$ treatment is done after detecting the cyclicity of the cows by rectal examination of the CL in the ovaries. Then the animals are inseminated as they show signs of estrus during 5 days. In the second method, all animals are given two injections of $PGF_{2\alpha}$ 11 days apart without considering the estrous cycle [13, 14]. It has been reported that after each injection of $PGF_{2\alpha}$ preparation, insemination of the animal can be done at fixed times at 72 hrs after the last injection [15, 16].

GnRH is a hormone responsible for the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary. GnRH is a tropic peptide hormone synthesized and released from GnRH neurons within the hypothalamus. The GnRH and $PGF_{2\alpha}$ method of estrus synchronization has proven to be successful in synchronizing estrus in cattle and buffaloes [17-19]. GnRH and its analogues, such as gonadorelin, buserelin, or lecirelin, are widely used to manipulate patterns of ovarian follicle development. It increases LH, FSH, and estradiol surges at any stage of the estrous cycle, which promotes the ovulation of a dominant follicle or the luteinization and/or atresia of pre-dominant follicles. Consequently, a new follicular wave emerges two to three days later [20].

Several studies have been carried out with $PGF_{2\alpha}$ alone or in combination with GnRH for synchronizing estrus successfully in domestic animals [21-23]. The administration of GnRH after $PGF_{2\alpha}$ injection increases the rate of synchronized ovulation in bovines [20]. They were the first to demonstrate that the administration of GnRH, followed by the administration of $PGF_{2\alpha}$ 7 days later, results in more precise control of ovarian follicle growth and estrus, and ovulation is assured by a second injection of GnRH 2 days after the $PGF_{2\alpha}$. This has led to a wider use of GnRH in estrus synchronization protocols in cattle, and such a protocol is popularly known as the Ovsynch protocol. This ovulation synchronization protocol employs the intramuscular administration of GnRH, $PGF_{2\alpha}$, and GnRH on days 0, 7, and 9 [20].

Progesterone is a steroid hormone naturally produced by the CL of mammalian ovaries. *In vivo*, progesterone is essential to maintain pregnancy. Progesterone provides a potent suppression of estrus, making it important for estrus synchronization in herds of animals [24]. It inhibits myometrial activity and improves nutrient utilization. Under the influence of progesterone, normal pituitary gonadotrophin output is inhibited, and the ovarian cycle is interrupted [24]. The utilization of progesterone or progestogens can be carried out by different routes of administration, such as intravaginal, auricular, or injectable. These protocols are particularly utilized in buffaloes out of the breeding season, since it has been largely demonstrated that progesterone is able to act on the hypothalamus-pituitary-ovary axis, leading to resumption of ovarian cyclicity in anestrus animals [25,26]. The CIDR is designed as a T-shaped nylon spine molded with a silicone rubber skin, which contains progesterone and is used in the dairy cow, buffalo, sheep, and goat [27]. CIDRs containing 9-12% progesterone are widely used for synchronizing estrus in domestic animals [28]. The progesterone is released at a controlled rate into the bloodstream after insertion. When CIDR is removed from the vagina of the animal, a rapid drop in concentration of systemic progesterone occurs in animals; thus, the estrus is synchronized in the herd.

To date, the most suitable protocol for water buffaloes remains unclear. Usually, the protocol developed for cattle is being used for synchronization of buffaloes. Thus, the success rate of estrus synchronization is lower in buffaloes than in cattle. Our previous report revealed that a single dose of $PGF_{2\alpha}$ induced estrus as well as conception more efficiently than its double dose protocol in buffaloes [30]. In another study, we showed that estrus response and conception rates did not differ significantly between PGF2α and GnRH protocols [31]. Here, estrus was synchronized in native buffaloes using $PGF_{2\alpha}$, GnRH, and CIDR to evaluate their comparative efficacy.

