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A study on plasma estradiol and progesterone profile at days 11, 12 and 13 post ovulation undergone embryo transfer at day 7-post ovulation in local Egyptian mares

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Abstract

This study was conducted on 28 recipient local mares after synchronizing and recovery of embryos of Arabian mares (donor mares) and transferring them to recipients on day 7 post ovulation, all mares were raised in the studs of police academy –Cairo Egypt under same circumstances in the breeding season from February till may of 2013. Seventeen recipient mares were pregnant and 9 mares were non pregnant when ultrasound pregnancy check was done on day 21 post ovulation, blood samples were taken on days 11,12 and 13 to detect the steroidal hormonal profile (estrogen and progesterone) via Elisa technique of the recipient mares plasma steroid level and its role in early pregnancy and maternal recognition. The results were significantly higher (P≤0.05) between pregnant (n=17) and non-pregnant (n=9) recipient mares in the plasma progesterone concentrations which were 10.99±0.16 vs 9.59±0.11, 12.69±0.16 vs 11.79±0.22 and 14.4±0.15 vs 13.78±0.23 ng/ml on days 11,12 and 13 post ovulation respectively, significant difference(P≤0.05) was observed when comparison between pregnant mare's plasma progesterone concentrations on days 11,12 and 13 post ovulation. Plasma estrogen concentration were significantly higher (P≤0.05) in non-pregnant and pregnant mares which were15.17±0.18 vs 14.84±0.14, 14.74±0.27 vs13.94±0.12 and 14.14±0.3 vs13.12±0.16 pg/ ml on days 11,12 and respectively, on the other hand when comparison between days11,12 and 13 plasma estrogen levels were significantly different (P≤0.05) in pregnant mares while no significant difference was found in the same days between non pregnant mares, thus might be the main reason for early embryonic death when detected in early pregnancy check via ultrasonography in 21 days post ovulation.

Keywords: Arabian mares, embryo transfer, estradiol, progesterone

Introduction:

The improvement of reproductive traits is of great importance for horse breeders in terms of breeding and economic considerations, however the genetic improvement of Arabian horses is one of the most demands of worldwide breeders (1), especially here in the Arab region where all breeders and stud holders pay much attention to purify the breeds they hold and this is done by using all reproductive techniques focusing on embryo transfer to prompt getting new generations of the pure Arabian mares. In the last decade Europe and America has relied on this process to reproduce its herds and depending on the process of cryopreservation of recovered embryos. Many factors were interfered with the process some are manual, management and experience handling the embryo and others are hormonal disturbances in the recipient mares which may fail to carry the embryo till term (foaling time). In equine breeding industry, big economic losses may occur and regardless of high fertilization rates, pregnancies are terminated within the first few weeks of conception (2). Because of the economic importance of maintaining

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pregnancies in valuable horses, the technique of embryo transfer was developed in the early 1970's and its use has steadily increased over the past two decades (3). Several factors are involved in pregnancy maintenance in Arabian mares. These factors include adequate progesterone levels (4). The conceptus is also an active partner in the successful establishment and maintenance of pregnancy (5). Progesterone is critical to embryonic survival; the cause-and-effect relationship between progesterone and spontaneous embryonic loss remains unclear (6,7). Reduced progesterone concentrations could be related to endometritis, failure of maternal pregnancy recognition, or luteal insufficiency (8). Several hormones play important roles in fertilization, implantation and maintenance of pregnancy in several animal species (9). In summary, a number of assisted reproduction techniques have been developed and practiced within the equine species. These techniques have allowed the equine industry to successfully breed valuable mares that undergo normal circumstances would be unable to conceive or maintain a pregnancy unassisted. As the current research on this topic progresses, more reliable and profitable methods are surely to be developed. This study focused on the hormonal profile on days 11, 12 and 13 post ovulation of transferred embryos to consider the hormonal status while the embryo are still wondering in the uterine cavity and the effect of plasma estradiol and progesterone concentration on maternal recognition and implantation. Here came the idea of this study that aims to determine the mare's hormonal profile (E2 and P4) in the period of 11,12 and 13 days post ovulation to conclude whether it plays or not in the role of process of pregnancy and end with a life foal.

Materials and Methods

1-Mare preparations

The study involved 26 recipient local mares after synchronizing with donor Arabian mares both were with excellent reproductive history, the experiment was done to increase the number of embryos recovered from the donor mares (increase the population of Arabian breed) was done in one stud farm within the breeding season inside the period starting in end of February till May of 2013, donor and recipient mares that have already foaled 2-4 times. Mares were maintained on winter pasture and fed 2 kg of grain mix per mare per day. Mares had constant access to mixed grass-alfalfa hay, water, and salt/ mineral blocks in the police academy, Cairo-Egypt.

