

Serum FSH, LH and Progesterone Hormones Concentrations in Relation to Hormonal Estrous Induced, Laparoscopic Insemination and Pregnancy in non-Breeding Season in Iraqi Goats

Ibrahim N S

Department of Surgery and Obstetrics – College of Veterinary Medicine, Baghdad University, Iraq

Abstract

This study was aimed to determine serum concentrations of FSH, LH and progesterone after induction of estrous in non-breeding season. This work was carried out on 16 female black local breed Iraqi does. Each doe treated with a sponge containing 20mg fluorogestone acetate (FGA) for 13 days and IM injection of 500 IU pregnant mare serum gonadotropin (PMSG) 24hrs before sponges withdrawal. After 24-36 hrs from estrous onset each doe was inseminated laparoscopic with 1ml of fresh diluted semen content at least 1×10^8 active fresh sperms directly into the uterine body. Blood samples were collected at the end of sponges treatment, after laparoscopic insemination and during pregnancy. The results revealed that all have shown signs of estrous (100%) after 24-72 hrs. with a mean time of 46.9 ± 4.90 hrs. after sponges removal. Estrous duration was 37.1 ± 1.91 hrs. Pregnancy diagnosis was performed by ultrasonography examination at 30th, 60th and 90th days post insemination. During laparoscopy insemination the numbers of follicles were found to be 1-3 follicles of different sizes on each ovary. Pregnancy was found in 14 does (87.5 %) 4 females had twin kids (28.56 %). The overall average values of serum FSH 2.26 ± 0.15 mIU/L, LH 1.18 ± 0.21 mIU/L and progesterone 48.89 ± 3.5 ng/mol/L were recorded during experimental period.

KEYWORDS: FSH; LH; progesterone in goat; estrous induction; doe; non-breeding season; laparoscopic insemination.

Introduction

Hormonal changes are studied during the gestation period which knock down doors that block understanding of the regulatory mechanisms accompanying fetal development and pregnancy maintenance; the most important stage of animal reproductive life. In goats, pregnancy is dependent on the corpus luteum (CL) with minimal contribution from placenta (Kandiel et al., 2010). Maintenance of progesterone secretion by the CL during pregnancy in the goat is luteinizing hormone (LH)-dependent; moreover, prolactin (PRL) is synergistic with LH in stimulating this function (Buttle, 1983). Prolonged postpartum anoestrus is one of the reasons for low reproductive efficiency and economic loss for animal breeders (Westwood et al., 2002).

The progesterone remains at basal levels during the postpartum anoestrus and increases with the resumption of postpartum cyclicity (Khanum et al., 2007). Serum concentrations of progesterone during postpartum period in goat were 0.27 ± 0.09 ng/ml at a day after parturition to 0.15 ± 0.05 ng/ml at day 28 after parturition (AL-Hozadet al., 1999). Progesterone levels remained at lower concentrations for 20-50 days of gestation then increased to reach to its maximum level (13.5 ± 0.279 ng/ml) in week 15th

of gestation (Talebi et al.,2012). In ewes the level of progesterone was recorded to be 2.97 ± 1.16 after 7 days after initiation of synchronization protocols and 0.41 ± 0.11 ng/ml at the end of synchronization, days11 (Naderipour et al.,2012).

Since prediction of the time and synchrony of ovulation after progesterone removal is important to ensure maximum fertility after mating or fixed-time AI (Greyling and Vander-Nest, 2000 and Naderipour et al.,2012).

The black Iraqi goat needed to demonstrate the sexual activity period which is necessary to control breeding interval, so the present study was done to compare the temporal relationships among the serum FSH, LH and progesterone hormones in goats synchronized with FGA and laproscopically inseminated in non-breeding season.

Materials and Methods

The experiment was conducted in non-breeding season (December 2010, January and February/2011, at the College of Veterinary Medicine, University of Baghdad (North $33^{\circ}18'$, $44^{\circ}35'$ East). Sixteen multiparous native black Iraqi does, weighing 40 – 50 kg were used in this trail.

The estrus cycles were synchronized by using progesterone sponges containing 20 mg of fluorogestoneacetate (Intervet, International BV. Boxmeer, Holland). Sponges were inserted for a period of 13 days and pregnant mare serum gonadotropine (PMSG) 500 IU single dose IM injected to each doe 24 hrs before sponges removal (day 0 = day of sponge removal).

