CIDR and Ultrasound-Fetometry Scan; Comparative Study Between Sheep and Goat in Egypt

A. Ahmed Ezzat¹, A. Sayed Abd El-Naeim², I. Kamel Sobhy¹

¹Dep. of Theriogenology, Faculty of Veterinary Medicine, South Valley University, Qena 83523, Egypt.
²Dep. of Theriogenology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia 41522, Egypt.

Abstract

The present study aims to evaluate the impact of controlled internal drug release (CIDR) in combination with prostaglandin F2α (PGF2α) and pregnant mare serum gonadotropin (PMSG) treatments on reproductive performance of dairy ewes and does besides, evaluation the accuracy of pregnancy diagnosis during the breeding season. Twenty female animals divided as Ossimi ewes (n=10) and Baladi does (n=10) were subjected to ovsynch regimen. Ultrasonographic examinations were conducted weekly using real-time B-mode ultrasound scanner by transrectal linear and transabdominal convex probes starting from day 14 post service. This study showed that estrus response was 100% in both ewes and does. The conception rates were 90% in ewes and 80 % in does. The incidence of twinning was higher in ewes when compared to that in does with incidence rate 67% and 50 in ewes and does, respectively. The accuracy of ultrasound examination for determining fetal number was 100% for single and twin pregnancy. The birth rate was 100 % for both species; and this was confirmed at lambing / kidding. In conclusion, our results indicate that using biotechnologies such as ovsynch regimen and pregnancy diagnosis by ultrasonography are useful to improve the management of reproduction in ewes and does.

Key words: CIDR, CRL, BPD, FOD, Progesterone, PMSG, Ultrasound.

Correspondence to:
Ahmed Ezzat
Ahmedahmed.ezzat@vet.svu.edu.eg.

1. INTRODUCTION

Sheep and goat are significant contributors in the global food and wool production. In Egypt, they constitute 5.60 and 4.13 million heads of sheep and goats, respectively (Egyptian Ministry of Agriculture, economic statistic book 2009). They serve as investment due to their higher fertility, short gestation interval, ability to produce under limited feed resources and harsh environmental conditions (Tsedeke, 2007). Sheep and goats are short-day breeders under natural environmental conditions having the regimen of 16 hrs darkness and 8 hrs light during the period of late winter and early spring (Hafez, 1952), and that reproductive property could be attributed to maintenance of levels of photo responsiveness (Gomez-Brunet et al., 2008). Reproductive performance including ovulation, fertilization, implantation, gestation and parturition is affected by environmental, health, management and genetic factors. Efforts are continuously paid to improve the fertility rates of sheep and goat through the assisted reproductive technologies (ART) such as estrus synchronization and diagnosis of early pregnancy diagnosis and gestational age by ultrasonography (Amiridis and Cseh, 2012). Estrus synchronization depends on either extending the luteal lifespan with exogenous progesterone (P₄) or by inducing premature luteolysis using prostaglandin F2α (PGF2α)
In that sense, hormonal treatment using some drugs such as pregnant mare serum gonadotropin (PMSG), P$_4$ and PGF2α either alone or in combination would be a good strategy to improve reproductive performance and fertility. The aims of the present study are to evaluate the impact of using the recent device of controlled internal drug release (CIDR) as a source of P$_4$ in combination with PGF2α and PMSG treatments on reproductive performance of ewes and does, and evaluate the fetometric measures for gestational age by using the transrectal ultrasonography in the pregnant ewes and does.

2. MATERIALS AND METHODS

2.1. Animals:
This study was carried out in the animal farm, Faculty of veterinary medicine, South Valley University, Qena city, Egypt. Twenty six animals were used; 10 postpartum Ossimi ewes (age, 1.5-2.5-years-old; body weight (b.wt.), 49.7 ± 1.1 kg), 10 Baladi does (age, 1.5-2.5-years-old; b.wt, 40.4 ± 1.2 kg), 3 healthy fertile rams (age, 3-4-years-old; b.wt, 65 ± 2.9 kg) and finally 3 healthy fertile bucks (age, 3-4-years-old; b.wt, 55.0 ± 2.9 kg). All animals were cyclic and used three months after lambing. Rams and bucks were living with the ewes and does. They were used for serving the estral ewes and does according the ovsynch regimen of the study. All ewes and does were non-pregnant before the start of the experiment and pregnancy status was confirmed using ultrasonography by two examinations with three weeks interval. All animals were free from parasites after periodical examination. They were kept in-door at night and had free access to natural grazing area for the most of daytime and fed mixed concentrated ration in adequate amounts with continuous source of water. During in-door animals were fed a diet of concentrated and liberal ration. Water and mineral licks are continuously available.

