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ECONOMIC METHODS FOR CONTROLLING THE ESTRUS WITH IMPROVING THE FERTILITY AND LITTER SIZE IN BARKI EWES

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SUMMARY

Cyclic Barki ewes (n=39), in good body condition, were assigned for 2 experiments. In experiment I, ewes were divided into 3 main groups, then each group was divided into 3 subgroups. Group I was injected intramuscularly (IM) with 3 ml lutalyse, 1 ml estrumate or 1 ml prosolvin. Group II were injected intra-vulvo-submucosa (IVSM) with 35-40% of IM dose. Group III were synchronized with the same dose and types of luteolytic hormones as group II, but the drugs were infused deep cervical (DC). The percentages of ewes that expressed estrus sign if icantly differ at P<0.05 (82.14, 96.55 and 86.67%) for IM, IVSM and DC, respectively. The interval times from PG injection to estrus were also significantly differ at P<0.01 (57.7, 52.5 and 60.35 hrs) for IM, IVSM and DC, respectively. The duration of estrus phases for IM, IVSM and DC were non significantly (31.43, 32.86 and 31.08 hrs, respectively). In experiment II, 36 Barki ewes were injected IVSM with 0.4 ml prosolvin, 19 (Group I) of these synchronized ewes were injected IM with 1 ml of receptal (GnRH) 48 hrs after PG injection and 17 ewes were used as control (Group II). All ewes were mated and observed for estrus 17-25 days of insemination. Seven-teen (89.47%) ewes that received GnRH and 15 (88.24%) in control group did not return to estrus up to day 25 of mating. Pregnancy rate at 2 months of mating were significantly differ at P<0.05 (76.47 and 89.47%, for control and GnRH group, respectively). The rate of twinning and litter size were also higher in GnRH ewes than control (36.84 Vs 17.6 %; P<0.09 and 1.23 Vs 1.44; P<0.05).

INTRODUCTION

Successful estrous synchronization can increase the probability that animals will be artificially inseminated during a designated period and contributed to improved reproductive efficiency (Ryan et al., 1995). Estrous cycle control can reduce management problems, especially those associated with daily monitoring to detect estrus in herds (Lehrer et al., 1992). In addition, the predetermine of the estrous time initiation and estrous detection could be reduced or even eliminated (Britt, 1987 and Romano, 1996). The forms of estrous cycle control will vary with the objective in relation to breeding management, but recent developments of original ones should be taken in account (MaCmillan and Peterson, 1993; Anderson and Day, 1994 and Ryan et al., 1995). The control of the estrous cycle is dependent on the manipulation of the hormonal events occurring during the normal ovarian/estrous cycle. The estrous cycle is frequently modified by forms of hormonal therapy which produce varied degrees of synchronized estrus (Macmillan and Peterson, 1993). Several hormonal treatments have been proposed to control the estrous cycle and induce ovulation in females (Cognie, 1990).

Prostaglandin $F_2\alpha$ (PG) and its derivatives were identified as the most potent luteolytic agents available for controlling the estrous cycle of sheep (McCracken et al., 1972) and cattle (Knickerbocker et al. 1986 and Ishwar and Pandey, 1992). These agents are commonly administered by IM route for the synchronization and induction of estrus. While intravulvosubmucosal (IVSM) administration in a small dose (10-25% of IM dose) is more economical for synchronization of estrus (Cardova et al., 1990 and Trivenidutt et al., 1995). The large variations in the estrous response observed after a synchronization treatment with prostaglandin and its analogue are the major factor in limiting conception rate in sheep. These variations can be attributed to intrinsic factors related to the ovarian status of the animals, beside the effects of extrinsic factors on the animal and related to forms of hormonal therapy and the route of injections (Roche, 1979 and Macmillan and Peterson, 1993).

