IMPACT OF USING CIDR-ECG PROTOCOL DURING OUT-OF-BREEDING SEASON ON REPRODUCTIVE SEASONALITY OF ANGLO-NUBIAN AND BALADI DOES

M.Y. El-Mokadem[1], A. M. A. Rashad[2] and T. A. Ramadan[1*]

1-*Animal Production Research Institute, Agricultural Research Center, 4 Nadi El-Said, 12311 Dokki, Giza, Egypt, 2- Animal Production Department, Faculty of Agriculture (El-Shatby), Alexandria University, Alexandria 22545, Egypt*
**** Corresponding author: (Tamer A. Ramadan), E-mail: tamereweda1174@yahoo.com***

IMPLICATIONS

The state of sexual inactivity in dairy goats from February to May causing cessations of estrus signs and vanish of fertile mating is known as reproductive seasonality. Using CIDR-eCG hormonal treatment successfully induced estrus behavior and enhanced conception rate, fecundity and prolificacy of Anglo-Nubian and Baladi does during out-of-breeding season. The success of CIDR-eCG protocol to prolong breeding season by eliminating reproductive seasonality in dairy goats represents a promising option to improve productivity and increase profits from dairy goats breeding.

SUMMARY

*This experiment was performed to explore the role of utilizing the controlled internal drug release **(CIDR)** device and equine chronic gonadotrophin **(eCG)** protocol on the reproductive seasonality of Anglo-Nubian and Baladi does and assess its efficiency to induce synchronized fertile estrus and evident reproductive activity during out of breeding season. Forty days after uterine involution (80 days after parturition), a total of thirty multiparous does, 15 Anglo-Nubian and 15 Baladi, received CIDR device for 19 days followed by intramuscular injection with 500 IU eCG on the time of CIDR removal. The experiment was performed during out of breeding season between February and May when estrus occurrence and fertile mating are abolished. Using CIDR-eCG protocol created a luteotrophic effect accentuated as the appearance of corpora lutea (CL) in an ascending ($P < 0.05$) in Baladi compared to Anglo-Nubian does. As the number of treatment days progressed, total number of follicles, small follicles, medium follicles and number of CLs tended to increase recording the largest ($P < 0.05$) values at day of CIDR-removal (Day 61). At day of mating (Day 63), number of large follicles and diameter of the largest follicle revealed the highest ($P < 0.05$) value which associated with disappearance of corpora lutea. Does of the two breeds, displayed estrus signs after CIDR removal and eCG injection. Conception rate, prolificacy and fecundity did not differ between both breeds. In conclusion, reproductive seasonality of Anglo-Nubian and Baladi does expressed as complete cessation of estrus and fertile mating from beginning of February to end of May was eliminated successfully using CIDR-eCG protocol which induced fertile estrus in both breeds.*

Keywords: *Goats, reproductive seasonality, CIDR-eCG protocol, conception rate, prolificacy, fecundity.*

INTRODUCTION

Goats are seasonal polyestrous spontaneous ovulators under temperate climatic conditions. However, reproductive seasonality is found in some local breeds adapted to or originated at subtropical latitudes as a depressed reproductive activity during spring months. In Egypt (31º N), the reproductive performance is completely abolished from December to May while the highest reproductive performance is achieved in summer and autumn from June to November in Anglo-Nubian does (El-Mokadem *et al*., 2017), Damascus and Baladi does raised in subtropical zone (Taha and El-Agamey, 2003).

Estrous synchronization proved to be a useful management practice employed to boost reproductive efficiency in does and ewes (Kusina *et al*., 2000). It establishes a chance for appropriate synchronized breeding and kidding. This, consequently, takes the advantages of seasonal green forage availability, photoperiod, sufficient labour resources and market abundance. Estrus synchronization could be achieved by using prostaglandin $F_{2\alpha}$ to reduce the span of the luteal phase of estrous cycle or by using progesterone or progestogens to extend estrus period artificially (Kusina *et al*., 2000). Since the prostaglandin $F_{2\alpha}$ production is abundant in the breeding season when *corpus luteum* (CL) is active, different methods for synchronization of estrus using progesterone have been utilized in goat (El-Mokadem *et al*., 2017) and sheep (El-Mokadem *et al*., 2018).

