The PGF2α, a less costly and invasive means than progestogens to manipulate the sexual activity in out-breeding season of the "Ouled Djellal" Algerian ewes

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Abstract

The sexual activity of 237 ewes of the most representative of Algerian sheep breeds (about 70%): the "Ouled Djellal", was studied during the anestrous season i.e. in spring (April 2016), in Constantine, a north-eastern Algerian province. A number of 50 ewes, chosen randomly from the flock, were subjected to a double injection of PGF2α at an 11 days' interval and the 187 females remaining have served as a control group. 80% of the ewes treated started mating about 60 hours after the release, with the whole flock, of 14 rams that have been previously separated from the flock but not sensory isolated. A lambing percentage of 90 spread over 23 days and 98.9 spread over 47 days occurred respectively in the treated and control group. Both treated and untreated ewes with PGF2α exhibited estrus (April and May), got pregnant and lambed within a period of less than 2 months. The use of PGF2α, through the luteolysis it induces during spring: first confirms the results of previous studies reporting the ability of "Ouled Djellal" ewe to exhibit sexual activity throughout the year, second shortens considerably the laming period and last and more importantly can be substituted to the use of the progestogens impregnated sponges, far more invasive, time consuming and more costly, as a means to manipulate the female sexual activity.

Key words: *non-seasonal sexual activity, PGF2α, sheep*

Introduction

Ewe's reproduction can be controlled and several motivations encourage breeders and / or researchers to synchronize or induce heat during the non-breeding season. Increasing the herd's productivity, organizing and planning reproduction, the possible use of artificial insemination and homogeneous batches for different experimental purposes may improve the annual productivity of the flock just like using the genetic or physiological pathway (Lamrani et al 2008.)

In Algeria, sheep farming is the most widely represented animal agricultural activity. It is the major source of red meat. The ministry of agriculture and rural development (MADR, 2017) has evaluated at around 28,4 million heads the national sheep population. The white breed sheep "Ouled Djellal" is the most representative; it accounts for 61% (Dehimi 2005), 63% (Boucif et al 2007) and 70% (Lafri 2013). Sheep farming has always been conducted in a traditional way which has limited its productive and reproductive potentials. In order to rationally exploit these latter, obtaining reliable scientific data on the cyclicity of ewes is a prerequisite for the control and improvement of the performances of this species. To this end, a study was carried out on the sexual seasonality of the white breed "Ouled Djellal" ewe in Constantine, a north-eastern province. PGF2α, a hormone known to be efficient only on ewes that cycle was used during the period reported to be a seasonal anestrous period (April 2016), in order to: firstly, confirm that the "Ouled Djellal" ewes are non-seasonal breeders as reported in previous research (Niar et al 2001; Taherti et al 2016) and secondly, if so, to use the PGF2α or its analogues as a substitute to progestogens which are more invasive (intravaginal progesterone impregnated sponges bring about local vaginal reactions; intramuscular injection of eCG), time consuming (12 to 14 days) and more costly (over twice the price of PGF2α). Moreover, PGF2α can be administrated intramuscularly which improves animal management and welfare and in addition, the production of chemical residues is decreased since it is rapidly and almost completely (99%), metabolized in the lungs (Abecia et al 2011).

Material and methods

Location, climate, period, study protocol and animals

The study was carried out in a public demonstrative farm located in El Khroub, 13km from the chief town Constantine. The climate of the study area is of the Csa type (Cs: warm temperate climate with Mediterranean dry summer, a: hot summer, mean temperature of the hottest month > 22° C) According to the Köppen-Geiger classification 1900. In El-Khroub, the average annual temperature is 15.6°C and the average annual rainfall is 540 mm /year. Fifty (50) ewes of the "Ouled Djellal" white breed, randomly selected from a flock of 237 females, aged 2 to 5 and that have at least lambed once, were treated with a synthetic analogue of PGF2α (Dalmazin® FATRO) in the form of a sterile aqueous solution containing 75 micrograms of D-Cloprosenol (dextrorotatory isomer) through two intramuscular injections of 1 ml each at an interval of 11 days during the spring season (April 8 and 19 of the year 2016); the rest of the females (187) were left untreated and served as the control group.

For an easy identification and to facilitate heat detection, the 50 treated ewes were rump marked.