Increasing the number of lambing could be a mean towards boosting lamb production in sheep (Fahmy and Lavallee, 1989). Several researchers have demonstrated that administration of GnRH in conjunction with traditional methods of estrous synchronization suppresses large follicles (McNeilly et al., 1986), initiate a new wave of follicular development and improves the number of ovulations (Cognie 1990) and may enhance the precision of estrous synchrony which may affect the fertility of synchronized animals (Bo et al. 1991 and Thatcher et al. 1993).

The aim of this study is to evaluate the use of a modified doses of lutalyse, estrumate and prosolvin at different sites of injection for estrus

synchronization, determination of onset and duration of estrus. It was also aimed to study if GnRH injection to synchronized Barki ewes improve pregnancy, lambing rates and litter size (number of lambs born per ewe).

MATERIALS AND METHODS

1) **Animals:** The present study was carried out on 39 Barki ewes aged 3 to 6 years old and bred at the experimental farm of the ARRI. Animals were housed in an open yard under natural light conditions. Ewes were fed on concentrate mixture containing 14% crude protein and 11% crude fiber. Barseem or darawa (green corn) were added during the green or dry season, respectively. Water and mineral blocks were offered ad-libitum.
2) **Drugs:** The medicaments used in the present study were:
   a) **Luteolytic hormones:-** *lutalyse* (Upjohn Co., Kalamazoo, USA), each ml contains 6.71 mg of dinoprost tromethamine salt, equivalent to 5 mg dinoprost; *estrumate* (Coopers Co., England), each ml contains 263 µg of cloprostenol sodium, equivalent to 250 µg cloprostnol and *prosolvin* (Intervet International, B. V., Holland), each ml contains 7.5 mg of luprostiol.
   b) **Gonadotrophic releasing hormone (GnRH):-** receptal (Hocchst Veterinary Gm bH, Germany), each ml contains 0.004 mg buserelin.
3) **Experimental design and procedure:** During the breeding season, all animals were observed at least for 2 successive estrous cycle before the start of the experiment, using a vasectomized ram of a good sexual desire.

Experiment I: In this experiment, ewes were divided into 3 main groups according to the route of luteolytic agents administred, then each group was divided into 3 subgroups according to the luteolytic hormone types. Group I were injected intramuscularly (IM) at days 10-12 of the cycle (day 0 was considered as day of estrus) with 3 ml lutalyse, 1.0 ml estrumate or 1.0 ml prosolvin. Group II were injected intra-vulvo-submucosa (IVSM) with 35-40% of IM dose, using an insulin syringe. Group III were synchronized with the same dose and types of luteolytic hormone as group II, but the route of administrated was deep cervical (DC). Ewes were lifted from hind limbs and by using small vaginal speculum and AI gun, the drugs were infused into the cervical canal deeply as possible and each ewe was raised at this position for about 5 min after drugs administration. The onset of estrus was detected with vasectomized ram 24 hrs after luteolytic hormone injection and every 8 hrs for 4 days. Ewes were considered in estrus when they stood to be mounted by the male. Estrous initiation was defined as the time between injection and the first accepted mount. Estrous duration was considered the time between the first and the last accepted mount. All ewes were used in more than one trial for estrous

synchronization after the elapse of 1-2 natural cycles. The time interval (hours) to estrus and estrous duration were recorded.

Experiment II: From the obtained results in experiment I, thirty-six Barki ewes were injected intra-vulvo-submucosa (IVSM) with 0.4 ml prosolvin. Nine-teen of this synchronized ewes were injected IM with 100µg of GnRH (1 ml of receptal) at the beginning of estrus and the others (n=17) were used as control. Ewes were naturally mated and observed for estrus 17-25 days after mating. Pregnancy diagnosis was performed 25 days after insemination, using sonography. Pregnancy state, lambing results and the number of lambs born per ewe were recorded. Pregnancy rate was defined as the percentage of synchronized ewes that became pregnant; lambing rate was defined as the percentage of synchronized ewes that lambed.

Statistical analysis: Data were analysed as 3x factorial design and Duncan's multiple test we used to compare between means of estrus onset and estrous duration. The data were analysed u ing Costat program; version 3.03, copyright 19 cottort software.