Synchronization of ovulation protocols, out of season, are commonly based on controlled internal drug release (CIDR), plus equine chorionic

gonadotropin (eCG) treatments (Abecia *et al.*, 2011) were it found effective to induce estrus and ovulation in goats during out of breeding season (Contreras-Villarreal *et al.*, 2015). The treatment of CIDR followed with eCG increased the rate of pregnancy in ewes, comparing to using CIDR only (Luther *et al*., 2007). eCG is used for initiation of ovulation and estrus during non-breeding season, and was found to increase ovulation rather than twinning rates in a dose-dependent manner (Jabbour and Evans, 1991).

The objectives of this work were to manipulate the reproductive seasonality of Anglo-Nubian and Baladi does and to evaluate the efficiency of the protocol of CIDR-eCG to generate synchronized fertile estrus and improve efficiency of reproduction in seasonally anestrus lactating Anglo-Nubian and Baladi does.

MATERIAL AND METHODS

The Institutional Animal Care and Use Committee of Alexandria University approved all experiments and the study protocol (ALEXU-IACUC no. AU-08211012384). This experiment was achieved at the Agricultural Experimental Station (31º 20′ N, 30º E), Faculty of Agriculture, Alexandria University, Alexandria, Egypt.

Animals and management:

Thirty multiparous does, 15 Anglo-Nubian weighing 30.31 ± 1.43 kg, and 15 Baladi weighing 34.17 ± 3.37 kg making their third to fourth parity were used in this study. Does were housed outdoors with shelter during the day and at night, and were kept in a semi-open barn. Roughage and concentrate supplements were offered according to their BW requirements (NRC, 2007). Also, each lactating doe received 1 kg/d concentrate mixture (16.04 % CP and 68 % TDN). Water was accessible all times. Animals were clinically normal and disease free with a healthy appearance.

Experimental design:

The present study was conducted during February and May, a period in which the reproductive performance is inert with severely deteriorated activities. The research was commenced 40 days after parturition when does become able to exert their first *post-partum* estrus as uterine involution was known to be completed by day 27 of parturition (Hafez and Hafez, 2000). All does were checked for estrus signs using teaser bucks daily throughout pre-treatment period which extended from day 40 to day 82 after parturition. At day 82 after parturition, all does were inserted with Eazi-Breed® CIDR® device (0.3 g of progesterone; Pfizer Animal Health, Auckland, New Zealand) for a period of 19 days then removed thereafter. At the removal of CIDR (day 101 after parturition), animals were injected intramuscularly with 500 IU eCG (Syncropart; CEVA Santé Animale, France). Estrus signs were detected daily using teaser bucks and those exhibited estrus were mated within 48 h of CIDR removal (Abecia *et al*., 2011). For all does, pregnancy was diagnosed ultrasonographically at day 35 after mating (day 138 after parturition). Experimental design layout is presented in Figure 1.