MATERIALS AND METHODS

Animal Selection and Management

A total of 93 female native buffaloes (*Bubalus bubalis*) of second to fifth parity with the body condition score from three to five were randomly selected for estrus synchronization during the autumn season. The animals were dewormed at regular intervals and vaccinated against common infectious diseases. The buffaloes were offered diets consisting of commonly available feedstuffs to fulfill their nutrient requirements as described previously [29]. All the experimental procedures were approved and supervised by the ethical standards of the research committee of the Bangladesh Agricultural University Research System (BAURES), Bangladesh Agricultural University, Mymensingh, Bangladesh (Reference number: BAURES/ESRC/121/AH/225).

Three different protocols, PGF2α, Ovsynch, and CIDR,of estrus synchronization were used for this comparative study. Briefly, in the $PGF_{2\alpha}$ group, estrus was synchronized by treating either 2 ml (500 µg cloprostenol) or 3.5 ml (875 µg cloprostenol) of Ovoprost (Renata PLC, Dhaka, Bangladesh). Before administering $PGF_{2\alpha,}$ the presence of CL was examined through rectal palpation. Buffaloes with palpable CL were selected for $PGF_{2\alpha}$ treatment (Figure **1**).

In Ovsynch protocol, estrus was synchronized by administering either 2 ml (200 µg gonadorelin) or 3.5 ml (350 µg gonadorelin) of GnRH (Ovorulin, Renata PLC) without considering the stage of the estrous cycle (day of GnRH treatment, day 0), followed by 3.5 ml (875 µg cloprostenol) of $PGF_{2\alpha}$ (Ovoprost) on day 7, and a second GnRH treatment (same dose) 48 hrs after $PGF_{2\alpha}$ (Figure **2**).

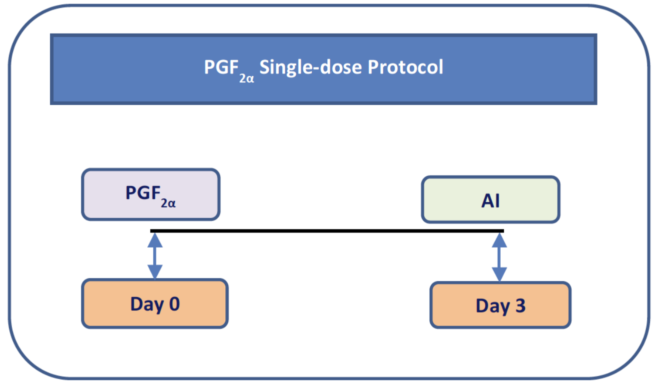

Figure 1: Synchronization protocol of estrus by $PGF_{2\alpha}$. Synchronization was initiated by injection of $PGF_{2\alpha}$ analogue (Day 0), and AI was performed at 72 hrs after $PGF_{2\alpha}$ administration.

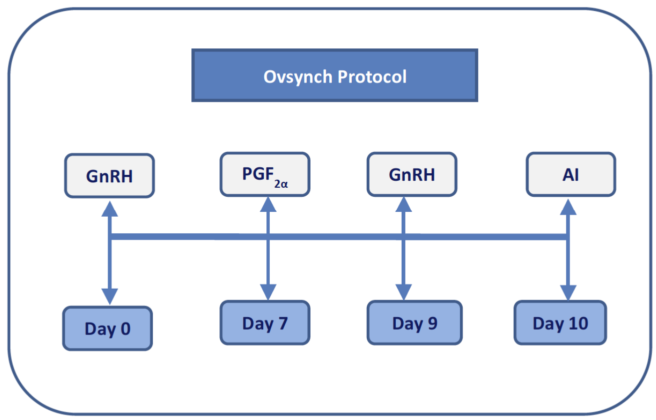

Figure 2: Synchronization protocol of estrus by Ovsynch. An initial dose of GnRH was administered on Day 0. The $PGF_{2\alpha}$ injection was given 7 days after the GnRH injection. The second GnRH injection was given 2 days after (Day 9) $PGF_{2\alpha}$. AI was performed at 16 hrs after the second GnRH injection.