RESULTS

Table (1) revealed that, 82.14% of Barki ewes group I expressed estrous symptoms and respon ed to synchronization treatment. This percenta reached 86.67% in ewes treated with luteoly drugs through DC infusion (group III) a reached a maximum percentage (96.55%) in ew of group II which were treated with luteoly drugs through intravulvosubmucosal (IVSM).

Table (1): Effect of PG treatments and its route of injection on the estrous synchronization and duration in Barki ewes (Mean ± SE):

Treated Groups	Type of drugs	No. of treated ewes	No. of ewes responded to estrus (%)	Onset time to estrus (hrs.)	Duration of estrus (hrs.)
Group I (IM)	Lutalyse	10	8 (80)	61.13± 2.94	32.00± 1.07
	Prosolvin	9	8 (88.89)	57.25± 3.36	32.13± 1.65
	Estrumate	9	7 (77.78)	54.29± 4.16	30.00± 0.95
Overall		28	23 (82.14)^{c}	57.70± 2.00^{ab}	31.43± 0.74^{a}
Group II (IVSM)	Lutalyse	10	10 (100)	52.5± 3.55	33.3± 1.16
	Prosolvin	9	8 (88.89)	49.5± 3.87	32.38± 1.41
	Estrumate	10	10 (100)	54.9± 3.13	32.8± 1.05
Overall		29	28 (96.55)^{a}	52.5± 1.99^{b}	32.86± 0.67^{a}
Group III (DC)	Lutalyse	10	9 (90)	67.00± 3.42	30.78± 0.83
	Prosolvin	10	10 (100)	51.7± 2.00	31.1± 1.28
	Estrumate	10	7 (70)	64.14± 3.48	31.43± 1.63
Overall		30	26 (86.67)^{b}	60.35± 2.13^{a}	31.08± 0.56^{a}

The difference between these percentages was significant ($P < 0.05$). The averag tim from PG administration to estrus were 57.7±2.00, 52.50±1.99 and 60.35±2.13 hours for IM, IVSM and DC route of injection, respectively. The duration of estrous phase were correspondingly 31.43±0.74, 32.86± 0.67 and 31.08±0.56 hours. Statistical analysis revealed significant differences ($P<0.01$) between the time to estrus and non significant for estrous duration (Table 1).

Occurrence of estrus after PG injection in different routes showed that, most treated ewes (78.26%) of group I (IM) expressed estrus after 43 up to 59 hours of administration. In group II (IVSM), 32.14% of treated ewes came in estrus after 35 up to 43 hours of PG administration, while, in ewes of group III (DC), 34.62% of them showed estrous behavior after 59 up to 67 hours of PG infusion (Table 2).

Irrespective to the route of PG injection, the mean time intervals from treatment to the onset of estrus were 59.89±2.22, 52.73±1.79 and 57.42±2.16 hours after administration of lutalyse, prosolvin and estrumate, respectively. Statistically, there was significant influence ($P<0.01$) of luteolytic drug types on this criteria. Concerning the length of estrous phase, it averaged 32.07±0.62, 31.81± 0.80 and 31.58±0.71 hours after application of lutalyse, prosolvin and estrumate, respectively. However, no significant differences regrading estrous duration (Figure 1) could be observed.

Analysis of variance revealed that, the time interval between PG injection and onset of estrus was affected ($P<0.05$) by both type and route of luteolytic drugs injection, while, the estrous duration did not significantly differ. However, results showed negative correlation ($r=-0.38$; $P<0.01$) between the time of onset estrus and duration.