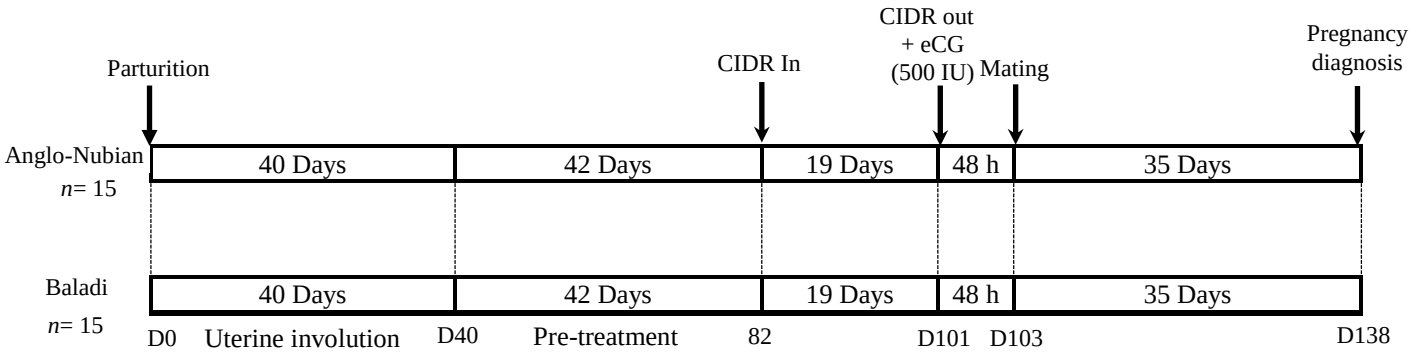

Figure 1. An experimental design to evaluate the CIDR-eCG -based estrus synchronization protocol in Anglo-Nubian and Baladi does during out-of-breeding season. CIDR: controlled internal drug release device (0.3 g progesterone); eCG, equine chorionic gonadotropin (500 IU Syncropart, intramuscular).

Ovarian activity and pregnancy diagnosis:

The ovarian response to CIDR protocol was achieved using the scanning procedure described by El-Mokadem *et al*. (2017). Ovarian activity including total number of follicles and follicle population were estimated for each doe (El-Mokadem *et al*., 2017) on days 40, 54 and 68 (after parturition) for determining the ovarian activity before insertion of CIDR device, on day 82 (insertion day), day 101 (CIDR withdrawal day) and (mating) day 103. The number and diameter of CL and diameter of the largest follicle were recorded. Pregnancy was diagnosed by examining the uterine contents at days 35 (day 138 of commencing the experiment).

Reproductive performance:

Reproductive performance was measured as:

Estrus rate: number of does displaying estrus/ number of synchronized does × 100; conception rate at day 35 after mating: number of does conceiving at day 35 after mating (based on ultrasonography)/ number of does exposed to males × 100; prolificacy

at day 35 after mating: number of fetuses / conceived does; and fecundity at day 35 after mating: no. of fetuses / number of does exposed to males × 100 (Olivera-Muzante *et al*. 2013).

Statistical analysis:

The statistical analysis of data was performed using PROC MIXED of SAS 8.0 for repeated measures. Data of the ovarian activity were analyzed as follow:

$$Y_{ijk} = ì + B_i + D_j + (B \times D)_{ij} + e_{ijk},$$

in which Y_{ijk} is the observed value of the dependent variable determined from a sample taken from each animal, ì is the overall mean, B_i is the fixed effect of the i^{th} breed, Anglo-Nubian or Baladi (i = 1:2), D_j is the fixed effect of the j^{th} day (j = 0 : 63) on ovarian activity. $(B \times D)_{ij}$ is interactions between breed and day, and e_{ijk} is the residual error. Significant differences among means were tested using least significant differences (LSD0.05) within each classification**.** The reproductive performance data were conducted to the chi-square-test. The confidence limits were tested at 5.0 % level.

RESULTS

Effect of CIDR-eCG protocol on ovarian activity

The effect of breed on ovarian activity of lactating Anglo-Nubian and Baladi does treated with protocol of CIDR-eCG during non-breeding season are displayed in (Table 1). Baladi does exhibited higher (P < 0.05) number of corpora lutea compared to Anglo-Nubian does. However, total number of follicles per doe, follicular population, diameter of largest follicle and diameter of corpus luteum were not affected by breed. With advancement of experimental days, all ovarian activities were significantly affected, except diameter of CL (Table 2). Total number of follicles including small and medium follicles, and CL increased gradually to record the greatest (P< 0.05) values at time of CIDR-removal (Day 101). At the day of mating (Day 103), the highest (P<0.05) large follicle number and the largest follicle diameter was accompanied with disappearance of CLs.

Table 1. Least square means ± SEM of ovarian activity of lactating Anglo-Nubian and Baladi does treated with CIDR-eCG protocol during out-of-breeding season

Parameter	Breed		SEM
	Anglo-Nubian	Baladi	
Total number of follicles per doe	3.12	3.14	0.19
Follicular population , number			
Small follicles, ≥ 2 to 3 mm	1.20	1.42	0.16
Medium follicles, > 3 to < 5 mm	1.09	0.83	0.15
Large follicles, > 5 mm	0.82	0.89	0.11
Diameter of largest follicle , mm	5.98	6.10	0.12
No. of corpus luteum	0.20^{b}	0.53^{a}	0.07
Diameter of corpus luteum , mm	9.80	9.74	0.23

[a , b] Within a row, within breed means with different superscript letters differ (P < 0.05).

