J. of Animal and Poultry Production, Vol. 1 (2): 35 - 44, 2010

EFFECTIVENESS OF DIFFERENT PROTOCOLS FOR INDUCING ESTRUS OF ARDY GOATS DURING THE NON-BREEDING SEASON.

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ABSTRACT

The experiment was conducted to determine the reproductive performance (kidding rate and litter size) of estrus synchronized local Ardy goats after different protocols and trans-cervical artificial insemination (TC-AI). This experiment was conducted at the beginning of their non-breeding season. Total of 90 Ardy does were randomly allotted into 6 similar groups of 15 in each and treated as the following: group 1: does were synchronized with a controlled intra-vaginal devices release (CIDR) for 14 days + injecting (i.m.) 600 i.u. of equine Chorionic Gonadotrophins (eCG) at withdrawal time; group 2: does were received two doses (10 mg/dose) of PGF2α at 11 days apart; group 3: does were treated with insertion of progesterone sponges for 14 days + injecting 600 i.u. of eCG at withdrawal time; group 4: does were treated with GPG method (day 1: the does were given a dose of 25 µg of GnRH (Cystorelin) ; each doe was injected with 10 mg of PGF2α on day 7 and at the 2nd

Keywords*:* Goats, estrous synchronization, CIDR, eCG, GnRH.

INTRODUCTION

Goats have tremendous potential for improvement and manipulation of reproduction due to their nature as a seasonal breeder and multiple ovulators (Amoah *et al.*, 1996; Powell *et al.*, 1996 and Zarkawi *et al.*, 1999). Their short gestation length, compared to cattle, theoretically allows two kiddings per year. Estrus synchronization is a valuable management tool that has been successfully employed to achieve this goal and enhance reproductive efficiency (Kusina *et al.,* 2000).

Goats are seasonally polyestrous animals and their reproductive season is affected by the change of day length. They reproduce during fall, winter and early spring when the day length is short, so they are called as "short day-breeder animals" (Amoah *et al.,* 1996 and Attwood, 2007).

The seasonality of reproduction exhibited by goats and some other farm animals results in inadequacy of their products supply during some months. This situation has created a strong interest among producers to induce estrus during the anestrous season.

Research has dramatically increased the number of synchronization options (Rahman *et al.,* 2008). The producers have many choices to pick a synchronization protocol for their operation, their production goals and their available labor. One of the oldest ways to synchronize estrus is a luteolytic agent, prostaglandin ($PGF_2α$) or one of its analogues, which causes the regression of the corpus luteum "CL" (Akusu and Egbunike 1984; Greyling and van Niekerk, 1986 and Kusina *et al.,* 2000).

Prostaglandin ($PGF_2α$) is only effective if administered after day 5 of the estrous cycle and has been used as a co-treatment in effective progestogen-based synchronizing protocols in goats (Kusina *et al.,* 2000; Pierson et al., 2001, 2003; Medan *et al.,* 2002 and Jackson *et al.,* 2003) sheep (Husein and Kridli, 2003), and cattle (Kojima *et al.,* 2000 and Lucy *et al.,* 2001).

Another way of creating estrus synchrony is using gonadotrophin releasing hormone (GnRH) or its analogue, which causes ovulation of the large follicle. Treatment with GnRH is combined with $PGF_2α$, as these hormones have different functions. Synchrony of estrus and fertility with a combination of GnRH and $PGF_2α$ (GPG method) are good for cyclic females, and some researches indicate that this combination may induce cyclicity in some females that experiencing postpartum anestrus. However, protocols using only GnRH and $PGF_2α$ are not advised for young females due to extremely variable response. A third method for synchronization of estrus is the use of progestins, which will maintain high levels of progesterone (P4) in the female's system even after the regression of the CL. Synchrony of estrus occurs 2 to 5 days following progestin removal (Wildeus,1999).