2.2. Experimental design:

2.2.1. Estrus synchronization and natural mating:
Estrus synchronization was performed using ovsynch regimen. The CIDR intravaginal devices (300 mg P$_4$) (EAZI-BREED CIDR, Pfizer, New Zealand) were used 12 and 14 days in ewes and does, respectively. At the day of CIDR removal, animals were intramuscularly injected with 400 IU of PMSG (Folligon, Intervet, Egypt) and 12.5 mg of PGF$_{2α}$ (Lutalyse, Pfizer, Egypt), as shown in Fig. 1. All animals were observed for estrus signs every 12 hrs for 3 days after the day of CIDR removal with the help of teaser ram or buck. Those animals exhibiting estrus were separated and allowed to natural mating by the male. The reproductive parameters; estrus response (%), time to onset of estrus (h), duration of estrus (h), and the pregnancy/conception rate (%) were evaluated within 72 h after CIDR removal. The time to onset of estrus was estimated as the interval (h) from CIDR removal till the time when ewe or doe start to express standing estrus (heat). Duration of estrus was estimated as the duration (h) from the start of standing estrus until the end of sexual receptivity, and these values expressed as mean ± SEM. Pregnancy/conception rate was calculated as the percentage of pregnant female animals - confirmed by ultrasonography 21 days post service - from the total number of synchronized ewes or does.

2.2.2. Ultrasonographic examination:
Ultrasonographic examination was conducted using real-time B-mode ultrasound scanner (Sonoscape A5V) by transrectal linear. The ewes and does were withheld from food and water for 12 h before
ultrasonography. The ultrasound scanning was applied weekly for 3 months starting from the day 21 post service. Briefly, the transrectal ultrasonography was performed as described by Raja-Khalif et al., (2014). The pregnancy was diagnosed, using one or more of these criteria such as fluid-containing uterine horns, embryonic vesicle, fetal body, heart beats, and/or placentomes. Depending on the status of pregnancy, ultrasonographic results were recorded and compared with the records of the upcoming time of parturition (Dursun et al., 2001). The fetometric measures including the crown-rump length (CRL), bi-parietal diameter (BPD) and the fetal orbit diameter (FOD) were also evaluated for detection of the gestational age.

2.2.3. Blood sampling and hormonal assay:
Blood samples (2.5-5 ml) were collected from the external jugular vein into anti-coagulant-(EDTA; ethylene-ediamine-tetra-acetic acid)-containing tubes (EDTA.K3 vacuumed tubes) as follow; just before CIDR insertion (day 1 or C1) and removal (day 12 (C12) or day 14 (C14) in ewes and does, respectively), and 3 days after CIDR removal (day 15 (C15) or day 17 (C17) in ewes and does, respectively), C for CIDR, followed by weekly blood sampling during pregnancy (P) starting from days 14 post service at these time points (P14, P21, P28, P35, P42, and P49.). Within 2 h after blood collection, samples were centrifuged for 20 minutes at 3000 r.p.m. and plasma was harvested into vials and stored at -20 °C till assayed for P4 by. Plasma P4 concentrations were measured by enzyme-linked immune sorbent assay (ELISA; Immunospec Corporation, U.S.A).

2.3. Statistical analysis:
All data were presented as the mean ± standard error of means (S.E.M). The statistical significance of differences in plasma P4 concentrations throughout the different days of study were determined by student t-test. All data were analyzed using the Graph-Pad Prism (Graph Pad Software, San Diego, CA, USA). The differences were considered significant at the *P<0.05 between each respective values.

3. RESULTS
3.1. Effect of ovsynch and P4 profile on reproductive parameters in ewes and does:
The estrus response after synchronization regimen was 100 %, in both ewes and does (Table 1). The time to onset of estrus ranged between averaged 25.1 ± 1.2 vs. 36.8 ± 1.6 hrs, in ewes and does, respectively. The duration of estrus averaged 32.5 ± 1.4 vs. 41.2 ± 3.1 hrs, in ewes and does, respectively. The conception rate was 90 and 80 % with twining rate of 67 and 50 % in in ewes and does, respectively. The birth rate was 100 % for both species. As shown in Table 2, Fig.2A, and Fig. 2B, the animals were out of the luteal phase before CIDR insertion (P4<1.0 ng/ml; 0.81 ± 0.05 and 0.69 ± 0.08 ng/ml in ewes and does, respectively). Just before CIDR removal, P4 concentrations showed a significant increase to 1.35 ± 0.09 and 1.50 ± 0.13 ng/ml in ewes and does, respectively. Those animals of lower P4 concentrations showed standing estrus state.