In the CIDR protocol, the CIDR implant (EAZI-BREED CIDR; Zoetis Inc., New Jersey, USA) containing progesterone was placed intravaginally for 12 days, irrespective of the estrous cycle stage of the animal (Day of CIDR device introduced count day 0). Estrus was synchronized by injecting either 2 ml (500 µg cloprostenol) or 3.5 ml (875 µg cloprostenol) of Ovoprost ($PGF_{2\alpha}$) at 24 hrs before the removal of the CIDR (Figure **3**). All the injections were administered intramuscularly on the back side of the animals, and the injection sites were thoroughly cleaned with an alcohol solution before administration.

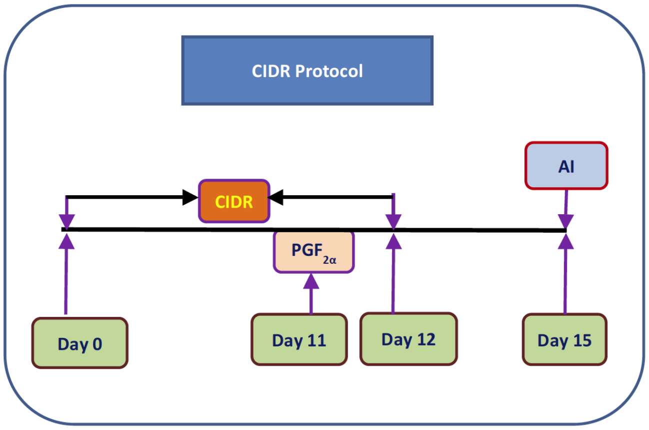

Figure 3: A CIDR implant containing progesterone was placed intravaginally for 12 days. PGF2α was administered (Day 11) at 24 hrs before the removal of the CIDR, and AI was performed at 72 hrs after the removal of the CIDR.

Estrus Detection, AI, and Pregnancy Diagnosis

Frequent urination, mucus discharge, and swollen vulva are the symptoms of estrus in buffaloes. Cervical mucus was aspirated from the cervix through the recto-vaginal method using a blue sterile sheath and a universal AI gun to detect estrus as we described previously [30]. AI was performed at 72 hrs after $PGF_{2\alpha}$ injection in the $PGF_{2\alpha}$ protocol, at 16 hrs from the second gonadorelin treatment in the Ovsynch protocol, and at 72 hrs from the removal of the CIDR implant in the CIDR protocol. Pregnancy diagnosis was carried out 60 days after AI by transrectal palpation.

Statistical Analyses

Data were presented as mean ± standard error of mean (SEM). Those were analyzed using one-way ANOVA followed by Duncan's multiple range test (IBM SPSS Statistics, version 22). Differences at $P< 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Estrus Response

A major limiting factor for optimum reproductive performance in buffalo farms is the failure to detect estrus in a timely and accurate manner. Several controlled breeding programs have been developed for synchronizing estrus in cows. Previously, we investigated the effect of single and double doses of $PGF_{2\alpha}$ on synchronization of estrus in buffaloes [30]. It was found that 875 µg of cloprostenol significantly increased the estrus rate in buffaloes compared to double doses of 500 and 875 µg of cloprostenol. In another study, we compared the effectiveness of $PGF_{2\alpha}$ and GnRH-induced estrus synchronization in buffaloes [31]. The results showed that estrus response and conception rates did not differ significantly between $PGF_{2\alpha}$ and GnRH protocols. In the present study, estrus was synchronized with three different protocols, i.e., $PGF_{2\alpha}$, Ovsynch, and CIDR, in water buffaloes. In the $PGF_{2\alpha}$ protocol, buffaloes were treated with either 500 or 875 µg of cloprostenol for estrus synchronization. In the Ovsynch protocol, the buffaloes were treated with either 200 µg or 350 µg gonadorelin for estrus synchronization with 875 µg of cloprostenol. In the CIDR protocol, buffaloes were treated with either 500 or 875 µg of cloprostenol for estrus synchronization. Efficacies in terms of estrus response, conception, and calving rates of these three protocols were examined. Here, CIDR induced estrus in all buffaloes in both doses of $PGF_{2\alpha}$. Estrus rate was