Table 2. Least square means ± SEM of the day effect after CIDR-eCG protocol on ovarian activity of lactating Anglo-Nubian and Baladi does during non-breeding season

Traits	Days after parturition						SEM
	40	54	68	82	101	103	
Total number of follicles per doe	1.54^{b}	2.08^{b}	3.38^{a}	4.13^{a}	3.67^{a}	3.97^{a}	0.30
Follicular population (*n*)							
Small follicles, ≥ 2 to 3 mm	0.83^{b}	0.63^{b}	1.46^{ab}	2.08^{a}	2.08^{a}	0.78^{b}	0.26
Medium follicles, > 3 to < 5 mm	0.38^{b}	0.75^{ab}	0.88^{ab}	1.44^{a}	1.47^{a}	0.86^{ab}	0.24
Large follicles, ≥ 5 mm	0.33^{cd}	0.70^{bc}	1.05^{b}	0.60^{bcd}	0.11^{d}	2.33^{a}	0.17
Diameter of largest follicles (mm)	6.20^{ab}	5.70^{bc}	5.67^{bc}	5.83^{b}	5.20^{c}	6.55^{a}	0.10
No. of corpora lutea	0.08^{cd}	0.36^{bc}	0.54^{ab}	0.46^{ab}	0.72^{a}	0.00^{d}	0.10
Diameter of corpus luteum (mm)	11.50	8.75	10.05	9.28	10.09	-	0.18

[a- d] Within a row, means with different superscript letters differ (p < .05).

Effect of CIDR-eCG protocol on reproductive performance

Although does of both breeds failed to exhibit estrus activity before CIDR insertion (throughout the pre-treatment period extending from day 40 after parturition till day 82 in which the CIDR was inserted), Anglo-Nubian and Baladi does recorded 100% estrus rate after CIDR removal and eCG injection (Table 3). In addition, conception rate, prolificacy and fecundity at day 35 of pregnancy were not affected by breed.

Table 3. Reproductive performance of Anglo-Nubian and Baladi does treated with CIDR-eCG protocol[1] during out-of-breeding season

Parameter	Breed	
	Anglo-Nubian	Baladi
Estrus rate	100 (15/15)	100 (15/15)
Conception rate (Day 35)	13% (2/15)	20% (3/15)
Prolificacy (Day 35)	2.00 (4/2)	1.00 (3/3)
Fecundity (Day 35)	26.67 (4/15)	20.00 (3/15)

[1] CIDR-eCG protocol: controlled internal drug release device contained 0.3 g of progesterone was inserted in out of breeding season and removed after 19 days and eCG (equine chorionic gonadotropin, 500 IU) was injected thereafter.

DISCUSSION

Effect of CIDR-eCG protocol on ovarian activity

Applying CIDR-eCG protocol to does during out-of-breeding season illustrated a superiority of the luteal function in terms of number of corpora lutea in lactating Baladi does compared to Anglo-Nubian. The developed ability of ovarian activity with the progress of experimental day suggested that higher total number of follicles at day 103 (day of mating) resulted from the number large follicles possibly under the effect of eCG administration at day 101 (day of CIDR-out) which was previously reported to increase the number of large follicles at estrus (Noël *et al.,* 1994). eCG enhances the small and medium follicles entry into the phase of large follicles but prevents the incidence of follicular atresia (Bister *et al.,* 1999). Thereafter, the number of small follicles decreased following eCG treatment to reach the lowest at estrus (day 103). Similar results have been reported previously on ewes (Kermani *et al.,* 2012). However, reduction in number of large follicles and the largest follicle diameter at CIDR removal time (day 101) was reported in dairy cattle by Honparkhe *et al.* (2011). Moreover, injection of eCG at CIDR removal time augmented the follicles growth rate causing an increase in the largest follicle diameter at estrus (Sá Filho *et al.,* 2010) associated with disappearance of corpora lutea (El-Mokadem *et al*., 2017).