In efforts to extend the lifespan of the CL for synchronizing estrus, various forms of progestogens and different methods of administration have been used in cycling does, as well as in seasonally anestrus does, to induce or synchronize estrus (Amoah and Gelaye, 1990; Wildeus, 1999 and Montlomelo *et al,* 2002). Progestogen administration is common, especially in seasonally anestrous animals and has been used with or without accompanying treatments such as gonadotrophins or prostaglandin analogs. In the United States, no approved progestogen product is being manufactured for use in goats (Rathbone *et al.,* 1998). However, in other countries such as Australia and New Zealand, commercially available progestogen sponges formulated for goats, including those containing fluorogestone acetate (FGA; Cronogest 45) and methyl acetoxy progesterone (MAP; Repromap and Veramix), can be used. Similarly, a controlled internal drug-releasing device (CIDR) in the form of a silicone intra-vaginal progesterone insert is also available for use in goats outside the United

States (Rathbone *et al.,* 1998). The CIDR is an intra-vaginal pessary containing P4 designed for synchronizing estrus in ruminants (Knights *et al.,* 2001a &b). Previously, norgestomet, an ear implant progestin product, marketed under the name SynchroMate-B (SMB), is available for use in sheep in the United States, and thus held promise for approval for use in goats. The successful use of SMB in goats has been reviewed previously (Wildeus, 1999) and has been documented in other countries such as Mexico and Brazil, (Mellado *et al.,* 2000; Oliveira *et al.,* 2001 and Kusina *et al.,* 2000).

Exposure to male after a period of isolation can be used for induction of estrus during the breeding and non-breeding seasons without additional treatments in goats (Wildeus, 1999; Kusina *et al.,* 2000; Ishwar and Pandey, 1992; Mellado *et al.,* 2000 and Delgadillo *et al.,* 2002). There are other methods of synchronizing estrus that were examined by many researchers (du Preez *et al.,* 2001 and Wuliji *et al.,* 2003).

Therefore, the aims of the present study are to investigate; (1) the benefit of using various estrus synchronization protocols on inducing estrus of goats out of their breeding season, and (2) the accuracy of carrying out early pregnancy, diagnosis on day 40 pos-breeding in goats.

MATERIALS AND METHODS

Animals and experimental outline

This study was conducted at a local private goat farm in Al-Qassim region, Saudi Arabia, during the beginning of the seasonal anestrous period from May till mid-February 2008. Ninety clinically sound, pluriparous Ardy goat does (2 – 3 years of age), kidding in February with an average live body weight of 47 kg were used in this experiment. Does were randomly assigned into six similar groups (n= 15 does/group) as the following: Group 1; does were synchronized with CIDR for 14 days + injecting 600 i.u. of equine Chorionic Gonadotrophins (eCG) at withdrawal time, group 2: does received two doses (10 mg/dose) of $PGF_2\alpha$ (Lutalyse: Pharmacia and Upjohn Co. Kalamazoo, MI) at 11 day- interval; group 3: progesterone sponges were intra-vaginally inserted (sponge/doe) for 14 days + injecting 600 i.u. eCG at withdrawal time; group 4: does were given GnRH-$PGF_2\alpha$-GnRH protocol (GPG). In GPG protocol, each doe was i.m. injected with 25 µg GnRH on day 1 (Cystorelin: Merial Ltd; Duluth, GA), on d 7; each doe was i.m. injected with 10 mg $PGF_2\alpha$, and on day 9, a second dose GnRH i.m of 25 µg was administered and trans-cervically inseminated, while group 5: does were synchronized naturally using male effect where a teaser buck was introduced to the does and left with them for 20 days. All does exhibiting estrus were exposed to Trans-cervical artificial insemination (TC-AI), while does in group 6 (control), does did not receive any hormonal treatment and were left with a tested fertile buck for 20 continuous days during the treatment period of the other groups. The experimental design is shown in Table (1).

GnRH dose on day 9; group 5: does were synchronized naturally by using male effect where a teaser ram was introduced to the does and left with them for 20 days, and group 6 (control): does did not receive any hormonal treatment and were left for 20 days with a tested fertile buck. All synchronized does were trans-cervically inseminated (TC-AI) with diluted, semen of Ardy bucks. The pregnancy was diagnosed by ultrasonic examination on 40 days post insemination. Results showed that does in G1 and G3 showed clear estrus approximately 36 hours post CIDR and sponge removal and were 8 and 12 hours earlier than those in G4 and G2, respectively. Percentages of does kidded were 53.33, 13.33, 40.00, 20.00, 6.67 and 00.00% in G1, G2, G3, G4, G5 and G6, respectively, and the differences were significant ($P<0.05$). Multiple birth percentages were 13.33, 00.00, 13.33, 6.66, 00.00 and 00.00% per does kidded in the respective groups. Injecting does with eCG in G1 and G3 at the time of pessary withdrawal was associated with fruitful improvements in the kidding rates. The kidding period in all hormonal-treated does was short (about 7 days) and this is an advantage for synchronizing the estrus in Ardy goats.