2-Donor Mare selection

Donor mares selection were experimental for embryo transfer used for this study had many previous foaling range 7-10 years of age and good reproductive history.

3-Recipient mare selection

Recipient mares were selected with excellent reproductive profile, which had at least 2 foaling during its life aging 5-9 years old.

3- synchronizing

Synchronizing both donor and recipient with a window synchrony of +2 days by injecting double dose of Estrumate two weeks apart, both donor and recipient mares came to estrus with 2 days apart (donors came to heat first).

4-Monitoring and treatment

Donor mares were monitored daily via trans rectal ultrasonography till dominant follicle became 37 mm in diameter, HCG was given intravenously 2500 I.U. and inseminated naturally from a well-known stallion with an excellent reproductive profile twice one day after the HCG injection 2 days apart.

5-Embryo collection and handling

All embryos were collected from donor mares in non-surgical recovery with a range of 1-3 flushing per mare at day 7-post ovulation. All embryos were examined and classified as excellent expanded morula and

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were transferred directly to the recipient mares non-surgically as well in the form of fresh transferred embryos. Recipient mares were examined at the time of transfer clinically by trans rectal ultrasound to determine the presence of corpus luteum and the absence of endometrial fluids, all mares showed clear uterus and mature C.L showing no fluids to less than 0.5 cm translucent fluid consistency in the endometrial lumen.

6-Blood sampling

Blood samples were collected daily at days 11,12 and 13 post ovulation. Plasma was immediately separated by centrifugation (3000 rpm) for 15 minutes and kept frozen until the time of assay.

7-Serological examination:

Estrogen and progesterone hormones in all experimental mares were assayed using ELISA according to (10).

9-Pregnancy diagnosis

Pregnancy diagnosis was achieved at day 21 to determine the pregnant and non-pregnant mares by trans rectal ultrasonography.

8-Statistical analysis

All data are presented as means± standard deviation (SD) of the means. Data were analyzed using SPSS (11).

Results:

Progesterone plasma concentrations showed significant difference between days of the study on days 11,12 and 13 (P≤0.05) the Mean± SD of 17 mares were 10.99±0.16 ,12.69±0.16 ,and 14.4±0.15 ng/ml respectively in 17 pregnant mares, while non-pregnant mare(n=9) showed significant difference in the same days which were 9.59±0.11 , 11.79±0.22 and 13.78±0.23 ng/ml respectively (P≤0.05). As shown in table and figure 1. Whereas day 11 showed significant difference in plasma progesterone level between pregnant and non-pregnant mares 10.99±0.16 and 9.59±0.11 ng/ml respectively (P≤0.05), day12 also showed significant difference between plasma progesterone levels in pregnant and non-pregnant mares 12.69±0.16 and 11.79±0.22ng/ml respectively (P≤0.05) as well as day 13 showed the same significant difference between pregnant and non-pregnant mares 14.4±0.15 and 13.78±0.23ng/ml respectively table and fig 1. While plasma estradiol levels in pregnant mares (n=17)were 14.84±0.14 , 13.94±0.12 and 13.12±0.16 pg/ ml on days 11,12 and 13 respectively showing significant difference (P≤0.05) on the other hand in non-pregnant mares were15.17±0.18 , 14.74±0.27 and 14.14±0.3 pg/ ml showing no significant differences on days 11,12 and 13 respectively, table and fig 2.

Table (1): Plasma progesterone levels ng/ml in pregnant and non- pregnant local mares in days 11, 12, 13 post ovulation.

Group	Day 11	Day 12	Day 13
Pregnant	10.99±0.16^{A} $_{a}$	12.69±0.16^{B} $_{b}$	14.4±0.15^{Cc}
Non-pregnant	9.59±0.11^{Ab}	11.79±0.22^{B} $_{c}$	13.78±0.23^{C} $_{a}$

***Capital letters refers significant differences in rows**
***Small letter refers significant differences in columns (P>0.05)**