Effect of CIDR-eCG protocol on reproductive performance

The success of CIDR-eCG protocol to induce 100% estrus activity during out-of-breeding season in both experimental breeds was in accordance with the findings reported previously on goats (Contreras-Villarreal *et al.,* 2015; El-Mokadem *et al*., 2017) and sheep (El-Mokadem *et al*., 2018). The similarity of the estrus cessation for lactating does of both breeds before CIDR insertion was suggested to be under the effect of prolactin which is oppositely associated with the goats pattern of reproductive activity (Mori *et al.,* 1985). It is worthy to note that does which came to heat by progesterone-eCG treatment showed only one estrus and if not conceive they did not come to heat again (Abecia *et al.,* 2011). These results indicated the effectiveness of P_4-eCG protocol to induce estrus signs and incidence of ovulation in goats during non-breeding season as was similarly reported previously by Contreras-Villarreal *et al.,* (2015).

P_4-eCG protocol succeeded to achieve fertile mating in both Anglo-Nubian and Baladi does. Progesterone boosts the epithelia differentiation and consequently, the secretory function of the endometrium (Cunha *et al.,* 2004). In addition, eCG enhances the oocyte to mature (Schmitt *et al.,* 1996). Hence, administering eCG to progesterone-treated ewes throughout the out of-breeding season had a favourable effect on pregnancy rates (Kaya *et al.,* 2013). It boosts the development of blastocyst and reduces estradiol and oxytocin, thus suppresses the of $PGF_{2\alpha}$ secretion and enhance the blastocyst to secrete interferon-tau (Nephew *et al.,* 1994) causing the growing of conceptus and leading to increases in placentation (Khan *et al.,* 2003).

CONCLUSION

The detrimental effect of seasonality on reproductive performance of Anglo-Nubian and Baladi does expressed as complete cessation of estrus activity during out-of-breeding season from February to May was eliminated by using CIDR-eCG protocol. The protocol induced estrus behavior successfully during out of breeding season in both breeds. The effectiveness of CIDR-eCG protocol was numerically apparent in Anglo-Nubian does which recorded greater prolificacy and fecundity compared to Baladi does during out-of-breeding season. However, CIDR-eCG protocol seems to be a feasible option to improve reproductive performance where fertile mating was successfully induced during out-of-breeding season in goats.

CONFLICTS OF INTEREST

None

REFERENCES

Abecia, J., F. Forcada and A. González-Bulnes, 2011. Pharmaceutical control of reproduction in sheep and goats. Veterinary Clinics of North America: Food Anim. Pract. 27: 167–179.

Bister, J., B. Noël, B. Perrad, S. Mandiki, J. Mbayahaga and R. Paquay, 1999. Control of

ovarian follicle activity in the ewe. Domes. Anim. Endocrin. 17:315–328.

Contreras-Villarreal, V., C. Meza-Herrera, R. Rivas-Muñoz, O. Angel-Garcia, J. Luna-Orozco, E. Carrillo, M. Mellado and F. Véliz-Deras, 2015. Reproductive performance of seasonally anovular mixed-bred dairy goats induced to ovulate with a combination of progesterone and eCG or estradiol. J. Anim. Sci. 87: 750–755.

Cunha, G., P. Cooke and T. Kurita, 2004. Role of stromal–epithelial interactions in hormonal responses. Archiv. Histo. Cyto. 67:417–434.

El-Mokadem, M.Y., A.N.M. Nour El-Din, T.A. Ramadan, A.M.A. Rashad, T.A. Taha and M.A. Samak, 2017. Manipulation of reproductive seasonality using melatonin implantation in Anglo-Nubian does treated with controlled internal drug release and equine chorionic gonadotropin during the nonbreeding season. J. Dairy Sci., 100: 5028–5039.

El-Mokadem, M.Y., A.N.M. Nour El-Din, T.A. Ramadan, A.M.A. Rashad, T.A. Taha, M.A. Samak and M.H. Salem, 2018. Effectiveness of controlled internal drug release device treatment to alleviate reproductive seasonality in anestrus lactating or dry Barki and Rahmani ewes during non-breeding season. Reprod. Domes. Anim. 53: 319-325.

