

Fetal adrenal cortex development with maternal serum Cortisol profiles in Relation to Estrous Induction, Laparoscopic Insemination in Iraqi goats out breeding season

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Summary

The objectives of this research were to determine the serum cortisol profiles and their temporal interrelationship during progesterone treatment, induced estrous, laparoscopic insemination and pregnancy (antepartum) period in goat, with histological fetal adrenal cortex development. The first part of the experiment included studying Hundred and sixty goat fetuses of different Crown Rump Length (CRL) were collected from slaughter house. The adrenal glands in the goat fetus displayed signs of cytological differentiation around the second half of intrauterine life, 20cm/CRL about 90 days old until full term. From histological observation and physical measurements the present study recorded that the adrenal cortex grow faster from 25 cm/CRL(100 days) to term. The majority of this growth take place due to hyperplasia in the adrenal cortex, zone fasciculate. This second part of the experiment conducted on 16 female local goats aged 2-4 years, weight 30-45Kg. Estrus was induced by application of impregnated sponges content 20 mg fluorogestone Acetate (FGA) for 13 days and an IM injection of 500 IU Pregnant Mare Serum gonadotropin (PMSG) 24hrs before sponges withdrawal. Within 24-36 hrs of estrous signs onset, each doe was inseminated laparoscopically with 1ml of fresh diluted semen contain at least 100 million active fresh sperms directly into the uterine body. Maternal blood samples were collected at day 10th of progesterone treatment, 24hrs after laparoscopic insemination and monthly during pregnancy. The results revealed that all does showed signs of estrous (100%) after 24-72 hrs with a mean time of 46.9±4.90 hrs after sponges removal. Estrous length was 37.1 +1.91 hrs. Pregnancy diagnosis was performed by ultrasonography examination at 30th, 60th and 90th days post insemination. Pregnancy was found in 13 does (81.22%), 3 of them had twin kids (23.01%). Serum cortisol levels shows the highest level during the 4th and 5th months of gestation periods compared to that of the others periods of this study with overall average 78.93±2.96 nmol/L, respectively.

keywords: cortisol, estrous induction, laparoscopic insemination, fetal adrenal.

Introduction

The regulatory mechanisms that operate to maintain the physiological status during gestation, parturition and postpartum are mainly aimed at homeostasis and homeorrhesis (1). Cortisol is one of the cortex adrenal hormones which is responsible for gluconeogenesis (2). Breen (3) reported that plasma cortisol interfere the follicular phase by suppressing the development of high frequency FSH and LH pulses. Elevations in glucocorticoids inhibit reproductive neuro endocrine activity in a variety of species. Maternal cortisol concentration for normal delivering goat with body condition score was 47.17±4.22 ng/ml (4).

Estrous induction and mating outside the breeding season could have a great impact on the animal showing reproductive seasonality.

Several estrous induction protocols are currently available with varying doses, duration, type and route of administration. And the most commonly protocols used are progesterone slow release intra vaginal devices contain fluorogestone acetate-impregnated sponges (FGA) (5) or medroxy progesterone acetate (MAP) (6).

All mammals have fetal adrenal gland of cortical and modularly regions which have different embryological origins and thus form two separate function and morphological structure within a single capsule (7). Recent experiments have underlined that the growth and functional activity of the fetal adrenal cortex is influenced by hypothalamic pituitary hormones (8). Within the fetus increasing level of corticosteroids cause an accelerated maturation of essential organs and prepared

the animal for life outside uterus (9 and 10). In addition to the event late in pregnancy, the fetal lamb adrenal is an active endocrine organs from as early as 50th days of gestation (11). Lack of the intact fetal pituitary lead to reduction in adrenal size and atrophy of the cortical tissue and prevent parturition at term in the sheep (9) goat (12) bovine (13). The object of this study was to follow the anatomical and histological development of adrenal fetal gland beginning at about 70 days (12 cm/CRL) of intra uterine life until term, Also, to increase the knowledge of some endocrinological parameter in Iraqi goats, as limited information is available concerning this species. It is important to determine whether there is a variation in pituitary adrenal axis function rating those which are related to the estrous induced (progesterone treatment), laproscopical insemination and antepartum period, as well as to improve the usage of sponges impregnated with 20 mg FGA, with a single injection of PMSG, and one fixed time laparoscopically insemination in non-breeding season on goat fertility.