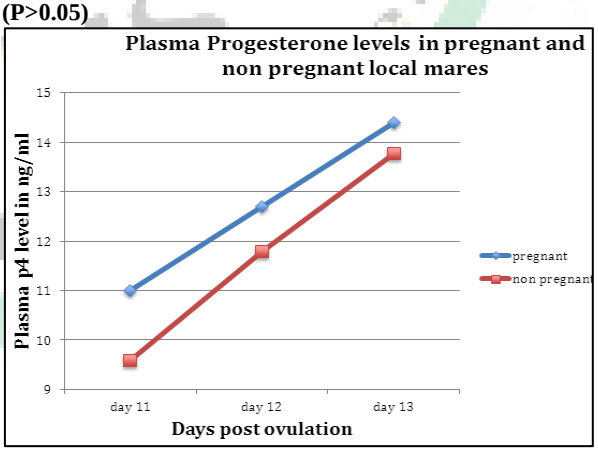

Figure (1): showing plasma Progesterone levels ng/ml in pregnant and non- pregnant local mares in days 11, 12, 13 post ovulation.

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Table (2): showing plasma Estradiol levels pg/ml in pregnant and non- pregnant local mares in days 11,12, 13 post ovulation

Group	Day 11	Day 12	Day 13
Pregnant	14.84±0.14^{Aa}	13.94±0.12^{Bb}	13.12±0.16^{Cc}
Non-pregnant	15.17±0.18^{Db}	14.74±0.27^{Dc}	14.14±0.3^{Da}

***Capital letters refers significant differences in rows**
***Small letter refers significant differences in columns (P>0.05)**

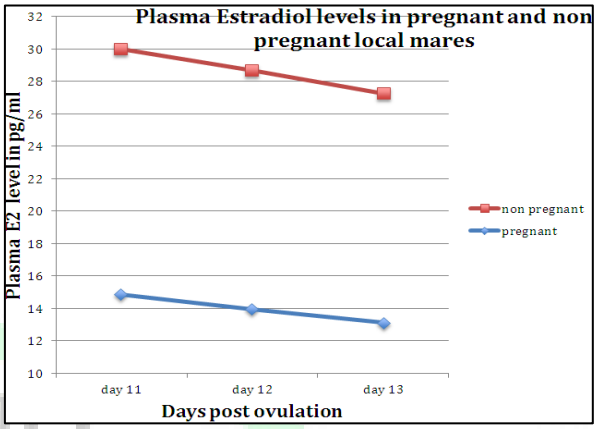

Figure (2) showing plasma Estradiol levels pg/ml in pregnant and non- pregnant local mares in days 11, 12, 13 post ovulation.

Discussion:

Progesterone levels were ascending in both pregnant and non-pregnant mares but starting with higher levels in pregnant mares showing significant difference between the2 groups in the same days this may be as(12) mentioned to the occurrence of luteolysis on further days at days 14 to 16 post ovulation despite the of existence of early embryo in the uterus, where (13) reported that maybe shortness of progesterone levels and primary CL insufficiency may lead to early embryonic death with in the first 3 weeks post ovulation and this would be likely one of the reasons which lead to miscarry the embryo. As its documented by (14) that in equine ovary, granulosa and theca interna are involved in the process of steriodogenesis, where thecal cells produces androgens and followed to convert to estrogens by granulosa cells which is called 2 cell theory may played an important role in the estrogen levels in the non-pregnant mares were estrogen levels were significantly higher than the pregnant group on the proportion of progesterone production in the non-pregnant mare at same subsequent days. While (15) proved that plasma estrogen concentration rapidly increases 2 days before ovulation and declines in the diestrus period within two days post ovulation, on the other hand(16,17 and 18) stated that estrogen concentrations during the first 30 days of pregnancy were not different in pregnant and non-pregnant mares and they proved that estrogen concentrations increased 2-3 folds between days 35 and 40 of pregnancy .while in this study it's found that estrogen concentrations were higher in non-pregnant in days 11,12 and 13 post ovulation. The significant difference in progesterone levels in the days of the experiment between the 2 groups might also be cause of endometritis as (19) mentioned especially the first 30 days of gestation when he found that almost 85% of mares had low concentrations of progestins at day 12 which were positive to pathogenic bacteria through culturing the uterine lavage. In my point of view I find that the early embryonic loss has occurred due to hormonal disturbance during the period of maternal recognition (day14-16 post ovulation) which is the day after of the experiment were (20,21) describes it the critical period and also suggested that this period involves a suppression of endometrial PGF2α secretion by the fetus factor and its migration in the first 16 days of gestation and thus prolongation of the luteal function which was significantly different in progesterone levels in both groups and also which(22) hypothesized the fixation of the vesicle occurs during accumulative thickening and

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uterine tone wall coupled with continuous growth of the developing vesicle, on the other hand (23) reported that estrogen concentration as an indicator of fetal demise in horses.

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