Table (2): Distribution of estrous onset in Barki ewes treated with PG at different routes of injections:

Treated Groups	Time to estrus (hrs)				
	35	43	51	59	67
	No. of ewes showing estrus (%)				
Group I (IM)	0 (0)	5 (21.74)	9 (39.13)	9 (39.13)	0 (0)
Group II (IVSM)	5 (17.86)	9 (32.14)	8 (28.57)	6 (21.43)	0 (0)
Group III (DC)	0 (0)	7 (26.92)	7 (26.92)	3 (11.62)	9 (34.62)

Figure (1): Effect of luteolytic drugs on the estrous synchronization and its duration in Barki ewes:

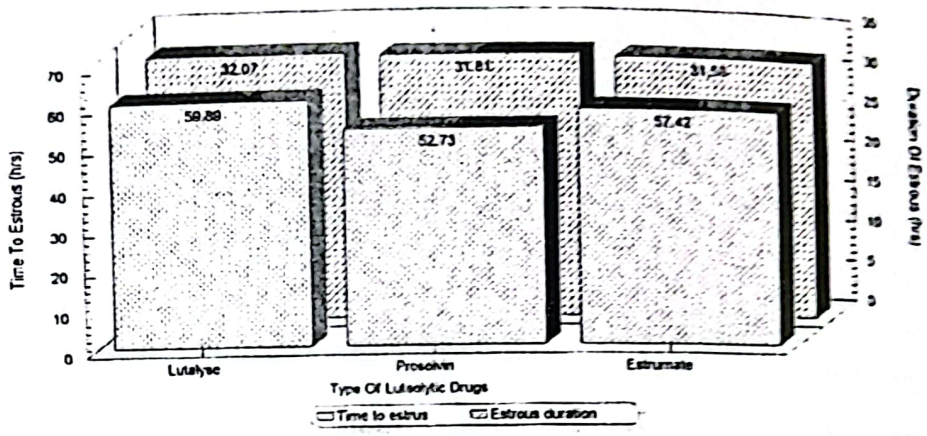

Table (3) showed the effect of GnRH injection on the pregnancy rate and litter size in ewes. Seventeen (89.47%) ewes that received GnRH and 15 (88.24%) in control group did not return to estrus up to day 25 of mating. Pregnancy diagnosis was confirmed using ultrasonography. Two ewes (11.76%) of control group showed early embryonic death between days 25 and 60 of gestation, while, nothing happened in GnRH treated group (this was determined by pregnancy diagnosis 2 months of gestation using ultrasonography). Pregnancy rate at 2 months of mating were significantly different at P<0.05 (76.47 and 89.47%) in control and GnRH group, respectively. However pregnancy rate was maintained unchanged up to the end of gestation in control group, but in GnRH group, one animal aborted after 2 months of pregnancy (Table 3). The rate of twinning and litter size were also higher in GnRH treated ewes than control ewes (36.84 Vs 17.6%; P<0.05 and 1.44 Vs 1.23; P<0.05).

Table (3): Influence of GnRH treatment on pregnancy rate and litter size in Barki ewes:

Treated Groups	No. Of ewes (%)			No. of ewes lambing (%)			
	Total	Non return to estrus	Pregnant at 2 months	Single	Twin	Total	Litter size
Control	17	15 (88.24)	13 (76.47)^{a}	10 (58.82)	3 (17.65)^{a}	13 (76.47)^{a}	1.23
GnRH	19	17 (89.47)	17 (89.47)^{b}	9 (47.37)	7 (36.84)^{b}	16 (84.21)^{b}	1.44

DISCUSSION

Several hormones have been shown to possess luteolytic activity when administered at various times throughout the estrous cycle (Hansel and Convey, 1983). $PGF_2\alpha$ and its analogues have proven to be the most practical means for inducing luteolysis and synchronizing estrus (Wiltbank et al., 1995). In this study PG was administrated as single dose at random stages of the estrous cycle, if the ewes did not respond to the first injection they were injected with a second dose, 11 days apart. The obtained results revealed that, the percentages of ewes that expressed estrous symptoms after the application of PG were 82.14, 96.55 and 86.67% for IM, IVSM and DC, respectively. Higher percentages of estrus were observed in ewes that received PG via IVSM and DC, since, the ability of PG to reach the uterine vein is faster and reaches to ovary directly via a counter current transfer mechanism in the utero-ovarian pedicle (McCracken et al., 1972), without passing through the pulmonary vascular bed, where it would be rapidly degraded to its inactive metabolites as in case of IM injection. Similarly, Cardova et al. (1990) suggested that effective estrus response to PG treatment through IVSM route may dependent on a unilateral pathway between the intravulvosubmucosa and ovary. During follicular maturation, intrafollicular PGF_2 and PGE_2 levels increase and reach maximum values approximately 64 hrs after injection of a luteolytic agents (Algire, 1989) during this time PGF_2 diffuses through the follicular wall (Espey, 1980) to activate the collagenolytic enzymes of the thecal fibroblasts in those follicles which have been selected to ovulate.