Goats in all groups were managed under the same conditions at the farm. Animals of treatment groups were kept in separate pens. All does were identified by plastic ear tags, kept under semi-shaded pens and fed approximately 0.7 kg alfalfa hay + 0.5 kg barley grains/ head/day. Water and salt licks were freely available all day.

Table (1): Experimental outline of goat does in different experimental groups.

Group	Synchronization method	Type of insemination
G1	CIDR + eCG	TC-AI
G2	Two doses of $PGF_2\alpha$ at 14 day-interval	TC-AI
G3	Sponge + eCG	TC-AI
G4	GPG protocol	TC-AI
G5	Male effect	TC-AI
6 (Control)	No hormonal treatment	Natural mating

TC-AI: Trans-cervical artificial insemination.

Insemination and reproductive performance:

Semen from four tested mature bucks was collected using an electro ejaculator. Semen was entirely tested visually and microscopically and only ejaculates of good sperm motility and morphology were used. The ejaculates from the four bucks were pooled and extended using IMV diluent (IMV-France). Semen was diluted to a final concentration of approximately 100 million motile sperm per ml. For each insemination, a volume of two ml containing approximately 200 million motile sperm was used. Diluted samples were subsequently examined microscopically to ensure that spermatozoa are of good quality. All does were artificially inseminated using trans-cervical AI technique (Wulster-Radcliffe *et al*., 2004). Reproductive performance was determined by measuring the percentage of does kidding to AI at the synchronized estrus (kidding rate) and by determining the number of kids born per female kidded (litter size).

Pregnancy diagnosis:

Ultrasonic examinations were done using an Aloka-SSD500 (Japan) equipped with a 5 MHz linear array abdominal transducer on day 40 post - breeding. Does were restrained in a regular chute under a shaded area and the ultrasonic examinations were done in the early morning before providing the diet. An obstetrical lubricant was applied just to the right of the area in front of the udder devoid of hair, to insure satisfactory acoustic coupling. The accuracy of pregnancy diagnosis was indicated on the basis of kidding results.

Statistical Analysis:

Data were analyzed by GLM procedure using SAS (2000) program to test the effects of synchronization protocol on studied traits. The fixed effect model was as follow:

$Y_{ij} = \mu + G_i + e_{ij}$

Where;

Y_{ij} = An observation taken on individual,

μ = Overall mean,

G_i = a fixed effect of the group (synchronization regime),

e_{ij} = Random error assumed to be independent and normally distributed with mean = 0 and variance = σ^2.

The significance among means was detected using Duncan's Multiple Range Test (Duncan 1955). The percentage values were subjected to arcsine transformation before performing the analysis of variance. Means were presented after being recalculated from the transformed values to percentages.

RESULTS AND DISCUSSION

Data of reproductive performance traits of the does as influenced by the estrous synchronization method are given in Table (2). Results show that does given CIDR+eCG (G1) significantly (P<0.05) exhibited the highest percentages of estrus incidence and pregnancy as compared to the other groups. Does in G3 (given sponges and eCG) ranked the second and did not differ significantly (P>0.10) from those of G1. However, the male effect (G5) expressed the lowest (P<0.05) percentages among all treated groups. It was anticipated that the control does (G6) did not exhibit any estrous activity during the treatment period (Table 2).

Does given CIDR + eCG (G1) and those given sponge + eCG (G3) showed clear estrous signs approximately 36 hours post CIDR and sponge removal and were 8 hours earlier than those given GPG (G4) and 12 hours earlier than the does given double doses of PGF2α (G2). Several researchers have reported the onset of estrus to occur within 24-144 hours following progesterone withdrawal. (Billing and Katz, 1999 and Dogan and Nur, 2006). The goats that received either CIDRs or sponges exhibited very clear estrous signs in comparison with the does given other treatments. Among those does that received either CIDRs or sponges, the rate of estrus response was not significantly (P>0.05) different, however, it was significantly (P<0.05) higher than the does in the other treatment groups.

Additionally, does given CIDR or sponge give higher pregnancy rates (Table 2). Although the addition of eCG to the estrous synchronization program concomitantly with the CIDR or sponge relatively elevated the costs per a head, our data showed a clear advantage of its use.

The insertion and withdrawal of vaginal devices were easy and no intra-vaginal devices were lost during the trial. Some investigators found that using sponges gave better results than using CIDR and this was due to the high retention rate of sponges in comparison to CIDRs (Knight and Hall, 1988). In the present study, the retention rate of sponges and CIDR was 100%, and this is in agreement with the observation of Romano (1998).

