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The Efficacy of Three Different Estrus Synchronization Protocols on Reproductive Performance in Chinese *Hu* Sheep

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ABSTRACT

Background: The estrous synchronization has become a worldwide available solution for modern management and large-scale cultivation of sheep. The objective of the present study was to compare the efficacy of three estrus synchronization protocols, CIDR9+$PGF_{2\alpha}$+eCG; CIDR14+eCG and GnRH+$PGF_{2\alpha}$, in *Hu* sheep.

Methods: This study was conducted on 80 multiparous ewes, which were assigned into four groups: CIDR9+$PGF_{2\alpha}$+eCG; CIDR14+eCG; GnRH+$PGF_{2\alpha}$ and control. Behavioral estrus was checked with teaser rams. Percentage of ewes behaving estrus at different time (0-24 h, 24-48 h, 48-72 h, respectively) was recorded. Pregnancy diagnosis was implemented on day 28 and confirmed on day 60 after insemination.

Result: The results suggested that the estrus rate, pregnancy rate, conception rate, lambing rate and prolificacy in three treatment groups have no significant difference compared with control group. Most notably, CIDR9+$PGF_{2\alpha}$+eCG and CIDR14+eCG have significantly shorter duration of pregnancy than control. While CIDR14+eCG and GnRH+$PGF_{2\alpha}$ did not dramatically change the fecundity of ewes, still CIDR9+$PGF_{2\alpha}$+eCG significantly decreased the fecundity of that compared with the control. Estrus responses in CIDR9+$PGF_{2\alpha}$+eCG, CIDR14+eCG and GnRH+$PGF_{2\alpha}$ groups rose gradually to attain their significantly higher percentages (56.3%, 52.9% and 66.7%, respectively) during 24-48 h post-estrous detection, afterwards, they fell down.

Key words: CIDR, GnRH, Reproductive performance, *Synchronous estrus*.

INTRODUCTION

Sheep is one of the most vital components of agribusiness economy of China. The superiority of *Hu* sheep, a non-seasonal breeder raised in Jiangsu and Zhejiang Province of China, is that its onset of estrus and gestation event works out in all seasons and multiparous trait (Yue, 1996), which makes it a desirable alternative for increasing reproductive performance. Significant improvement in reproductive performance can be accomplished via application of exogenous hormones including gonadotropins, progestogens and luteolytic agents to induce secretion of endogenous hormones (Oliveira *et al.*, 2001; Abecia *et al.*, 2012; Menchaca *et al.*, 2018). The critical theory of such methods is to manipulate luteolysis and the corpus luteum lifespan (Titi *et al.* 2010). The purpose of application of estrous synchronization protocols is to control the reproductive system, which has become a worldwide available solution for modern management and large-scale cultivation of sheep (Boscos *et al.*, 2002). Additionally, compared with natural mating, artificial insemination could rise the numbers of descendant per ram and allow a spatial and temporal (in term of frozen-thawed semen) separation between collection of spermatozoa and fertilization, as well as saving time (Leboeuf *et al.*, 2000; De *et al.*, 2015). The application of CIDR dispenser and/or injection of exogenous hormones, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), equine chorionic gonadotropin (eCG), was considered as a section of the protocol (Nogueira *et al.*, 2011; Kulaksiz *et al.*, 2013). Another protocol for controlling the estrus cycle is based on the lysis of the corpus luteum using $PGF_{2\alpha}$ (Fierro *et al.* 2013). $PGF_{2\alpha}$ treatments have the advantage of being applied by intramuscular injection and be rapidly and almost completely (99%) metabolized by the liver, which was always combined with GnRH agonist (Hashem *et al.* 2015). It has been reported that the implement of intravaginal devices may induce several problems, such as the production of chemical residues and vaginitis that can threaten to public health (Alavez *et al.* 2016).