Hafez, B., and E.S.E. Hafez, 2000. Reproduction in farm animals, 7th edition. Lippincott Williams and Wilkins, Philadelphia, USA.

Honparkhe, M., P. Ghuman, J. Singh and G. Dhaliwal, 2011. A CIDR-based AI protocol establishes pregnancy in repeat breeder dairy cattle. The Indian J. Anim. Sci. 81: 340-343.

Jabbour, H.N., and G. Evans, 1991. Ovarian and endocrine responses of Merino ewes to treatment with PMSG and/or FSH-P. Anim. Reprod. Sci. 26: 93-106.

Kaya, S., C. Kaçar, D. Kaya and S. Aslan, 2013. The effectiveness of supplemental administration of progesterone with GnRH, hCG and PGF2α on the fertility of Tuj sheep during the non-breeding season. Small Rumin. Res. 113: 365–370.

Kermani, M. H, H. Kohram., A. Zareh Shahneha and T. Saberifar, 2012. Ovarian response and pregnancy rate following different doses of eCG treatment in Chall ewes. Small Rumin. Res. 102: 63– 67.

Khan, T.H., P.M. Hastie, N.F.G. Beck and M. Khalid, 2003. hCG treatment on day of mating improves embryo viability and fertility in ewe lambs. Anim. Reprod. Sci. 76: 81–89.

Kusina, N.T., F. Tarwirei, H. Hamudikuwanda, G. Agumba and J. Mukwena, 2000. A comparison of the effects of progesterone sponges and ear implants, PGF2alpha, and their combination on efficacy of oestrous synchronization and fertility of Mashona goat does. Theriogenology 53: 1567-1580.

Luther, J.S., A.T. Grazul-Bilska, J.D. Kirsch, R.M. Weigl, K.C. Kraft, C. Navanukraw, D. Pant, L.P. Reynolds and D.A. Redmer, 2007. The effect of GnRH, eCG and progestin type on estrous synchronization following laparoscopic AI in ewes. Small Rumin. Res. 72: 227–231.

Mori, Y., K. Maeda, T. Sawasaki and Y. Kano, 1985. Photoperiodic control of prolactin secretion in the goat. The Japanese J. Anim. Reprod. 31: 9-15.

NRC., 2007. Nutrient Requirements of Small Ruminants. National Academy Press, Washington, DC, USA.

Nephew, K., H. Cardenas, K. McClure, T. Ott, F. Bazer and W. Pope, 1994. Effects of administration of human chorionic gonadotropin or progesterone before maternal recognition of pregnancy on blastocyst development and pregnancy in sheep. J. Anim. Sci. 72:453–458.

Noël, B., J. Bister, B. Pierquin and R. Paquay, 1994. Effects of FGA and PMSG on follicular growth and LH secretion in Suffolk ewes. Theriogenology 41: 719–727.

Olivera-Muzantea, J., J. Gil, C. Viñolesb and S. Fierroa, 2013. Reproductive outcome with GnRH inclusion at 24 or 36 h following a prostaglandin F2α-based protocol for timed AI in ewes. Anim. Reprod. Sci. 138: 175– 179.

Sá Filho, M., H. Ayres, R. Ferreira, Marques, E. Reis, R. Silva, C. Rodrigues, E. Madureira, G. Bó and P. Baruselli, 2010. Equine chorionic gonadotropin and gonadotropin-releasing hormone enhance fertility in a norgestomet-based, timed artificial insemination protocol in suckled Nelore (Bos indicus) cows. Theriogenology 73:651-658.

Schmitt, E., C. Barros, P. Fields, M. Fields, T. Diaz, J. Kluge and W. Thatcher, 1996. A cellular and endocrine characterization of the original and induced corpus luteum after administration of a gonadotropin-releasing hormone agonist or human chorionic gonadotropin on day five of the estrous cycle. J. Anim. Sci. 74:1915–1929

Taha, T., and E. El-Agamey, 2003. Fertility-associated protein profile in the seminal plasma of sheep and goats throughout one year. Alexandria J. Agricul. Res. 48:17–28.