Does were detected for estrous, using fertile males, twice daily for 30 days and pregnancy diagnosis were also performed by using Ultrasonic methods (Ultrasonography; equipment, prop 5-MHz; Welld, China) before sponges insertions. Laparoscopic Insemination was performed 24-36 hrs after estrous onset using 1ml of fresh diluted semen contains at least 1×10^8 live active sperms (Laparoscope equipment, Carl storz company, Germany). During post insemination period all does were checked for estrous returning by bucks for 30 consecutive days. Ultrasonography was used for pregnancy diagnosis at days 30th, 60th and 90th post insemination. Daily checking was performed to pregnant does for any obstetrics or gynecological problems.

Venous blood samples were collected in evacuated tubes (10 ml) without any additives from v. caudalis mediana according to the protocol between 9 and 11 a.m., to avoid daily fluctuations in analytes. Goats were sampling at day 10th of progesterone treatment, 24 hrs following laproscopical insemination and at mid of the 1st, 2nd, 3rd, 4th and 5th months of pregnancy. Blood centrifuged at 2500 rpm for 15 minutes to serum separation which stored in $-20^{\circ}C$ until hormonal assayed. Serum hormones concentrations were determined by Radioimmuno assay (BioMeriux kits, Marcy-I Etoile, France).

SPSS (2008) was used to calculate one way-ANOVA and to estimate the differences between treatments. Duncan Test was used (Duncan, 1955) to detect differences among different periods for hormones levels.

Results and Discussion

The FAG sponges did not fall off; no doe exhibited estrus while being treated with progesterone, indicating that the 20 mg intravaginal FAG was adequate to suppress estrus activity. Romano (1988) confirming that progestagen has ability to inhibit estrus in goat via the negative feedback.

In present work the total percentage of does in estrus was 100% and the interval of estrus and duration of estrus were 46.9±4.90 and 37.1±1.91hrs respectively, reflected the efficiency of the protocol in inducing estrus. Ahmed, *et al.*,(1998) reported estrous onset was 53 hrs in goats.

LH surge was recorded at the hours of 41st – 51st after progesterone removal (Pierson *et al.*,2001). Cameron *et al.*(1988) reported an ovulation occurred between 36 – 48 hrs. The interval from FGA removal to estrus was found in most critical and, hence, the factor predicting the occurrence of LH surge and ovulation (Martinz-Alvarez *et al.*,2007), as well as indirectly initiated the endogenous GnRH peak which resulted in the LH surge (Cameron *et al.*,1988). In this study it is indicated that the estrous synchronization protocols maybe useful when precise timing of ovulation is required, by improving the synchrony of LH surge–ovulation. It will facilitate implementation of fixed time breeding and AI in non-breeding season. By injection of 500 I.U.PMSG led to the presence of 1-3 follicles of different sizes on goat ovaries and to increase estrogen secretion and estrus emergence in 100% during non-breeding season, the crucial factor in the continual development of the ovulatory follicle its ability to synthesize oestradiol (Chao *et al.*,2008), oestradiol is responsible for day to day fluctuation in concentrations of FSH which determine, the emergence of follicular waves (Baril,1993 and Widayati *et al.*,2011).Martinz-alvarez *et al.*, (2007) reported the peak of estrogen occurred at the 48hrs post progesterone removal, estrogen peak close correlated with maximum size of pre-ovulatory follicle.

The overall pregnancy rate recorded in this study was 87.5% while, Fonseca *et al.* (2005) reported that 81% pregnancy rate in goat. And superior to that reported by Barilet *et al.*(1993) which were found the pregnancy rate was 59% in goats treated for 11 days with FGA intra vaginal sponges. Iraq goats have very good responses to the protocol applied during non-breeding seasons.

Table – 1: The temporal relationships between the different variables of the (M±SE) serum FSH,LH and Progesterone in goat serum.

Treatment		FSH	LH	Progesterone
During treatment		B 1.922 ± 0.184	AB 0.877 ± 0.182	A 79.444 ± 8.509
Post treatment		B 2.166 ± 0.214	A 2.388 ± 1.160	A 72.666 ± 6.251
Pregnancy	1 st month	B 1.640 ± 0.120	B 0.500 ± 0.083	B 44.000 ± 9.523
	2 nd month	B 1.977 ± 0.121	B 0.500 ± 0.079	B 38.444 ± 6.564
	3 rd month	B 1.957 ± 0.370	AB 0.885 ± 0.206	A 44.333 ± 5.494
	4 th month	A 2.533 ± 0.274	AB 1.066 ± 0.216	B 37.000 ± 7.123
	5 th month	A 3.311 ± 0.723	A 1.700 ± 0.467	C 21.000 ± 2.968
Total		2.264 ± 0.150	1.184 ± 0.212	48.894 ± 3.500
		xx	x	xx

Different letters showed significant among different means.

x=P<0.05 xx=P<0.01

The mean serum FSH, LH and progesterone(P4) concentrations profiles are illustrated in table 1.