Applicable synchronization protocol could significantly change the onset and interval and duration of estrus as well as the rates of pregnancy and lambing, thus increasing productivity and fertility of livestock (Kulaksiz *et al.* 2013). It has been reported that CIDR-based estrous synchronization protocols in goats have included short (5-9 days) (Oliveira *et al.* 2001; Nogueira *et al.* 2011; Inya *et al.* 2013) and long duration (12-14 days) (Motlomelo *et al.*, 2002; Romano,

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2004; Abecia *et al*., 2012) with the combination applications of $PGF_{2\alpha}$ and eCG at or prior to insertion removal. Promising results were obtained by application of progestogen for 9 days (Nogueira *et al.* 2011), 8 (Greyling *et al.* 1979), 7 (Fitzgerald *et al.* 1985) or 5 (Beck *et al.* 1993) in combination with $PGF_{2\alpha}$, or the analogue cloprostenol, at withdrawal time. Martinez *et al.* (2018) observed that the ewes received the long-term CIDR-based treatment for 14 days could lead to higher fertility yields (70%) compared with that of long-term sponge-based treatment for 14 days (45%). Ataman *et al.* (2006) found that ewes received intramuscular injection of GnRH analogue and treatment of $PGF_{2\alpha}$ analogue 5 day later were proved to be more effective in the synchronization of the estrus than those received given twice $PGF_{2\alpha}$ at 9 day interval, intramuscular injection of $PGF_{2\alpha}$. However, the comparisons in the efficacy of CIDR-long treatment, CIDR-short treatment and GnRH-$PGF_{2\alpha}$ treatment to synchronize estrus have yet not been reported in sheep. Different species of female livestock has her own estrous cycle and proper mode for estrus. Hence, the objective of the present study was to compare the efficacy of three estrus synchronization protocols, CIDR9+$PGF_{2\alpha}$+eCG; CIDR14+ eCG and GnRH+$PGF_{2\alpha}$, in *Hu* sheep.

MATERIALS AND METHODS

Eighty fertile and multiparous *Hu* sheep, ageing 2.5±0.30 years, with a mean body condition score of 3 (scale ranging from 1=emaciated to 5=obese) described by Russel *et al.* (1969), were conducted in this study during November 2017 through May 2018. Ewes were placed in yards under a roof in a ventilated barn at the commercial farm of Jiangsu Qianbao animal husbandry Co., LTD located in Yancheng of Jiangsu Province of China. During the period of the experiment, the diets of animals was based on chopped green maize, bean pulp, rapeseed meal and bran as roughages and concentrate supplements at a daily level of 500 g/ewes (15.05% crude protein) to meet their daily energy and protein requirements and had free access to clean water and mineral salt. The experiments on the administration and handling of sheep were assessed and approved by the Animal Ethics Committee of Experimental Animal of Yangzhou University (Yangzhou, China; Code No. AW 20171015).

Synchronization protocols

According to the protocols of estrous synchronization, ewes were randomly allocated into four groups (n=20 per group). Briefly, CIDR9+$PGF_{2\alpha}$+eCG-group ewes were received short-treated CIDR constructed with silicone elastomer impregnated with 0.4 g natural P4 for 9 days period combination with intramuscular first injection of 30 μg dose of $PGF_{2\alpha}$ on 8 day followed with the second injection of 350 IU dose of eCG at CIDR withdrawal time on 9 day. CIDR14+eCG-group ewes were treated with long-treated CIDR for 14 days and injection of 350 IU dose of eCG at CIDR withdrawal. GnRH+$PGF_{2\alpha}$ group ewes were conducted by intramuscular administration of 6-μg GnRH and injection of 30 μg $PGF_{2\alpha}$ on 8 day. The fourth group was control group received no further treatment. CIDR or GnRH treatment applied in treated-group was defined as day 0, the experimental design is shown in Fig 1. Before experiment, normal estrus cycle of the ewes had been confirmed twice and they had not been fertilized in order to ensure the animals employed in our experiment didn't have ovarian disease.

