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Oestrous response and characterization of the ovulatory wave following oestrous synchronization using $PGF_{2\alpha}$ alone or combined with GnRH in ewes

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ABSTRACT

Effects of two oestrous synchronization protocols (Ovsynch vs double $PGF_{2\alpha}$ injection) on oestrous activity, characterization of the ovulatory wave and fertility were studied in ewes during the breeding season. The Ovsynch-treated ewes (GPG-group, $n = 14$) received a GnRH analogue (4 μg buserelin; i.m.) followed by a $PGF_{2\alpha}$ analogue (10 mg dinoprost tromethamine; i.m.) on day 7 and a second injection of a GnRH analogue 48 h later. The double $PGF_{2\alpha}$-treated ewes (2PGF-group, $n = 14$) received a double injection of a $PGF_{2\alpha}$ analogue (10 mg dinoprost tromethamine; i.m.), 11 days apart. Follicular dynamic was ultrasonographically monitored for four consecutive days, starting at the day of the last $PGF_{2\alpha}$ treatment in both groups (day 0). Oestrous response and fertility traits were also recorded. The total number of follicles and the number of small follicles did not differ between the two regimes, while the number of medium follicles increased ($P = 0.05$) in the 2PGF-group. During four consecutive scanning days (0, 1, 2 and 3), the number of the large follicles increased significantly in both groups from day 0 to day 2. This increase continued until day 3 in the 2PGF-group, whereas it decreased sharply in the GPG-group. Oestrous rate and time to onset of oestrus were lower ($P < 0.05$ and $P = 0.1$; respectively) in the GPG-group (42.85%; 34.0 ± 2.1 h) than in the 2PGF-group (78.57%; 50.4 ± 7.3 h). Treatment with GPG-protocol reduced ($P < 0.05$) the interval from the second $PGF_{2\alpha}$ treatment to ovulation compared with the 2PGF-protocol (85.8% of ewes ovulated within 60.0 ± 3.1 h vs 78.6% of ewes ovulated within 84.4 ± 3.1 h). Conception rate and litter size did not differ between the two regimes. In conclusion, a tighter synchrony of ovulation could be obtained following application of Ovsynch-protocol in sheep.

1. Introduction

Application of oestrous synchronization protocols to control reproduction in sheep is a worldwide practice (Boscos et al., 2002). However, a double prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) treatment at an interval 9–12 days is commonly used for oestrous synchronization during the breeding season, it does not provide an adequate tight synchrony of oestrus and/or ovulation (Martemucci and D'Alessandro, 2010). Generally, the response for $PGF_{2\alpha}$ based protocols depends on the presence of an active corpus luteum; also time interval for onset of oestrus depends on the follicular status at the time of treatment (Menchaca and Rubianes, 2004). Several studies reported that protocols those include GnRH agonist and $PGF_{2\alpha}$ such as a GnRH-$PGF_{2\alpha}$-GnRH treatment sequence, known as "Ovsynch" or "GPG" protocol, tend to provide a full control of ovarian functions (Holtz et al., 2008). This protocol has been used successfully for oestrous/ovulation synchronization in dairy cows (Wiltbank and Pursley, 2014). However, conflicting results have been reported in small ruminants. Ali et al. (2008) obtained more scattered ovulation and less oestrous rate when the GPG-protocol was used in Farafra ewes. On the other hand, Deligiannis et al. (2005) and Riaz et al. (2012) obtained adequate synchrony of ovulation in ewes and goats; respectively. Accordingly, more understanding for the effect of such protocol on ovarian activity and oestrous response is required. The objective of the present study was thus to evaluate the follicular dynamic of the ovulatory wave and reproductive performance following application of two simple oestrous synchronization protocols depends on a $PGF_{2\alpha}$ alone or combined with a GnRH in Barki × Rahmani crossbred ewes during the breeding season.