Serum progesterone levels started significantly increased (P<0.01) in blood samples taken from progesterone treated does and during estrus compare to pregnancy samples table – 1, this could be due to long treatment with good quality of the better progesterone (P4) absorption, as well as, slow excretion of progesterone hormone from goat body after FGA removal. The high progesterone level did not affect the follicular growth which stimulated by PMSG injected 24 hrs before FGA effect removal. Similar results were recorded by(Naderipouret al.,2012 and Turk et al.2008).

During pregnancy (first month) blood serum P4 declined after the source of progesterone was removed and P4 concentration declined gradually to parturition (Table - 1).

The overall increase in P4 levels during gestation and decline towards parturition and parturition observed in Iraqi native breed (Alwan,2011), also resembled with other breed (Kadzere et al.,1997and and Khanum et al.,2008). The prepartum decline in the progesterone levels was correlated with onset of parturition (Laura et al.,2004).Progesterone returned to its basal value within 1-2 weeks before parturition (Talebi et al.,2012).

In the present study the mean peak LH during progesterone treatment and induced estrus (table -1) could be with the aid of intra vaginal FGA sponges for 13 days and PMSG administration. Similar suggestion was reported by Pierson et al. (2001) whom reported a 24 hrs interval between LH peak and ovulation after synchronizing estrus with the aid intravaginal MAP for 10 days and eCG administration at the time of sponges removal.

LH concentration showed significant (P<0.05) higher during the last two months of gestation. Kandielet al.,(2010) reported almost the similar LH levels during estrous and gestation periods in goat .Also it was confirm the suppressing effect of progesterone on the pituitary secretion of LH through a negative feedback mechanism. Serum LH values drop to reaches its lowest value by 130 days of gestation similar finding in goat (Kandiel et al.,2010).

FSH concentration was high around the onset of estrus and decreased thereafter and remained to the low level near the basal levels during the monitored period of gestation and then increased gradually from 4th month and reached its highest level during the 5th month of pregnancy (table1). Similar FSH pattern was reported by Kandielet al. (2010).

In conclusions: Levels of serum FSH, LH and progesterone hormones were influenced by hormonal treatment, laparoscopical insemination and pregnancy. Estrous could be induced in Iraqi goats successfully in non breeding season using 20 mg FGA impregnated sponges with PMSG.

References

Ahmed, M.M.; Makawi, A.S.andJubara, A.S.(1998). Synchronization of estrus in Nubian goats. Small Rum.Res.,30: 113 –120.

Al-Hozad, A.; Salem, H.A, an dAmer, H.A.(1999). Postpartum levels of serum progesterone ,oestradiol, prolactin and thyroxin in native goat of Saudia Arabia. Pakistan Vet. J., 19:32–34.

Alwan, A.F.; Amin, F.A. and Ibrahim, N.S.(2010). Blood progesterone and estrogen hormones level during pregnancy and after birth in Iraqi sheep and goat. *Basrah J. Vet. Res.*, 9: 153 – 154.

Baril, G.;Leboeuf. B. and Saumande,J.(1993). Synchronization of estrus goats: the relationship between time of occurrence of estrus and fertility following artificial insemination.*Theriogenology*, 40: 621 – 628.

Buttle, H.L.(1983). The luteotrophic complex in hysterectomized and pregnant goats. *J. Physiol.*, 342: 399-407.

Cameron, A.W.;Battye, K.M. and Trounson, A.O.(1988).Time of ovulation in goats (*Capra hircus*) induced to super ovulate with PMSG. *J. Reprod.Fertil.*, 83: 747–752.

Chao, L.M.; Takayama, K.; Nakanishi, Y.;Hamana, K.; Takagi, M.; Kubota, C.and Kojima, T.(2008). Luteal lifespan and fertility after estrus synchronization in goats. *J. Vet. Sci.*, 9: 95 – 101.