Detection of estrus and artificial insemination

Estrus activity was detected using teaser rams (one ram per approximately 15 ewes) equipped with harnesses beginning with 12 h after CIDR removal or injection of exogenous hormone and checked every 12 h, twice a day until the end of the signs of estrus. Expressing a sign of standing estrus ewes was recorded.

$$\text{Estrus rate} = \frac{\text{The number of ewes showed estrus responses}}{\text{The number of synchronized ewes}} \times 100$$

$$\text{Percentage of ewes behaving estrus at different time} = \frac{\text{The number of ewes showed estrus responses within the same time estrus detection period}}{\text{The number of synchronized ewes}} \times 100$$

0-24 h, 24-48 h, 48-72 h, respectively) in each treatment group was recorded.

Three proven healthy and fertile rams were chosen for ejaculated sperm collection by artificial vagina. Obtained semen was immediately evaluated macroscopically and

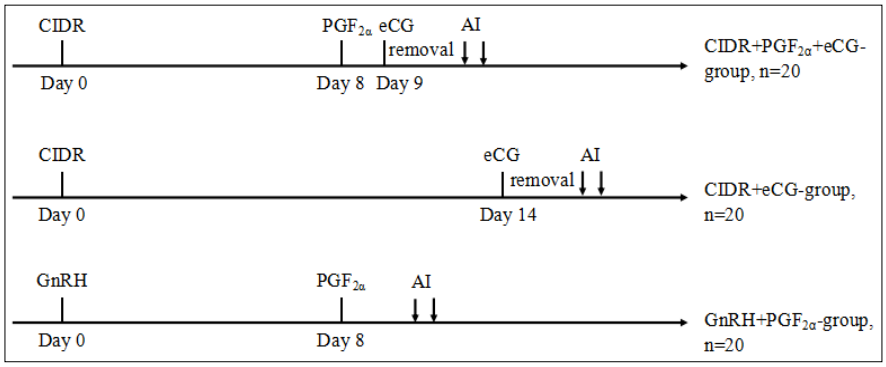

Fig 1: Schematic frame work of the protocols for estrus resynchronization in *Hu* sheep implemented in experiment. Day 0 = Day of the administration of CIDR in the two former groups and the injection of GnRH in the third group (CIDR = Controlled internal drug release devices, $PGF_{2\alpha}$= Prostaglandin $F_{2\alpha}$, eCG= Equine chorionic gonadotropin, AI= Artificial insemination).

microscopically. A total of 6 fields of view were quantified per chamber by electron microscope (OLYMPUS, TOKYO 163-0914, JAPAN). Approximately 500 sperms were analyzed and done in duplicate. The motility of sperm (forward progressive sperm / the total analyzed sperm) is up to 0.8, the smell is slightly fishy and the color and luster looks like milk white, which could be used for artificial insemination (AI). The semen was diluted for 200×10^6 live normal motile sperm per 0.1 mL with commercial extender (Eigelbfreies Verdunnerkonzentratfur Bullensamen androMed, Minitube, Germany). Diluted semen was stored at 4°C and used within 3 days. At the time of AI, Sperm motility was reanalyzed and confirmed to be up to 0.8. Ewes were submitted to laparoscopic AI with 0.3 mL diluted semen.

Pregnancy diagnosis

Pregnancy diagnosis was carried out on day 28 after insemination and confirmed on day 60 through transrectal B-mode real ultrasonography using a dual-frequency (3.5/5.0 MHz) convex abdominal probe (NL219M/DFLM36 Tinga Vet 50 s, Yum medical in Italy). The purpose of such regular detections performed was to identify the number of fetuses and to confirm the amount of lambing.

$$\text{Pregnancy rate} = \frac{\text{Number of pregnant ewes}}{\text{Number of synchronized ewes}} \times 100$$

$$\text{Conception rate} = \frac{\text{Number of pregnant ewes}}{\text{Inseminated ewes}} \times 100$$

Duration of pregnancy = Time between insemination to delivery of ewes, days)

$$\text{Lambing rate} = \frac{\text{Lambed ewes}}{\text{Inseminated ewes}} \times 100$$

$$\text{Fecundity} = \frac{\text{Newborn lambs}}{\text{Inseminated ewes}} \times 100$$

$$\text{Prolificacy} = \frac{\text{Newborn lambs}}{\text{Pregnant ewes}} \times 100$$

Statistical analysis

Estrus detection rate, pregnancy rate, conception rate, twining rate, lambing rate, fecundity and prolificacy were analyzed by Chi-square. The other statistics were evaluated by ANOVA as repeated-measures with the Tukey HSD multiple comparison test in all measured parameters, using SPSS 22.0. A probability of $P<0.05$ was regarded as significant difference. Date represented in duration of pregnancy and hormones levels except percentages are expressed as the mean±standard error.