Based on the mean interval from PG injection to onset of estrus, the ewes of IVSM group expressed estrus 5 and 8 hours earlier than IM and DC ewes, respectively. The mean time intervals in the present study were influenced by the route of PG injection and its types, these intervals were 57.70±2.00, 52.5±1.99 and 60.35± 2.13 hours for IM, IVSM and DC group and were 59.89±2.22, 52.73±1.79 and 57.42±2.16 hrs, for lutalyse, prosolvin and estrumate, respectively. Different results were recorded by many authors. Bindon et al (1979) found the interval time from PG injection to onset estrus were 36.00±1.9 to 44.00± 1.8 hrs with 31.00±2.9 to 52.7±24 3.1 hrs duration of estrus. The time to estrus was significantly shorter in PG than progesterone synchronized ewes (Hayat, 1996). In accordance Mutiga and Mukasa-Mugerwa, (1992) found that the onset of estrus ranged from 43.5± 1.61 to 45.3±1.43 hrs by $PGF_2\alpha$, while it was 49.33±2.1 to 52.67±1.61 hrs by spongy removal. Also, Beal (1996), found the average interval from injection of PG to estrus is usually 60 to 72 hrs. This interval depends on the phase of follicular development at the time of PG injection, animals that possess dominant follicles that are still growing will show estrus in 48 to 60 hrs, while animals with follicles at the plateau stage or regressing phase will take more than 3

days (Bo et al., 1994 and Pinheiro et al., 1998). Others explained the variation in the timing of estrus to differences among animals in the rate of regression of the CL following treatment (Beal, 1996).

Analysis of variance showed non significant differences between ewes in groups I, II and III regrading duration of estrus (31.43±0.74, 32.86±0.67 and 31.08±0.56 hrs, respectively). However, negative correlation (r=-0.38; P<0.01) was found between estrous duration and interval to estrus. This result is in accordance with that recorded by Gallo et al. (1992) who reported that, neither progesterone nor duration of treatment influenced estrous duration. This indicates that there was an adequate surge of endogenous gonadotropins to initiate the sequence of hormonal events resulting in estrus and ovulation. In addition, Hanrahan and Quirke (1975) found a negative correlation within all breeds between the duration of estrus and the interval from spong withdrawal to the onset of estrus. Moreover, they found a negative relationship between the duration of estrus and ovulation rate. On the contrary Land (1970) and Bindon et al (1979) found a positive relationship between duration of estrus and fecundity as well as litter size.

Gonadotrophin releasing hormone (GnRH) has been used extensively to improve fertility and for the treatment of reproductive problems (Leslie et al 1984). Several researchers have demonstrated that treatment with GnRH to regress the dominant follicle and initiate a new wave of follicular development in conjunction with traditional methods of estrous synchronization may enhance the precision of estrus synchrony and may affect the fertility of synchronized animals (Bo et al., 1991 and Thatcher et al., 1993). Moreover, Schmitt et al. (1996) stated that $PGF_2\alpha$ injection followed by GnRH analogue (24 hrs) produced a high incidence (35%) of short return -to- service intervals. In the current study, the influence of GnRH on the pregnancy rate and litter size in Barki ewes was investigated. Pregnancy rate at 25 days in both control and GnRH groups were 88.24 and 89.47%, respectively. Although the differences between the two percentages were not significantly at 25 days of insemination but were significant (P<0.05) at 2 months of gestation, 76.47 and 89.47% for control and GnRH group, respectively. In the same line the present results revealed that, the early embryonic death between 25 days and 2 months of gestation was higher (11.76%) in control ewes than GnRH treated group. Although the factors causing embryo loss in ewes have yet to be established, but, the percentage of embryonic death which occurs as a result of an inappropriate relationship between the embryo and uterus in the absence of other obvious anti-fertility agent remains speculative (Ashworth, 1992). This may be due to the fact that ovine and bovine conspi tuses secrete proteins, prostaglandin's and ste oids which together with ovarian steroids modi uterine biochemistry and morphology which ma