Table 1: Estrus Rates of Buffaloes Synchronized using $PGF_{2\alpha}$, Ovsynch, and CIDR

Protocols	Treatments	Number of buffaloes examined	Estrusrates(%; Mean ± SEM; n=4)
$PGF_{2\alpha}$	500 µg Cloprostenol 875 µg Cloprostenol	20 20	$15(75.0\pm3.9^{b})$ $17(85.0\pm0.8^{ab})$
Ovsynch	200 µg Gonadorelin + 875 µg Cloprostenol	11	$8(75.0\pm14.4^{b})$
	350 µg Gonadorelin + 875 µg Cloprostenol	16	$13(82.2\pm9.6^{ab})$
CIDR	CIDR Device + 500 µg Cloprostenol	13	$13(100.0\pm0^{a})$
	CIDR Device + 875 µg Cloprostenol	13	$13(100.0\pm0^{a})$

Means with uncommon superscripts in the same row differ significantly ($P< 0.05$).

significantly higher (P=0.035) in the CIDR protocol than in the $PGF_{2α}$ and Ovsynch protocols when a lower dose of $PGF_{2α}$ and GnRH was injected (Table **1**). Higher doses of $PGF_{2α}$ and GnRH did not result in any significant increase in estrus response in buffaloes. These rates of estrus responses in buffaloes were comparable to our previous reports [30,31]. A similar $PGF_{2α}$-induced estrus response in water buffalo was also reported by Atabay *et al*. [32]. It has been reported that exogenous hormone administration stimulates ovarian activity that ultimately increases the estrus expression of buffaloes [33].

Conception and Calving Rates

The conception rates of buffaloes treated with different estrus synchronization protocols are described in Table **2**. The conception rates of buffaloes did not differ significantly (P=0.823) among the protocols, i.e., $PGF_{2α}$, Ovsynch, and CIDR. The higher dose of $PGF_{2α}$ and GnRH did not increase conception rates in buffaloes. Several factors are known to influence the fertility of livestock, including health conditions of animals, synchronization protocols, and overall reproductive management [34]. The fertility of ruminants is affected by their response to estrus synchronization and AI treatments [35]. Livestock fertility is also affected by genetics, animal age, parity, health conditions, and feed management [36]. The hormone used for estrus synchronization can help the formation of the CL and increase the concentration of progesterone, thus helping embryo implantation and placental development, supporting embryo survival to increase the pregnancy rate of buffaloes.

Importantly, the calving rates were higher (P=0.278) in buffaloes synchronized with CIDR than $PGF_{2α}$ and Ovsynch groups (Table **3**). The reasons behind this

Table 2: Conception Rates of Buffaloes Synchronized using $PGF_{2α}$, Ovsynch, and CIDR

Protocols	Treatments	Number of buffaloes examined	Conception rates (%; Mean ± SEM; n=4)
$PGF_{2α}$	500 µg Cloprostenol 875 µg Cloprostenol	20 20	9(45 ± 6.91) 11(55 ± 6.91)
Ovsynch	200 µg Gonadorelin + 875 µg Cloprostenol	11	4(38.88 ±2 0.03)
	350 µg Gonadorelin + 875 µg Cloprostenol	16	7(42.22 ± 13.51)
CIDR	CIDR Device + 500 µg Cloprostenol	13	5(35.83 ± 13.14)
	CIDR Device + 875 µg Cloprostenol	13	7(52.50±7.25)

Table 3: Calving Rates of Buffaloes Synchronized using $PGF_{2α}$, Ovsynch, and CIDR