RESULTS AND DISCUSSION

Detection of reproductive performance

Ewes failed to keep CIDR devices in the vagina were eliminated from our experiment (n=1 both in CIDR-based protocol groups). Statistics shown in Table 1 illustrate that the estrus rate (84.21%, 89.47%, 90%, respectively), pregnancy rate (47.37%, 63.16%, 65.00%, respectively), conception rate (56.25%, 70.59%, 72.22%, respectively), lambing rate (56.25%, 70.59%, 72.22%, respectively) of three treatment groups have no significant difference compared with control (75.00%, 60.00%, 80.00%, 80.00%, respectively). It is noted that even though the estrus rate between CIDR14+eCG (89.47%) and GnRH+$PGF_{2\alpha}$ (90.00%) were almost equal, the implement of intravaginal devices may trigger several problems, including the production of chemical residues and vaginitis that may threaten to female health, which encourage the usage of $PGF_{2\alpha}$ as an alternative, clean and safe for estrus synchronization.

Most notably, CIDR+$PGF_{2\alpha}$+eCG and CIDR+eCG (145.78±1.56, 144.42±0.67, respectively) have significantly shorter ($P<0.05$) duration of pregnancy than control (147.20±1.52), but GnRH+$PGF_{2\alpha}$ (146.31±1.55) has no effect on duration of pregnancy compared with control. Furthermore, CIDR+eCG (144.42±0.67) has shorter ($P<0.05$) duration of pregnancy than the remaining treatment groups. This may due to the serum levels of P4 increased in CIDR-treated (14 days) ewes at 0 hour (Swelum *et al.* 2015); The increasing P4 level during early pregnancy, which can decreases the lost of embryo and increases pregnancy rate and fertility (Ataman *et al.* 2013). Therefore, during the later of pregnancy, there will not enough nutrition and space for the lamb to growth, which leading to shorter duration of pregnancy. With respect to fecundity, CIDR+eCG and

Table 1: The influence of CIDR9+$PGF_{2\alpha}$+eCG, CIDR14+eCG and GnRH+$PGF_{2\alpha}$ estrus synchronization protocols on reproductive performance of *Hu* sheep.

Variables	CIDR9+$PGF_{2\alpha}$+eCG	CIDR14+eCG	GnRH+$PGF_{2\alpha}$	Control
Estrus rate,% (*n*)	84.21 (16/19)	89.47 (17/19)	90.00 (18/20)	75.00 (15/20)
Pregnancy rate,% (*n*)	47.37 (9/19)	63.16 (12/19)	65.00 (13/20)	60.00 (12/20)
Conception rate,% (*n*)	56.25 (9/16)	70.59 (12/17)	72.22 (13/18)	80.00 (12/15)
Duration of pregnancy, days	145.78±1.56^{b}	144.42±0.67^{c}	146.31±1.55^{ab}	147.20±1.52^{a}
Lambing rate, % (*n*)	56.25 (9/16)	70.59 (12/17)	72.22 (13/18)	80.00 (12/15)
Fecundity, (*n*)	1.38^{b} (22/16)	1.82^{ab} (31/17)	1.50 ^{ab} (27/18)	2.33^{a} (35/15)
Prolificacy, (*n*)	2.44 (22/9)	2.58 (31/12)	2.08 (27/13)	2.92 (35/12)

Duration of pregnancy are presented as mean±standard deviation.

^{abc}Values with different superscripts differ within the rows ($P<0.05$).