lead to embryo mortality (Ashworth, 1992). There is evidence to suggest that luteal inadequacy is one of factors which lead to early embryonic death (Dowing 1980). Luteal inadequacy may result from environmental factors such as stress ornutrition (Wilmut et al., 1986). Others (Ashworth and Bazer, 1989) suggested, that even in species having only one or two ovulations, progesterone concentrations may induce changes in the uterine environment rendering it inappropriate for embryo development. Moreover, they found that, pregnancies in which embryo death occurs have different progesterone profiles during the first 2 weeks after mating than successful pregnancies. However in order to ensure successful pregnancy, it is essential that these conceptus products are secreted in appropriate quantities at the appropriate time.

The incidence of twinning and litter size in GnRH treated ewes present in this study were higher ($P<0.05$) than control group (36.84 Vs 17.65% and 1.44 Vs 1.23, respectively). Our results are supported by Walter et al. (1989); Kumar et al (1991); Rowe and East (1996) and Zenhom and Daghash (1997). Walton and Stubbings (1986) suggested that GnRH has the ability to release LH and FSH from the pituitaries of the treated animals and as result the secretion of LH is elevated during the preovulatory follicular stage. This LH surge, is essential for proper follicle development and ovulation process (McNatty et al., 1981). It is highly probable that the later LH surge resulting in later time of ovulation in the PG treated ewes avoided the problem of ovum aging and subsequent poor ovum development that can occur following delayed insemination (Scudamore et al., 1991), thus GnRH treatments improved embryo survival (Beck et al., 1994).

In conclusion, the present results indicate that, small dose (35-40% of IM dose) of PG or its analogues is effective for estrous synchronization in Barki ewes when injected IVSM and DC, but IVSM route is more simple and easy than deep cervical. Barki ewe is a breed of known low fecundity and to increase the pregnancy rate and litter size, GnRH administration, 48 hrs after PG injection is more effective.

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REFERENCES

Algire, J. E. (1989): Prostaglandins in follicular development and ovulation in cattle. M.Sci. Thesis, McGill Univ., Montreal, Quebec, Canada.

Andreson, L. H. and Day, M. L. (1994): Acute progesterone administration regresses persistant dominant follicles and improves fertility of cattle in which estrus was synchronized with melengestrol acetate. J. Anim. Sci., 72: 2955-2961.

Ashworth, C. J. (1992): Synchrony embryo-uterus. Anim. Reprod. Sci., 28: 259-267.

Ashworth, C. J. and Bazer, F. W. (1989): Interrelationships of proteins secreted by the ovine conceptus and endometrium during the periattachment period. Anim. Reprod. Sci., 40: 425-433.

Beal, W. E. (1996): Application of knowledge about corpus luteum function in control of estrus and ovulation in cattle. Theriogenology, 45: 1399-1411.

Beck, N. F. G., Peters, A. R. and William, S. P. (1994): The effect of GnRH agonist (buserelin) treatment on day 12 post mating on the reproductive performance of ewes. Anim. Prod., 58: 243-247.

Bindon, B. M., Blance, M. R., Pelletier, J., Terqui, M. and Thimonier, J. (1979): Periovulatory gonadotrophin and ovarian steroid patterns in sheep of breeds with differing fecundity. J. Reprod. Fert. 55, 15-25.

Bo, G. A., Adams, G. P., Pierson, R. A., Caccia, M., Tribulo, H. E. and Mapletoft, J. (1994): Follicular wave dynamics after estradiol-17B treatment of heifers with or without a progestagen implant. Theriogenology, 41: 1555-1569.