2. Materials and methods

The present study was conducted at the Agricultural Experimental Station (31°20′ N, 30° E), Faculty of Agriculture, Alexandria University, Alexandria, Egypt,

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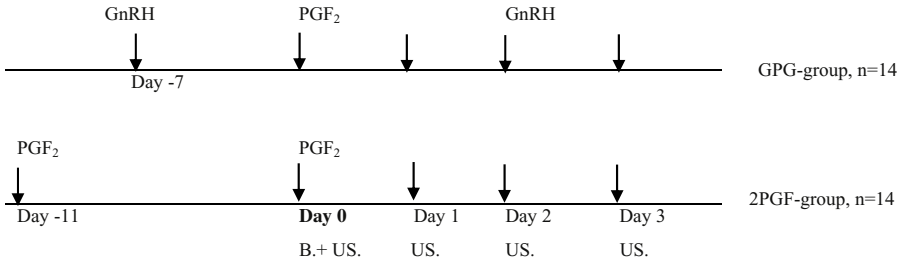

Fig. 1. Schematic frame work of the study showing times of blood sampling and ultrasonography examinations. Day 0 = day of the last $PGF_{2\alpha}$ treatment in both groups (GnRH = gonadotropin releasing hormone, $PGF_{2\alpha}$ = prostaglandin $F_{2\alpha}$, B. = bleeding and US. = ultrasonography scanning).

during the period from July to December (breeding season). All procedures and experimental protocols were conducted according to the Guide for the Care and Use of Agricultural Animals in Research and Teaching, Federation of Animal Science Societies (FASS, 2010).

2.1. Animals and management

Twenty-eight fertile, multiparous, non-lactating Barki × Rahmani crossbred ewes, ageing 2.5 ± 0.30 years, weighing 45.2 ± 2.3 kg and with a mean body condition score of 3.1 ± 0.2 (scale ranging from 1 = emaciated to 5 = obese; Jefferies, 1961) were used. Animals were kept outdoors with shade and shelter during the daytime, and were housed in semi-open barns at night. Animals were fed a chopped green maize as a roughage, and concentrate supplements at a daily level of 500 g/ewes (63% TDN and 14% CP), according to their body weight requirements (NRC, 1985). Ewes had free access to clean water during the experimental period. Ewes were diseases-free and clinically normal with a healthy appearance.

2.2. Synchronization protocols

Ewes were divided into two equal homogenous groups ($n = 14$) with respect to their body weights and parity. Ewes in the first group (GPG-group) received an i.m. injection of 1 mL GnRH analogue (4 μg buserelin/mL, Receptal, Boxmeer, Holland), followed by an i.m. injection of 2 mL $PGF_{2\alpha}$ analogue ($PGF_{2\alpha}$, Lutalyse, 5 mg dinoprost tromethamine/mL, Pfizer) on day 7, and a second i.m. injection of the GnRH analogue 48 h later. Ewes in the second group (2PGF-group) received a double i.m. injection of 2 mL prostaglandin $F_{2\alpha}$ analogue, 11 days apart. Day at which the last $PGF_{2\alpha}$ treatment was applied in both groups was defined as day 0, the experimental design is shown in Fig. 1.

2.3. Detection of oestrus and mating management

Oestrus was observed twice a day beginning one day after the last $PGF_{2\alpha}$ treatment (day 0) in both groups using teaser rams. Ewes exhibiting overt signs of oestrus in both groups were hand-mated with proven fertile rams and every 12 h until the end of the signs of oestrus. Time to onset of oestrus was estimated to be the halfway between the beginning of the oestrous detection time and the first positive overt signs of oestrus. Duration of oestrus was estimated to be the halfway between the last negative and the first positive overt signs of oestrus.