3.2. Ultrasonographic examination for pregnancy diagnosis and fetometry measures:
The average increasing rate of the CRL, BPD and FOD were 13.98 ± 2.11, 4.58 ± 0.14 and 2.82 ± 0.36 mm week in ewes, and 13.90 ± 2.31, 4.46 ± 0.2 and 2.80 ± 0.47 mm/week in does (Fig. 3, 4). The CRL appears to be the most one highly correlated with the fetal age followed by the FOD and the BPD in order (Table 3). The weekly transrectal ultrasound scanning (Freq. 7.5 MHZ) of the fetometric measures are shown in Fig. 3 and 4.

Table 1: Reproductive parameters after using CIDR-synchronization regimen in ewes and does showing the estrus response, time to onset, duration of estrus, and conception rate.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ewes (n=10)</th>
<th>Does (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrus response (%)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Time to onset of estrus (h)</td>
<td>25.1 ± 1.2</td>
<td>36.8 ± 1.6</td>
</tr>
<tr>
<td>Duration of estrus (h)</td>
<td>32.5 ± 1.4</td>
<td>41.2 ± 3.1</td>
</tr>
<tr>
<td>Conception rate (%)</td>
<td>90</td>
<td>80</td>
</tr>
<tr>
<td>Incidence of twining (%)</td>
<td>67</td>
<td>50</td>
</tr>
</tbody>
</table>

Table 2: Progesterone (P₄) concentrations are demonstrated before insertion and removal of controlled internal drug release (CIDR) device, and during estrus in ewes and does. At the day of CIDR removal, animals were intramuscularly injected with 400 IU of pregnant mare serum gonadotropin (PMSG; Folligon, Intervet, Egypt) and 12.5 mg of prostaglandin-F₂α (PGF₂α) (Lutalyse, Pfizer, Egypt). All values were expressed as mean ± SEM for nine and eight pregnant ewes and does, respectively. Letters; a and b denote the significant difference at P<0.05.

<table>
<thead>
<tr>
<th>Sampling time</th>
<th>Plasma P₄ (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ewes (n=10)</td>
</tr>
<tr>
<td>Before CIDR insertion</td>
<td>0.81 ± 0.05 b</td>
</tr>
<tr>
<td>Before CIDR removal</td>
<td>1.35 ± 0.09 a</td>
</tr>
<tr>
<td>During estrus</td>
<td>0.54 ± 0.05 b</td>
</tr>
</tbody>
</table>

Table 3: The multiple correlation matrixes among fetal age, crown-rump length (CRL), biparietal diameter (BPD) and the fetal-orbit diameter (FOD) stated during the period from 56 to 91 days of gestation.

<table>
<thead>
<tr>
<th>SHEEP</th>
<th>F. Age</th>
<th>CRL</th>
<th>BPD</th>
<th>FOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>F. Age</td>
<td>0.99*</td>
<td>0.97*</td>
<td>0.97*</td>
<td>0.99*</td>
</tr>
<tr>
<td>CRL</td>
<td>0.99*</td>
<td>0.79*</td>
<td>0.98*</td>
<td>0.96*</td>
</tr>
<tr>
<td>BPD</td>
<td>0.97*</td>
<td>0.79*</td>
<td>0.97*</td>
<td>0.97*</td>
</tr>
<tr>
<td>FOD</td>
<td>0.99*</td>
<td>0.98*</td>
<td>0.96*</td>
<td>0.97*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>GOAT</th>
<th>F. Age</th>
<th>CRL</th>
<th>BPD</th>
<th>FOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>F. Age</td>
<td>0.99*</td>
<td>0.98*</td>
<td>0.98*</td>
<td>0.98*</td>
</tr>
<tr>
<td>CRL</td>
<td>0.99*</td>
<td>0.97*</td>
<td>0.97*</td>
<td>0.99*</td>
</tr>
<tr>
<td>BPD</td>
<td>0.98*</td>
<td>0.97*</td>
<td>0.97*</td>
<td>0.97*</td>
</tr>
<tr>
<td>FOD</td>
<td>0.99*</td>
<td>0.99*</td>
<td>0.97*</td>
<td>0.97*</td>
</tr>
</tbody>
</table>
Fig. 1: Protocol of ovsynch regimen showing time of CIDR device insertion, time of PMSG and PGF2α administration, time of CIDR removal, and period of estrus detection and natural mating in ewes and does.