Bo, G., Pierson, R. A. and Mapletoft, R. J. (1991): The effect of estradiol valerate on follicular dynamics and superovulatory response in cows with synchromate-B implants. Theriogenology, 36: 169-183.

Britt, J. A. (1987): Induction and synchronization of ovulation. In E. S. E. Hafez (Editor), Reproduction in farm animals. Lea&Febiger, Philadelphia, 507-516.

Cardova, S. A., Jimenez, F. K. and Villa-Godoy, A. (1990): Intravulvo-submucosal injection of luprostiol may reach CL by a local unilateral pathway in cattle. Theriogenology, 33: 207.

Cognie, Y. (1990): Current technologics for synchronization and artificial insemination of sheep. World sheep and wool Congress. C: 16: 207-216.

Costat computer program copyright (1986): Version 3.03 Cottort Software.

Downing, J. M. (1980): Studies on the attainment of puberty and reproductive performance in Clun Forest ewe lambs. Ph. D., Dissertation, Univ. Wales.

Espey, L. L. (1980): Ovulation as an inflammatory reaction a hypothesis. Biol. Reprod., 22: 73-106.

Fahmy, M. H. and Lavallee, D. (1989): Reproductive performance of polypay, Dorset and their F1 cross under two accelerated breeding systems. Small Rumin. Res., 269-279.

Gallo, G. F., Algire, J. E., Srikandakumar, A. and Downey B. R. (1992): Effects of a prostaglandin F2 alpha analogues on the ovulatory response of superovulated heifers. Anim. Reprod. Sci., 27: 83-90.

Hanrahan, J. P. and Quirke, J. F. (1975): Repeatability the duration of estrus and breed differences in the relationship between duration of estrus and ovulation rate sheep. J. Reprod. Fert., 45: 29-36.

Hansel, W. and Convey, E. M. (1983): Physiology of the trous cycle. J. Anim. Sci., 57: 404-424.

Hayat H. Mohamed (1996): Some biochemical studies during estrous cycle and after synchronization in B ewes. M. V. Sci., Thesis (Zagazig Univ., Benha bra Egypt.

Ishwar, A. K. and Pandey, J. D. (1992): Oestrous synchronization and fertility in Black Bengal goats following ministration of progesterone/prostaglandin and gona rophins. Res. Vet. Sci., 52: 141-146.

Knickerbocker, J. J., Thatcher, W. W., Bazer, F. W., Drost, M., Barron, D. H., Fincher, K. B. and Roberts, R. M. (1986) Protein secreted by day 16 to 18 bovine conceptuses extends corpus luteum function in cows. J. Reprod. Fert., 17: 381-391.

Kumar, J., Osborn, J. C., Cameron, A. W. (1991): Luteinizing hormone and follicle stimulating hormone induce premature condensation of chromatin in goat oocytes. Reprod. Fertil., Dev., 3: 585-591.

Land, R. B. (1970): A relationship between the duration of estrous, ovulation rate and litter size in sheep. J. Reprod. Fert., 23, 49-53.

Lehrer, A. R., Aizinbud, E. and Lewis, G. S. (1992): Oestrus detection in cattle. Recent developmwnt. Anim Reprod. Sci., 28: 355-362.

Leslie, K. E., Doig, P. A., Bosu, W. T., Curtis, R. A. and Martin, S. W. (1984): Effect of gonadotrophin releasing hormone on reproductive performance of dairy cows with retained placenta, Can. J. Med. 48, 354-359.

Macmillian, K. L. and Peterson, A. J. (1993): A new intravaginal progesterone releasing device for cattle (CIDR-B) for estrous synchronization, increasing pregnancy rates and the treatment of post-partum anoestrus. Anim. Reprod. Sci., 33: 1-25.

McCracken, J. A., Baird, D. T. and Goding, J. R. (1972): Factors affecting the secretion of steroids from the transplanted ovary in the sheep. Rec. Prog. Horm. Res., 27: 537-582.