None of the does showed estrus while the intra-vaginal devices were in place. Therefore, it can be accepted that the dose of P_4 in the CIDR and Sponge devices absorbed from the vagina during the treatment was sufficient to suppress the preovulatory discharge of pituitary gonadotrophins (Moeini *et al*., 2007). The source of progestin did not influence the efficiency of estrus synchronization rate in CIDR *vs*. sponges (80% *vs.* 73%). Moreover, CIDRs did not cause bad smell in comparison with sponges. However, the source of progestin used for estrous synchronization and AI in small ruminants may still need further investigation.

Table (2): Reproductive performance traits of Ardy does subjected to various estrus synchronization regimes.

Group (Treatment)	Estrus exhibition (%)	Pregnancy rat (%)	Multiple birth per doe (%)	Pregnancy duration (day)
G1 (CIDR + eCG)	80.00±0.106[a]	53.33±0.13[a]	13.33±0.09[a]	149.38±0.53
G2 (Two doses of $PGF_2α$)	33.33±0.125[c]	13.33±0.091[bc]	0.00±0.00[c]	152.00±1.00
G3 (Sponges +eCG)	73.33±0.118[ab]	40.00±0.13[ab]	13.33±0.09[a]	150.00±0.93
G4 (GPG Method)	46.67±0.133[bc]	20.00±0.106[bc]	6.66±0.06[b]	148.67±0.88
G5 (Male effect)	20.00±0.106[cd]	6.67±0.066[c]	0.00±0.00[c]	149.00±0.0
G6 (Control)	0.00±0.00[d]	0.00±0.00[c]	0.00±0.00[c]	0.00±0.00

a, b, c and d: Means in the same column with different superscripts are significantly different at P<0.01.

Using two doses of PGF2α at 11 day-interval did not give the expected results, since five does (33.33%) displayed estrus and only two (13.3%) of them became pregnant who gave single births. Also, GPG protocol did not give encouraging results where only seven does (46.67%) showed unclear estrus signs from which three (20%) became pregnant. There might be more beneficial effects for $PGF_2α$ in GPG protocol over the use of PGF2α alone (G2 and G4) during the reproductive season when there are developing follicles and active corpora lutea (CLs). The absence, or by other words, the very low number of CLs reduced the effect of PGF2α in these two treatments.

Male exposure in G5 did not give good induction of estrus and this does not agree with the results reported by Velize *et al*. (2002) when they introduced active bucks to a group of prepared does. They found that more than 90% of the does displayed estrus behavior in the first 15 days following the introduction of those males. The variation of male effect might be due to the breed of treated goats (Nuti *et al.,* 2003). This also would be attributed to the effect of male pheromone glands. The failure of teasing outside this limited period may be due to the inability of the female to respond during the beginning of the seasonal anestrous period, resulting from refractoriness of the female to the male stimulus. Alternatively, it may be due to inadequate stimulation from the male. Indeed, male behavioral and physiological activity tends to decrease during the period of female seasonal anestrous in both sheep and goats (Chemineau, 1983). In the control group no doe came into estrus during the period of observation (zero estrus and pregnancy).

When ultrasound scanning was used for diagnosing pregnancy at approximately 40 days post breeding, all the positive diagnosed does gave births. The accuracy of diagnosing multiple pregnancies was 100%. Results presented in Table (2) show also that the multiple births percentages were 13.33, 00.00, 13.33, 6.66, 00.00 and 00.00% per doe kidded in the respective treatment groups. Injecting does with eCG in G1 and G3 at the time of pessary withdrawal was associated with fruitful improvements in the kidding rate. This finding is in agreement with those found in beef cattle given different doses of eCG (Zeitoun, 1990). The kidding period in all hormonal-treated does was short (about 7 days) and this is an advantage for synchronizing the estrus in Ardy does.

Although the fertility results can be affected by many factors such as: type of intra-vaginal device, dose and timing of eCG injection, semen type and dose, time and number of AIs, breed and age of does, body condition, management, season and others. However the fertility results after hormonal treatment are contradictory.

In conclusion, results from this study indicate that goats treated with vaginal CIDRs or sponges accompanied with the injection of eCG showed higher percentage of estrus expression and fertility during the non-breeding season of Ardy goats. There still many studies are need to be conducted as to follow the ovarian follicular dynamics during out of the breeding season in goats.