Fig. 2: Profiles of plasma P₄ concentrations in ewes (Fig. 2A) and does (Fig. 2B) are demonstrated throughout the ovsynch regimen; before CIDR insertion (C1), before CIDR removal (C12 or C14), 3 days after CIDR removal (C15 or C17), and weekly starting from day 14 post service (P14, P21, P28, P35, P42, and P49). Values are shown as mean ± SEM. a, b, c, d, ab, ac, and bc on the bars denote the significance difference at (P<0.05).
**Fig. 3:** The increase rate in the fetal crown-rump length (CRL) for 5 weeks, the biparietal diameter (BPD) for 8 weeks and the fetal-orbit diameter (FOD) (mm) for 5 weeks of pregnancy in ewes and does.

It had revealed that the crown rump length (CRL; 28<sup>th</sup> to 63<sup>rd</sup> days) (Fig. 4A), the bi-parietal diameter (BPD; 35<sup>th</sup> to 91<sup>st</sup> days) (Fig. 4B) and the fetal orbit diameter (FOD; 56<sup>th</sup> to 91<sup>st</sup> days) (Fig. 4C) were steadily increasing in both the ewes and does (Fig. 3, 4), either. The early pregnancy diagnosis by transrectal approach was more suitable until 49<sup>th</sup> day post service in sheep and goat. The embryonic vesicle and signs of fetal vitality like heart beating and fetal movability parallel to the fetometric measures of the gestational age are the most signs confirming pregnancy (Fig. 5). The embryonic vesicle was detected as a non-echogenic black structure. It could be identified as early as the day 21<sup>st</sup> post-service. Combination of the fetus itself, fetal heart, uterine wall and amniotic fluid was detected as early as the Day 28<sup>st</sup> in both ewes and does.
4. DISCUSSION