Protocols	Treatments	Number of buffaloes examined	Calving rates(%; Mean ± SEM; n=4)
$PGF_{2α}$	500 µg Cloprostenol 875 µg Cloprostenol	20 20	2(10 ± 5.20) 3(15 ± 0.79)
Ovsynch	200 µg Gonadorelin + 875 µg Cloprostenol	11	2(19.44 ± 10.01)
	350 µg Gonadorelin + 875 µg Cloprostenol	16	3(18.88 ± 11.60)
CIDR	CIDR Device + 500 µg Cloprostenol	13	4(27.50± 16.00)
	CIDR Device + 875 µg Cloprostenol	13	6(44.16±6.58)

Table 4: Cost Analysis of Estrus Synchronization using $PGF_{2\alpha}$, Ovsynch, and CIDR Protocols

Protocols	Treatments	Number of buffaloes examined	Cost (BDT) per buffalo								
			In estrus			Conceived			Calf		
			Hormone	Technician	Total	Hormone	Technician	Total	Hormone	Technician	Total
$PGF_{2\alpha}$	500 µg Cloprostenol	20	187	133	320	311	1055	1366	1400	4750	6150
	875 µg Cloprostenol	20	288	118	406	445	955	1400	1633	3500	5133
Ovsynch	200 µg Gonadorelin + 875 µg Cloprostenol	11	722	412	1134	1444	1825	3269	2887	3650	6537
	350 µg Gonadorelin + 875 µg Cloprostenol	16	905	369	1274	825	1614	2439	3920	3767	7687
CIDR	CIDR Device + 500 µg Cloprostenol	13	1187	300	1487	3960	2080	6040	3858	2600	6458
	CIDR Device + 875 µg Cloprostenol	13	1288	300	1588	2920	1486	4406	2791	1733	4524

higher rate of calving in CIDR-induced buffaloes might be due to a lower rate of pregnancy loss in CIDR-implanted buffaloes compared to other protocols. CIDR releases progesterone into the bloodstream of animals after insertion. It has been reported that supplementation of progesterone during the development of the ovulatory follicle reduces the risk of pregnancy loss [37]. Withdrawal of the intravaginal progesterone-releasing device caused a sudden fall in progesterone levels and induced the emergence of a new follicular wave [38, 39]. The CIDR-$PGF_{2\alpha}$ protocol will provide new insights to address reproductive disorders in buffaloes, including anestrus, silent heat, and low conception rates.

Several reports revealed the successful regulation of estrus of domestic animals using different protocols. A limited number of reports demonstrated the cost involvement of protocols of estrus synchronization. It has been reported that the costs of $PGF_{2\alpha}$ and Ovsynch protocols were almost similar [40]. Their results showed that costs per pregnancy were euro 235.43 (BDT 33,454.6) and euro 235.08 (BDT 33,404.8) for the Ovsynch and the PGF group, respectively, which are significantly higher than the expenses per buffalo in the present study. Here, the costs of the materials (hormones) and technicians involved in hormone or CIDR administration, as well as in AI, were compared among $PGF_{2\alpha}$, Ovsynch, and CIDR (Table **4**). The costs of materials per buffalo synchronized, conceived, and calved were higher in the CIDR protocol and lower in the $PGF_{2\alpha}$ than in others. However, considering all the expenses and calving rates, costs per buffalo calves were cheaper in the CIDR group with a higher dose of $PGF_{2\alpha}$.

CONCLUSION

The results demonstrated that CIDR induced estrus and calving rates more efficiently than prostaglandin and Ovsynch protocols in buffaloes. CIDR can be applied to increase the reproductive efficiency of buffaloes.

CONFLICT OF INTEREST

The authors declare that no conflict of interest could be perceived as prejudicing the impartiality of the research submitted.

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LIST OF ABBREVIATIONS

ANOVA = Analysis of Variance

ARTs = Assisted reproductive technologies

CIDR = Controlled Internal Drug Release

CL = Corpus Luteum

GnRH = Gonadotropin Releasing Hormone

IBM = International Business Machines

$PGF_{2\alpha}$ = Prostaglandin $F_{2\alpha}$

SPSS = Statistical Package for Social Sciences

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