2.4. Ultrasound examination and progesterone analysis

A real time B-mode scanner (Dynamic Imaging Concept MLV, Livingston, Scotland) equipped with (5 and 7.5 MHz) linear array probe was used to monitor the ovarian activities of synchronized ewes. The scanning procedure described by Gonzalez-Bulnes et al. (2010) was used. Briefly, the probe was fitted to a plastic rod (1 × 30 cm) as an adaptor to enable the insertion of the probe into the rectum while the ewes in standing position. The probe was lubricated by a hydrosoluble gel and sheathed with PVC pipe (2 × 35 cm) to avoid damage of the rectal mucosa, and was gently inserted about 20 cm through the rectum after faeces removal until the anechoic content of the bladder was visible on the screen, then the probe was rotated 90° clockwise and 180° counterclockwise across the reproductive tract until the uterine horns and both ovaries were scanned. Ovarian activity was daily monitored for four consecutive days starting at day 0 (day of the last $PGF_{2\alpha}$ treatment in both groups). Number of corpora lutea (CLs) observed on day 0 was recorded (taken as an indicator for the effect of first injection in both protocols on ovulation induction). All follicles greater than 2 mm in each ovary were counted and calibrated, and then were classified into three categories according to their sizes (small follicles: ≥2 to 3 mm; medium follicles: >3 to <5 mm; large (ovulatory) follicles: ≥5 mm; Hashem et al., 2015). Time needed for ovulation was presumed as the interval between the first detection of the ovulatory follicle at day 0 (day of the last $PGF_{2\alpha}$ treatment in both groups and the first ultrasonography examination, Fig. 1) and the half-interval from the last detection of the ovulatory follicle to its disappearance (ovulation was confirmed by the disappearance of the largest ovulatory follicle). Pregnancy diagnosis was carried out by scanning the uterine contents at 32 days post-mating. The blood samples were collected by a jugular venipuncture on day 0 at morning. Serum progesterone (P_4) concentration was measured using solid-phase enzyme immunoassay kit obtained from Callbiotech Inc, USA. The lower limit of detection (95% B/B°) was 0.3 ng/mL, the intra- and inter assay coefficient of variations (CV) were 7.1% and 2.4%; respectively.

2.5. Statistical analysis

Square root transformation was used to approximate normal distribution of total number of follicles and their population (according to diameter) before subjecting to analysis of variance (Harvey and Damon, 1987). Data observed once a time including progesterone concentration on day 0, onset of oestrus, duration of oestrus, time of ovulation and litter size were analyzed by generalized linear model (GLM) procedure (SAS, 2004) using the following model: $y_{ij} = \mu + T_i + e_{ij}$ in which y_{ij} is the observed value of the dependent variable, μ is the overall mean, T_i is the fixed effect of the ith treatment ($i = 1{:}2$) and e_{ij} is the residual error. To study the effects of oestrous synchronization protocol on total number of follicles and follicle population. The mixed procedure for repeated measurements (SAS, 2004) was used as shown in this model: $y_{ijk} = \mu + T_i + D_j + (TD)_{ij} + e_{ijk}$ in which y_{ijk} is the observed value of the dependent variable determined from a sample taken from each animal, μ is the overall mean, T_i is the fixed effect of the ith treatment ($i = 1{:}2$), D_j is the fixed effect of the jth day of scanning ($j = 1{:}4$), $(T \times D)_{ij}$ is the interaction between treatment and day of scanning, and e_{ijk} is the residual error. Differences among the treatment groups were detected using Duncan's new multiple range test. Categorical data were analyzed using chi-square test. All the results were expressed as the mean (±SEM). The statistical significance was accepted at $P < 0.05$.

3. Results

3.1. Characterization of the ovulatory wave

Data shown in Table 1 and Fig. 2 illustrates the effect of oestrous synchronization protocols on the ovulatory wave characterization during the scanning period (four consecutive days). Percentages of active CLs and concentrations of serum P_4 on day 0 were in the similar trend in both protocols. Total number of follicles and number of small follicles were not affected by the treatment, day of scanning or the interaction between them. On the other hand, the overall mean number of medium follicles was significantly affected ($P = 0.05$) by the treatment, the number of medium follicles was two-fold higher in the 2PGF-group (0.88) compared with the GPG-group (0.44). Statistical analysis showed that the number of large follicles was affected ($P < 0.05$) by the treatment by day of scanning interaction (Table 1). During four consecutive days (0, 1, 2 and 3) of ultrasonography examination, the number of the large follicles increased significantly with time advancement in both groups (from day 0 to day 2). This increase continued until day 3 in the 2PGF-group, whereas it decreased sharply in the GPG-group at the same time (Fig. 2). Growth rate of the ovulatory follicles was faster ($P < 0.05$) in the GPG-group compared with the 2PGF-group (Table 1). Percentages of ewes those had ovulated did not differ between the two groups, however time from the last $PGF_{2\alpha}$ treatment (day 0) to ovulation was shorter ($P < 0.05$) in the GPG-group (Table 1).

3.2. Oestrous response and fertility traits

As set out in Table 2, percentage of ewes displaying oestrous behaviour following the end of each protocol was significantly