In the present study, estrus response was 100% in both ewes and does in agreement with those who stated that estrus response following treatment with CIDR plus equine chorionic gonadotrophin (eCG) was high with incidence more than 84%, at least (Dogan et al., 2004; Omontese et al., 2010). Variations in estrus response could be attributed to age, parity, nutrition, breed, location, and climate (Kausar et al., 2009). The time to onset of estrus reported in the present study ranged from 25 to 36 h in ewes and 36 to 48 h in does following CIDR.
removal that is coincident with Didarkhah and Mesgaran (2013) who mentioned that the time to onset of estrus ranged from 28 to 55 h following CIDR removal. In this regard, Jackson et al., (2014) reported that the time to the onset of heat in short term CIDR synchronization in ewes and does was 60-108 hours. Our results were coincident with Hashemi et al., (2006) who found that long term synchronization protocol was associated with shorter interval required to onset of estrus compared to those of shorter term of CIDR in ewes and does. In our study, the PMSG treated ewes and does showed shorter time to onset of estrus after CIDR withdrawal, it could be attributed to follicle-stimulating hormone (FSH) and luteinizing hormone (LH) like activity of PMSG when injected at CIDR removal would stimulate the follicular growth and enhance the time of ovulation (Leboeuf et al., 1998). In response to the stimulatory activity of PMSG following CIDR withdrawal, longer durations of estrus observed in the treated ewes and does; 36-48 and 48-72 h, respectively which supports the findings of Omontese et al., (2012). In our study, the high pregnancy rates coincide with Amer and Hazzaa (2009). The incidence of twining in both ewes and does was in agreed to previous results stated that injection of PMSG after P4 treatment increases the multiple births from the induced ovulation (Knights et al., 2001). All treatment groups showed abrupt rise in P4 levels after CIDR implantation and sharp decline to basal values prior to CIDR withdrawal. Hussein and Ababneh (2008) reported that this phenomenon would reset the hypothalamic-pituitary-ovarian to promote the regression of the persistent follicles and recruit new healthy follicles. In the present study, transrectal ultrasonography was useful and accurate in early diagnosis of pregnancy in both ewes and does. Ultrasonography has been used with great deal of accuracy as a mean for pregnancy diagnosis and estimation of fetal numbers in ewes and does (Martinez et al., 1998; Gonzales et al., 2004). In the present study, the first sign of pregnancy in ewes and does was the presence a circular or elongated echogenic structure located in uterus cranial to the urinary bladder on day 21 post service. These results agreed with the previous findings which has been reported by Karen et al., (2001) who remarked anechoic round or oval structures on the uterine lumen on the Day 19th to 26th post-service just dorsal and cranial to the urinary bladder and defined as the earliest detectable sign of pregnancy by both transrectal or transabdominal ultrasonography either. Those studies explained that the body of the uterus was imaged usually over the urinary bladder but it was also found anterior or dorsal to the bladder in the non-gravid or early pregnant ewes. In the present study, combination of fetus, fetal heart, uterine wall, and amniotic fluid was detected as early as 28 days of gestation in both ewes and does. These results were in accordance with those of Fadel et al., (2000) and Raja-Khalif et al., (2014). Previous studies indicated that fetal heart and embryo proper were detected as earlier as the Day 21st of gestation (Martinez et al., 1998). Presence of the embryonic vesicle and the signs of fetal vitality, like heart beating and the fetal movability are the most signs of confirming pregnancy. The fetal parameters which were measured by transrectal ultrasound scanning were the crown rump length (CRL), biparietal diameter (BPD) and the fetal orbit diameter (FOD). The CRL was measured weekly from 28 to 63 days (4th to 9th week) of gestation in pregnant ewes and does (7.5 MHz freq.) that would be nearly consisted with Schrick and Inskeep (1993) who had measured the CRL of the ovine fetus from
the day 20 to 40 of gestation and described the relationship between the CRL and the gestational age reporting a high correlation ($r=0.94^*$) between the CRL and gestational age from the days 19 to 48 of gestation (Gonzalez et al., 1998). The measurement of the CRL had shown a steadily increase in both ewes and does weekly, and the average increase in the CRL were 13.98 ± 2.11 and 13.90 ± 2.31 mm /week in both ewes and does, respectively. The most increase in the CRL were from the 8th to 9th week of pregnancy, this may suggested that a high increase in calcium, phosphorus and protein content intake and in blood in addition to the higher increase in size of internal organs like stomach, liver, lung and other organs. The lowest increase in the CRL were from 7th to 8th week of gestation may refer to the slowly growth of fetus in that period. The obtained results for CRL measurement recommend it as a main parameter for prognosis of gestational age in from the day 28th of pregnancy. The BPD was measured weekly from the 35th to 91st days (5th to 13th week) in pregnant ewes and does according to Gonzalez et al., (1998) who used the transrectal ultrasonography to measure the BPD in pregnant ewes from 32 to 90 days of gestation to find a high correlation ($r= 0.96^*$) between the measured diameters and the gestational age. The BPD had shown a steadily increase in both ewes and does weekly. The average increase in the BPD was 4.58 ± 0.14 and 4.46 ± 0.20 mm /week in both ewes and does, respectively. The most increase in the BPD were from 6th to 7th week of pregnancy, this may suggest that in younger age the head of the fetus be large size then nearly get slow in growth, since the lowest increase in the BPD were from 8th to 9th week of gestation. The obtained results for BPD measurement recommend it as a parameter for detection of gestational age from the Day 35th of gestation in both species. The FOD, as that BPD, was measured weekly from the 56th to 91st days (8th to 13th week) of gestation in pregnant ewes and does, nearly the same as Gonzalez et al., (1998) and conducted with their findings that the ovine fetal orbit increased in diameter from 2 mm at the Day 36th to 17 mm at the Day 90th of gestation giving a high correlation ($r= 0.92^*$) with the fetal age. The FOD showed the average increase of 2.82 ± 0.36 and 2.80 ± 0.47 mm /week in both ewes and does, respectively. The most increase in the FOD was observed from the 6th to 7th week of pregnancy that could correspond to the skull growth. However, the lowest increase in the FOD was observed also from the 8th to 9th week of gestation the same as BPD. The obtained results for FOD measurement is recommended as another parameter for gestational age detection from the day 56th up to the 91st of pregnancy. Our results displayed that there were non-significant difference (P<0.05) between the CRL, BPD and the FOD in the pregnant ewes with each respective parameters in the pregnant does.

5. CONCLUSION
The ovsynch regimen using CIDR and PMSG as followed in our study showed higher conception rates especially in sheep rather than goat and all treated animals had shown clear estrus characteristics and reached the full term of pregnancy till parturition. The weekly increase in the CRL was nearly three times as that increasing values of BPD, and the later was twice higher than that of the FOD, and all the fetometric parameters are significantly correlated with the gestational age.

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7. REFERENCES