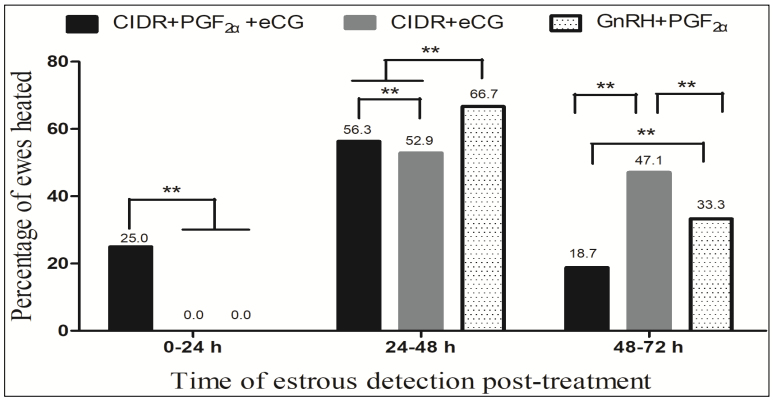

Fig 2: Effect of CIDR9+$PGF_{2\alpha}$+eCG (*n*=20), CIDR14+eCG (*n*=20) and GnRH+ $PGF_{2\alpha}$ (*n*=20) treatments, time of estrous detection post-treatment and their relationship on the percentage of *Hu* sheep showing standing estrus. Asterisks indicate significant differences, ***P*<0.01.

GnRH+$PGF_{2\alpha}$ did not dramatically change the fecundity of ewes, while CIDR+$PGF_{2\alpha}$+eCG significantly decreased (*P*<0.05) the fecundity of that compared with the control.

Detection of estrus of various times

Effect of three treatments on percentage of ewes behaving estrus responses within various time of estrus detection post-treatment, 0-24 h, 24-48 h, 48-72 h, was descripted in Fig 2. It suggested that estrus responses in CIDR9+$PGF_{2\alpha}$+eCG, CIDR14+eCG and GnRH+$PGF_{2\alpha}$ groups rose gradually to attain their significantly higher percentages (56.3%, 52.9% and 66.7%, respectively) during 24-48 h post-estrous detection, afterwards, they fell down. Besides, GnRH+$PGF_{2\alpha}$ would cause the estrus cycle more synchronized and the estrus time is focused at the 24-48 hours after $PGF_{2\alpha}$ treatment with the estrus rate up to 66.7%. This in line with the results, reported by Evans (1988), Menchaca and Rubianes (2004), Zeleke *et al.* (2005), that both the use of equine chorionic gonadotropin (eCG) and gonadotropin releasing hormone (GnRH) treatments could provide a more compact ovulation in ewes, thereby providing the potential to increase the pregnancy rates following AI in sheep. From the investigations on sheep folliculogenesis, it has been known that a follicle is in its active growing phase for the following four days to reach its maximum diameter at the fifth day after the insertion of a CIDR (Martinez *et al.* 2018). It should be noted that the differences in size between dominant and subordinate follicles are very small and periods of effective dominance are very short (Gonzalez *et al.* 2004). Furthermore, when sheep treated by GnRH+$PGF_{2\alpha}$, the luteal tissue that forms as a result of the GnRH administration is responsive to $PGF_{2\alpha}$ and is capable of undergoing luteolysis (Husein and Kridli, 2003). However, the response to GnRH is dependent upon the stage of the cycle at which it is administered. There have been reported that about half of the ewes had active corpora lutea at the time of GnRH administration (Alminer *et al.*, 2005; Titi *et al.*, 2010). Accordingly, GnRH+$PGF_{2\alpha}$-group appear to delay the phenomenon of ovulation, onset of oestrus.

CONCLUSION

Although CIDR-based short-term (9 days), CIDR-based long-term (14 days) and GnRH+$PGF_{2\alpha}$ estrus syn chronization protocols are all efficient in Hu sheep, taking the discussion above into account, GnRH+$PGF_{2\alpha}$ based protocol would result in the estrus cycle more compact synchronization in *Hu* sheep and the estrus time is concentrated at the 24-48 hours after $PGF_{2\alpha}$ treatment with the estrus rate up to 66.7%.

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Conflict of interest: None.

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