Their body condition score (BCS) was assessed on the 1st and 2 nd ultrasound exam using the method described by Russel (1984) and Lloyd (2005). On a scale of 1-5, a score ranging from 1 (extremely thin) to 5 (extremely fat) was assigned to each individual and the marks were frequency classified in the following pre-defined weight ranges: **< 2, ≥2<3, ≥3**.

In order to evaluate the BCS, the lumbar region that starts from the last rib, is localized; the spinal and transverse processes of the lumbar vertebrae are then palpated so as to evaluate the amount of muscle (far = longissimus dorsi) and the degree of adiposity that covers them.

The ewes grazed morning and afternoon, received a concentrated supplement and a mixture of hay of vetch and oat, and had access to water twice a day. The 14 breeding rams, after having been separated but not sensory isolated from the flock, were released with the 237 ewes (treated and untreated) about 60 hours after the 2nd PGF2α injection.

A very experienced shepherd was charged to report the number of covered ewes on a period of 3 days beginning from the day of rams' release. The rams remained 38 days within the flock before being removed so that the non-pregnant ewes could be covered at least twice (after the first estrus induced, 17 days and 34 days later). A first ultrasound examination was done 36 days on the 50 ewes treated, to diagnose the females having been fertilized at around 2 to 4 days after release of the rams and a second ultrasound examination was realized 37 days after their withdrawal, i.e. 49 days after the first ultrasound examination and 85 days after the rams' release to diagnose the pregnant females fertilized during the 2nd and 3rd estrus, i.e. .around 17 and 34 days after the release of the rams.

Results

After ram release, about 60 hours after the 2nd injection of PGF2α, with all ewes making up the flock (237), forty of the fifty ewes treated (rump marked with yellow paint) were covered and none of the187 untreated ones was, according to the shepherd in charge of heat detection. 80% of ewes, as a result of PGF2α heat inducing, were covered. Following the 1 st ultrasound examination of the fifty animals treated, that was performed 36 days after ram release, a pregnancy rate of 10% was recorded, while at the second ultrasound examination of the 40 remaining non- pregnant ewes or whose pregnancy stage did not allow an accurate diagnosis (i.e. less than 25 days of gestation) 35 of them were pregnant (table 1).

Table 1. Outcome of the pregnancy diagnosis of the 1st and 2nd ultrasound examination of the treated group.

Ultrasound examination	**1st**		**2nd**	
Results	**N**	**%**	**N**	**%**
+	10	20.00	35	87.5
- (?)	40	80.00	5	12.5
Total	50	100.00	40	100.00

(?): represents ewes whose gestation stage does not allow

diagnosis (<25 days).

Table 2 gives the total number of ewes in the flock, the number of ewes treated with PGF2α and the number and percentage of lambing as a result of natural estrus and PGF2α induced estrus.

Table 2. Number and percentage of lambing of treated and untreated ewes.

	Untreated group		**Treated group**		
	N	**Lambing**	**N**	**Lambing**	**Total**
Number	187	185	50	45	230
%	78.9	98.9	21.1	90.00	97.05

It should be noted that: a / five ewes from the treated flock remained non-pregnant. b / two non-pregnant ewes in the control group died.

The figure 1 summarizes the various steps from the first PGF2α treatment to the last lambing of all treated and untreated ewes constituting the flock.

PLG1: Lambing period (in days) of the untreated ewes **G1**. PLG2: Lambing period (in days) of the treated ewes **G2**.

Figure 1. Schematic representation of periodic events from the first PGF2α treatment until lambing of the last ewe of the untreated and treated group.

The table 3 and figure 2 show the monthly distribution rate of lambing of the treated and control group ewes.

Table 3. Monthly distribution rate of lambing of the untreated and treated ewes.

	Group				
	Treated		**Untreated**		
Period	**N**	**%**	**N**	**%**	**Total**
September	3	6.66	13	7.00	16
October	42	93.33	162	87.5	204
November	00	00	10	5.5	10
Total	45	100.00	185	100.00	230

Figure 2. Graphical representation of the monthly lambing distribution rate of the untreated and treated group of ewes.

Figure 3. Distribution of lambing of treated ewes according to the 1st and 2nd ultrasound pregnancy diagnosis.

It should be noted that one ewe lambed during September while it was diagnosed pregnant during the 2nd ultrasound examination and that another one lamed beyond October, the seventh while diagnosed pregnant during the first ultrasound exam (figure 3).