تأثير استخدام بروتوكول التحاميل المهبلية المصحوب بالهرمون الكريوني الخيلي المنبه للغدد التناسلية علي موسمية التناسل في إناث الماعز الأنجلونوبيان والبلدي خلال موسم عدم التناسل

مروان يوسف المقدم[١]، عمرو محمد رشاد[٢]، تامر عوض رمضان[١]*

١ ـ معهد بحوث الأنتاج الحيواني، مركز البحوث الزراعية، الدقي، الجيزة، جمهورية مصر العربية، ٢ ـ قسم الإنتاج الحيواني، كلية الزراعة (الشاطبي)، جامعة الاسكندرية، الاسكندرية، جمهورية مصر العربية

هدفت هذه الدراسة الي تقيم دور التحاميل المهبلية المصحوبة بحقن الهرمون الكريوني الخيلي المنبه للغدد التناسلية علي موسيمة التناسل لعنزات الأنجلونوبيان والبلدي و تحديد كفاءتها لإحداث الشياع المخصب وتحسين الكفاءة التناسلية خارج موسم التزاوج. خلال أربعين يوم لاستعادة الرحم لوضعة الطبيعي (٨٠ يوم بعد الولادة), تم إستخدام عدد (٣٠) عنزة متعددة الولادة، قسمت إلى مجموعتين (١٥) إنجلونوبيان و(١٥) بلدي. وتم معاملة كل عنزة بتحاميل مهبلية لمدة ١٩ يوم مصحوبه بحقن الهرمون الكريوني الخيلي المنبه للغدد التناسلية في العضل بتركيز ٥٠٠ وحدة دولية في يوم نزع التحاميل المهبلية. أجريت هذه الدراسة خارج موسم التزاوج والتي إستمرت ما بين شهر فبراير حتى شهرمايو والتي تتميز بعدم ظهور علامات الشياع وكذلك تلاشي التزواج المخصب. إستخدام بروتوكول التحاميل المهبلية المصاحبة بالهرمون الكريوني الخيلي المنبه للغدد التناسلية قام بتحفيز تكوين الجسم الأصفر والتي ظهرت في صورة زيادة (باحتمال ٠.٠٥) في عدد الأجسام الصفراء في عنزات البلدي مقارنة بعنزات الأنجلونوبيان. وبإستمرارأيام المعاملة, وجد أن العدد الكلي للحويصلات وكذلك عدد الحويصلات الصغيرة والمتوسطة وعدد الأجسام الصفراء إتجهت الي الزيادة لتسجل أعلي قيم للحويصلات الكبيرة (باحتمال ٠.٠٥) في يوم إزالة التحاميل المهبلية (اليوم ٦١). فضلا عن ذلك, وجد في يوم التزاوج (اليوم ٦٣) أن عدد الحويصلات الكبيرة وكذلك قطر الحويصلات الكبيرة سجلت أعلي قيم لها (باحتمال ٠.٠٥) وكان ذلك بمصاحبة عدم ظهور الجسم الأصفر. التأثير الضار لموسمية التناسل في عنزات الأنجلونوبيان والبلدي والتي تمثلت في غياب سلوك الشياع والتزاوج المخصب خلال موسم عدم التناسل تم تخفيفه بإستخدام برتوكول التحاميل المهبلية مع حقن بالهرمون الكريوني الخيلي المنبه للغدد التناسلية. فضلا عن ذلك , فإن إستخدام برتوكول التحاميل المهبلية مع حقن بالهرمون الكريوني الخيلي المنبه للغدد التناسلية أدي ظهور الشياع يوم إزالة التحاميل المهبلية لكلا من عنزات الأنجلونوبيان والبلدي كما لوحظ أن كل من معدل الحمل ونسبة الخصوبة ونسبة التوأمية لم تختلف معنويا بإختلاف السلالتين. الخلاصة أن التأثير المفيد لإستخدام بروتوكول التحاميل المهبلية المصاحبة بالهرمون الكريوني الخيلي المنبه للغدد التناسلية نجح في إحداث شياع وتزاوج مخصب في كل من عنزات الأنجلونوبيان والبلدي خارج موسم التزاوج خلال الفترة من شهر فبراير الي نهاية شهرمايو.