McNatty, K. P., Gibb, M., Dobson, C. and Thurley, D. C. (1981): Evidence that changes in luteinizing hormone secretion regulate the growth of the preovulatory follicle in the ewe. J. Endocr. 90, 375-389.

McNeilly, A. S., Jonassen, J. A. and Fraser, H. M. (1986): Suppression of follicular development after chronic LHRH immunoneutralization in the ewes. J. Reprod. Fertil., 76: 481-490.

Mutiga, E. R. and Mukasa-Mugerwa, E. (1992): Effects of the method of estrus synchronization and PMSG dosage on estrus and twinning in Ethiopian Menze sheep. Theriogenology, 38: 727-734.

Pinheiro, O. L., Barros, C. M., Figueiredo, R. A., do Valle, E. R., Encarnacao, R. O. and Padovan, C. R. (1998): Estrous behaviour and the estrus-to- ovulation interval in Nelore cattle (Bos indicus) with natural estrus or estrus induced with PGF2 alpha or norgestomet and estradiol valerate. Theriogenology, 49: 667-681.

Roche, J. F. (1979): Control of estrus in cattle. World Review anim. Prod.. 15: 49-76.

Romano, J. E. (1996): Comparison of fluorgestone and medroxyprogesterone intravaginal pessaries for estrous synchronization in dairy goats. Small Ruminant Research, 22: 219-223.

Rowe, J. D. and East, N. E. (1996): Comparison of two sources of gonadotropin for estrous synchronization in does. Theriogenology, 45: 1569-1575.

Ryan, D. P., Snijders, S., Yaakub, H. and O'farrell, K. J. (1995): Effect of programmed recruitment and ovulation of a healthy follicle on estrous detection and pregnancy rates in lactating dairy cows. J. Reprod. Fert. Abst., Ser., 15: 23.

Schmitt, E. J., Diaz, T., Drost, M. and Thatcher, W. W. (1996): Use of a GnRH Agonist or hCG for timed insemination in cattle. J. Anim. Sci., 74: 1084-1091.

cudamore, C. L., Robinson, J. J. and Aitken, R. P. (1991): The effect of timing of laparoscopic insemination in superovulated ewes with or without sedation on the recovery of embryos, their stage of development and subsequent viability. Theriogenology, 35: 907-914.

Thatcher, W. W., Drost, M., Savio, J. D., MacMillan, K. L., Entwistle K. W., Schmitt, E. I. and De la Sota R. I. (1993): New clinical uses of GnRH and its analogues in cattle. Anim. Reprod. Sci., 33: 27-49.

Trivenidutt, Kharche,S. D., Ansari, M. R. and Taneja, V. K. (1995): Superovulation with folltropin, estrous synchronization/induction using intravulvo-submucosal administration of luprostiol and embryo transfer in crossbred cows. Indian J. Anim. Sci., 65(9): 990-992.

Walker, S. K., Smith, D. H., Frensham, P. J., Ashman, R. J. and Seamark, R. F. (1989): The use of synthetic gonadotropin releasing hormone treatment in the collection of sheep embryos. Theriogenology, 31: 741-752.

Walton, J. S. and Stubbings, L. (1986): Factors affect the yield of viable embryos by superovulated Holstein Friesian cows. Theriogenology. 26: 167-177.Wilmut, I. Sales, D. I. and Ashworth, C. J. (1986): Maternal and embryonic factors associated with prenatal losses in mammals J. Reprod. Fert., 76: 851-864.

Wiltbank, M. C., Shiao, T. F., Bergfelt, D. R. and Ginther O. J. (1995): Prostaglandin F2alpha receptors in the early bovine corpus luteum. Biol. Reprod., 52: 74-78.

Zenhome, M. and Daghash, H. A. (1997): Response of infertile ewes to injection with gonadotropin releasing hormone (GnRH-analogue). Egyptian Soc. Anim. Repr. Fertil., 9th annual Cong. 91-102.