The analysis via the Kh2 test (table 4) of the relationship between the BCS and the rate of pregnancy showed no significant effect ($p>0.05$) as to the first ultrasound examination whereas it became significant at the time the second ultrasound examination was performed ($p<0.05$).

Table 4. Pregnancy rate according to the BCS of ewes recorded at the 1st and 2nd ultrasound examination.

BCS	**< 2**				**≥ 2 < 3**				**≥3**			
Ultrasound	**1st**	**%**	**2nd**	**%**	**1st**	**%**	**2nd**	**%**	**1st**	**%**	**2nd**	**%**
Number	7	14.00	5	11.1	9	18.00	9	22.5	34	85.00	26	65
+	1	14.3	3	60.00	1	11.1	0	00	8	23.5	24	92.3
-(?)	6	85.7	2	40.00	8	88.9	9	100.00	26	76.5	2	07.7
Total Number / %	10	20.00	35	87.5								
X^2	*: $p<0.05$.											

Discussion

Eighty percent of the ewes (80%) treated at least were covered after the release of rams with all ewes of the flock about 60 hours after the 2nd PGF2α injection (table1). This showed that these animals had a sensitive corpus luteum, which argues in part for the absence of an extended seasonal anestrus. This protocol can be applied throughout the year as for the tropical breeds, where ewes have a continuous estrous cycle and no seasonal anoestrus (Godfrey et al 1997, 1999). In addition, according to the observation made out of the monthly lambing distribution curve established for the control group, drawn from the data of tables 2 and 3, figures 1 and 2, this statement is to be enhanced since the per cent of lambing recorded was similar between the two ewe groups. It is worthy of note that the lambing period in the treated group was shorter, half of that of the control group (23 vs 47 days), most probably as a result of PGF2α heat synchronization which led to the fertilization of the treated group during the 1st and 2nd estrus.

The ram effect may have not been involved, since the rams were only separated but not sensory isolated from the flock, although it has been reported that ewes in contact with the male at the end of the estrus synchronization treatment ovulate earlier than those deprived of this contact (Ungerfeld et al 2003**,** Hawken et al 2007) and that the ram effect was reported to be more effective during a larger part of the year in the non-seasonal breeds as they are more sensitive, and less effective in seasonal breeds during the seasonal anestrus period (Lindsay et al 1975, Hawken et al 2007).

The high percentage of ewe covering obtained during April, a month usually considered as an out breeding season, enhances the non-seasonal sexual activity of the "Ouled Djellal" ewe (as reported by Chemineau et al 1992, Karsch et al 1984,Lincoln and Short 1980).The ewes were able to cycle during this period despite the presence of rams.

Studies have also shown that ewes who express sexual activity before the introduction of the ram remain on their cyclical rhythm and are mainly fertilized between the introduction of the ram and the 17th day of the cycle (Raes 2010). These statements argue for the cyclicity of all ewes of the flock despite the proximity of the rams.

Our results are in agreement with those reported by Niar et al (2001) in a study carried out on the sexual activity of three different Algerian sheep breeds (Ouled Djellal, Hamra and Rembi) during the period of seasonal anestrus, which showed that these breeds are capable of expressing estrus all year around. They also agree with what was recently reported by Taherti et al (2016), in a study carried out on the monthly variations of the sexual activity of "Ouled Djellal" ewes reared in Chlef , a north-western city of Algeria. These authors showed that these animals expressed serum progesterone levels high enough to prove that they are capable of cycling all the year around.

According to the outcome of the second ultrasound examination (figure 3), the ewes diagnosed pregnant should have normally lambed some time later than the last lambing of the ewes diagnosed pregnant during the first ultrasound examination, but according to the lambing dates one ewe declared pregnant at the 2^{nd} ultrasound examination lambed at the same time as the ewes declared pregnant at the 1^{st} ultrasound examination. This case could have been a false negative because the stage of pregnancy of this ewe was probably too early to detect or the ewe was fertilized between the first and second estrus. Similarly, 4 ewes declared pregnant during the first ultrasound examination lambed at the same time as the ones declared pregnant during the 2^{nd} ultrasound examination. This could have been due to false positives, early embryonic mortality, error in date of lambing and/or an extended pregnancy period.