ACKNOWLEDGEMENTS

The author is grateful to the Research Center of Al-Qassim University for providing the funds and supporting this research. Many thanks to the people of Rokany Goat Farm for giving me the chance to do this experiment at their farm. Thanks go to Prof. Magdy M. Abdulsalam for his assistance and advises in statistical analysis and appreciation to Prof. Moustafa M. Zeitoun for reviewing the manuscript.

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كفاءة استخدام طرق مختلفة لإحداث الشياع في ماعز العارضي خارج موسم التناسل

خالد بن عبد الله السبيل
قسم إنتاج الحيوان وتربيته كلية الزراعة و الطب البيطري - جامعة القصيم المملكة العربية السعودية

أجريت هذه التجربة لدراسة الأداء التناسلي (نسب الحمل ومعدل الولادات وحجم البطن) للماعز العارضى التي تم تنظيم شياعها بواسطة المعاملات الهرمونية المختلفة والتلقيح الاصطناعي.
تم استخدام 90 عنزة عارضي خارج موسم التناسل وقسمت الحيوانات إلى 6 مجاميع كل منها احتوت على 15 حيوان وعوملت الحيوانات كما يلي، المجموعة الاولى: تم تنظيم الشبق للماعز باستخدام CIDR لمدة 14 يوم + الحقن بـ 600 وحدة دولية من هرمون eCG عند إزالة الـ CIDR مباشرة، المجموعة الثانية: أُعطيت الماعز جرعتين بالعضل (10 ملجم / جرعة) من هرمون البروستاجلاندين PGF2α بينهما 11 يوم ،المجموعة الثالثة: تم استخدام إسفنجة البروجستيرون لمدة 14 يوم + الحقن العضلي بـ600 وحدة دولية من هرمون eCG عند ازالة الاسفنجة، المجموعة الرابعة: تم فيها استخدام نظام الـ GPG (في اليوم الأول يعطى الحيوان 25 ميكروجرام هرمون GnRH ثم اليوم السابع حقنة من البروستاجلاندين PGF2α وفى اليوم التاسع يعطى الحيوان حقنة أخرى 25 ميكروجرام هرمون GnRH ثم يتم التلقيح اصطناعيا- الحقن كله عضلي)، المجموعة الخامسة: تم فيها تنظيم الشبق طبيعيا باستخدام تأثير الذكور حيث يتم وضع تيس كشاف للماعز لمدة 20 يوم والإناث التي ظهرت عليها علامات الشبق تم تلقيحها اصطناعيا، المجموعة السادسة: استخدمت كمجموعة ضابطة حيث أن الحيوانات لم تعطى اى معاملة هرمونية وتركت 20 يوم مع ذكور مخصبة مختبرة. وكل الماعز المنظمة تم تلقيحها اصطناعيا، تم جمع السائل المنوي المستخدم في التلقيح من 4 تيوس عارضى مختبرة باستخدام طريقة التنبيه الكهربي، وتم تخفيف السائل المنوي بواسطة مخفف IMV وكان حجم التلقيحات المستخدمة 2 مل تحتوى على 200 مليون حيوان منوي حي متحرك، وتم تسجيل معدل الحمل والولادات وتم تشخيص الحمل بواسطة جهاز الموجات الفوق صوتية بعد 40 يوم تقريبا من التلقيح.
وأوضحت النتائج أن المجموعة الأولى والثالثة أظهرت أعلى معدل استجابة لحدوث الشبق (80 و 73) على الترتيب و المجموعة الأولى والثالثة أظهرت شبق واضح بعد 36 ساعة من إزالة الـ CIDR و الإسفنجة وقبل 8 و 12 ساعة في المعاملة الرابعة والثانية على التوالي ، وكانت الاختلافات بين المعاملات في معدل الحمل وعدد المواليد مرتفعة معنوياً وكانت النسبة المئوية لمعدل المواليد 53.33 ، 13.33 ، 40 ، 20 ، 6.67 ، صفر% في المعاملات الأولى والثانية والثالثة والرابعة والخامسة والسادسة على التوالي. بينما كانت النسبة المئوية لمعدل التوائم 13.33، صفر، 13.33 ، 6.66 ، صفر ، صفر% لكل عنزة على التوالي المعاملات.
الإناث المحقونة بـ eCG في المعاملات الأولى والثالثة أظهرت تحسن جيد مصاحب لمعدل الولادات ، وكانت فترة الولادة في كل الإناث المعاملة هرمونيا قصيرة (7 أيام) وهذا يعتبر ميزة هامة في تنظيم الشبق في الماعز العارضى

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