Duncan, D.B.(1955). Multiple range and multiple F – Test. *Biometrix* ., 11:1-4.

Fonseca, J.F.; Torres, C.A.; Costa, E.P.; Maffili, V.V.;Carvalho, G.R.;Alves, N.G. and Rubert, M.A.(2005). Progesterone profile and reproductive performance of estrous-induced Alpine goats given hCG five days after breeding. *Anim.Reprod.*, 2: 54 – 59.

Greyling, J.P. and Vander-Nest, M.(2000). Synchronization of oestrus in goats: dose effect of progestagen. *Small Rum. Res.*, 36:201–207.

Kadzere, C.T.; Llewelyn, C.A, and Chivandi, E.(1997). Plasma progesterone, calcium, magnesium and zinc concentrations from estrous synchronization to weaning in indigenous goats in Zimbabwe.*Small Rum. Res.*, 24: 21–26.

Kandiel, M.M.; Watanabe,G.; Sosa,G.A.; Abou El-Roos,M.E.; Abdel-Ghaffar,A.E.; Li, J.Y.; Manabe, N.; El-Azab, A.S.and Taya, K.(2010).Profiles of circulating steroid hormones, gonadotropins, immune-reactive inhibin and prolactin during pregnancy in goats and immune-localization of inhibin subunits, steroidogenic enzymes and prolactin in the corpus luteum and placenta.*J.Reprod.Dev.*,56: 243-250.

Khanum, S.A.; Hussain, M. and Kausar,R.(2007). Assessment of reproductive parameters in female Dwarf goat (*Capra hircus*) on the basis of progesterone profiles. *Anim.Reprod.Sci.*, 102: 267-275.

Khanum, S.A.;Hussain,M.andKausar,R.(2008).Progesterone and Estradiol profiles during estrus cycle and gestation in dwarf goats (*Capra hircus*). *Pakistan, Vet. J.*, 28: 1-4.

Laura, A.S.; Kumar, M.S. and William, G. and Sandra, L.A. (2004). Predicting the onset of parturition in the goat by determining progesterone levels by enzyme immunoassay. *Small Rum. Res.*, 52: 203-209.

Martinez- Alvarez, L.E.; Hernandez-Ceron, J.; Gonzalez-Padilla, E.; Perera-Marin, G. and Valencia, J. (2007). Serum LH peak and ovulation following synchronized estrus in goats. *Small Rum. Res.*, 69: 124 – 128.

Naderipour, H.; Jafar, Y.; Ali, G.S. and Mohammad, A.S. (2012). The effects of three methods of synchronization on estrus induction and hormonal profile in Kalkuhi ewes: A comparison study. *African J. Biotech.*, 11:530–533.

Pierson, J.T.; Baldassarre, H.; Keefer, C.L. and Downey, B.R. (2001). Seasonal variation in preovulatory events associated with synchronization of estrus in dwarf goats. *Theriogenology*, 56: 759-769.

Romano, J.E. (1988). Comparison of fluorogestone and medroxy progesterone intravaginal pessaries for estrous synchronization in dairy goats. *Small Rum. Res.*, 22: 219–223.

SPSS. (2008). Statistical Package for Social Science version ,16;17. (Win/Mac/Linux) Users SPSS Inc-Chicago 2008;USA. Website. <http://www.spss.com>.

Talebi, J.; Moghaddam, A.; Souri, M. and Mirmahmoudi, R. (2012). Steroid hormone profile of Markhoz does (Iranian Angora) throughout estrous cycle and gestation period. *Trop. Anim. Health Prod.*, 2012; 44:355-405.

Türk, G.; Gür, S.; Sönmez, M.; Bozkurt, T. and Aksu, E.H. Aksoy, H. (2008). Effect of exogenous GnRH at the time of artificial insemination on reproductive performance of Awassi ewes synchronized with progestagen-PMSG-PGF2alpha combination. *Reprod. Domest. Anim.*, 43:308-313

Widayati, D.T.; Sunendar, K.S.; Asuti, P. and Junaidi, A. (2011). The effect of body condition score on hormonal and vaginal histological changes during estrus of synchronized Etawah cross bred does. *World Acad. Sci. Engin. Techn.*, 53-63.

Westwood, C.T.; Lean, I.J. and Garvin, J.K. (2002). Factors influencing fertility of Holstein dairy cows: a multivariate description. *J. Dairy Sci.* 85: 3225–3237.