During the first ultrasound examination made 36 days after the rams' release, 20% of the ewes were diagnosed as pregnant and 80% as non-pregnant or were at an undetectable pregnancy stage (table 1). The pregnancy rate during the first ultrasound examination could thus have been higher than that recorded when referring to the date and lambing period of the ewes treated (figure 3). This low pregnancy rate (20%) could have been due to several factors, including an error in the detection of estrus, an early embryonic mortality or fertilization failure, some ewes may had been in true anestrus or had a too low body condition score (table 4).

The analysis via the X2 test of the relationship between the BCS and the presence or the absence of pregnancy at the first ultrasound examination which took place 36 days after the rams' release showed that there was no significant effect ($p > 0.05$) of the BCS on the pregnancy rate (table 4); however, the same analysis performed during the second ultrasound examination, 49 days later, showed a significant effect ($p<0.05$) of the BCS on the pregnancy rate (table 4). It is difficult, though, to quantify this effect since a certain number of ewes were pregnant during the first ultrasound examination but could not be detected in view of their early pregnancy stage. This may also explain why out of the 3 non pregnant ewes in the treated group 2 had a BCS, at both ultrasound examinations, of less than 2 on a scale of 5.

Robinson et al (2002), report that the effect of the BCS can attenuate that of the photoperiod, this concords with our results particularly at the 1 st ultrasound examination which did not prove any effect of the BCS on the cyclicity as shown through the pregnancy rate obtained (table 4) as a result of the BCS of the ewes (14.3%, 11.1% and 23.5% for a BCS <2, ≥ 2 <3 and ≥3, respectively). This suggested that the photoperiod has little or no influence on the reproduction of the sheep followed-up which confirms the non-seasonal sexual activity.

However, the results obtained at the second ultrasound examination revealed a significant effect of the BCS on the pregnancy rate (60%, 0.0% and 92.3% for a BCS <2, ≥ 2 <3 and ≥3 respectively). This may well have accounted for the better percentage of laming following the second ultrasound examination.

The difference between the pregnancy percentages of 14.3% vs 60% for BCS <2 in the 1st and 2nd ultrasound examinations respectively could be due to several factors including the short period between the last lambing and the treatment, a gain of weight for two ewes during the second ultrasound examination or the stage of lactation. Goulet (2000) reported a negative effect of sucking on reproductive performance associated with a rise of prolactin known to inhibit the secretion of LH (Pope et al 1989) resulting in acyclicity (Mandiki et al 1990).

The difference between the gestation rates recorded: 0.0% vs 92.3% for ewes included in the frequency class of BCS> 2 <3 and ≥ 3 respectively at the second ultrasound examination may be due to an overstepping of the BCS of some ewes, an error of BCS data consignment and/or that some ewes whose BCS were > 2 <3 were in anestrus. Our results are in concordance with those of other researchers (Folch et al 2000, Thimonier et al 2000, Atti et al 2001, Lassoued and Khaldi 1993, Molina et al 1994, Cheminaux et al 1996 and Robinson et al 2002**)**, who reported that, the sexual activity as well as the duration and intensity of ewe anestrus is not only due to the photoperiod but also to the BCS and those of Benyounes et al (2013),who reported that the anestrus was shorter in "Ouled Djellal" ewes with a BCS ⩾ 3 and that their seasonal sexual inactivity was less marked when the nutritional level was respected. They suggest that the term "seasoning" should be replaced by an extended anestrus following an inadequate diet. This was true as far as the BCS was concerned since the followed-up ewes had a better fertilization rate at the second ultrasound exam when their body reserves were higher (table 4).

Conclusion

- The most important Algerian white sheep breed: "Ouled Djellal" showed a true luteal activity when treated by means of a PGF2α analogue during the period usually corresponding to the seasonal anestrus (spring).

- An 80% estrus rate, as determined by the number of covered ewes after ram's release about 60 hours after PGF2α estrus synchronization was achieved. Moreover, the BCS more than the photoperiod was effective to favor sexual activity. In addition, an overall lambing rate of 97% was noted over 23 and 47 days for treated and untreated ewes respectively.

- The use of PGF2α, apart from the fact that it induced luteolysis, reduced considerably the breeding and lambing period.

- These results show that the "Ouled Djellal" Algerian white breed sheep has a continuous sexual activity most of the year, and that this ability can be used for a better management of sheep breeding to improve their reproductive performances.

- The PGF2α, either to induce, synchronize heat or to shorten the lambing period, could be substituted to the use of progestogens which are invasive, more costly and time consuming.

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