Materials and Methods

The first part of study conducted on A hundred and twenty two (122) pregnant local goat genitalia were collected from slaughter house, of them 84 with single and 38 with twin fetuses, goat Fetuses (160) in various stages of gestation were included in present work. Fetuses were weighted after separation from the placenta. Crown rump length (CRL) was recorded following the technique of (14).

Fetal adrenal glands were quickly dissected free of connective tissues and weighed, on a sartorius 1212Mp balance then fixed in Bovines Fixative and embedded in paraffin. Cross section (5 nm thickness) through the centre of the gland. The histogenesis of the adrenal cortex was done by used, protocol for Mallory-Heidenhain stain(M-H), according to the technique of (15) and examined by light microscope.

The other part of the experiment was conducted out - breeding season (December 2010, January and February/2011), at the College of Veterinary Medicine, Baghdad University. The site is situated at longitude and

latitude (North 33.18, 44.35 East). The mean annual temperature is around 10 C.

Sixteen multi parous native black Iraqi does, weighing 40-50 kg were used in this trail. All animals were provided with water and manually fed alfalfa hay, supplemented with grain pellets 500 g/ day/animal.

Detection of estrous in the does was performed before application of vaginal sponges using a apronized buck, twice daily for 30 days and pregnancy diagnosis were performed by using Ultrasonic methods (Ultrasonography; equipment, prop 5-MHz; Welld ,China) .The estrus cycles were synchronized by using progesterone sponges containing 20 mg of fluorogeston acetate (Intervet, International BV.Boxmeer, Holland). Sponges were inserted for a period of 13 days and pregnant mare serum gonadotropine (PMSG) 500 IU (i.m) was injected to each doe 24 hrs. Before sponges removal (day 0 = day of sponge removal).

The does were checked for estrus emergence by a pronized buck during progesterone treatment. Laparoscopic Insemination were performed 24-36 hrs after estrous onset using 1ml of fresh diluted semen contains at least 100 million live fresh active sperm(Laparoscope equipment, Carl storz company, Germany).

During post insemination period all does were checked for estrous returning by a pronized bucks for 30 consecutive days. Ultrasonography was, also, used for pregnancy diagnosis at days 30th 60th and 90th post insemination.

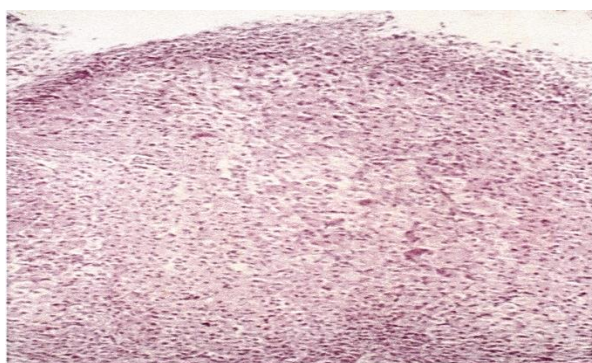
Blood samples were collected at day 10th of progesterone treatment, 24hrs following laproscopical insemination and at mid of the 1st ,2nd ,3rd ,4th and 5th months of pregnancy. Blood centrifuged at 2500 rpm for 15 minutes to serum separation which stored in -20C until hormonal assayed. Serum hormones concentrations were determined by Radioimmuno assay (RIA), Kits purchased from BioMeriux, Marcy-I Etoile, France).

Statistical Analysis: SPSS (16) was used to calculate one way-ANOVA and to estimate the differences between treatments. Duncan Test was used to detect the differences among different group means (17).

Results and Discussion

The mean body weight of the fetuses at different fetal CRL represented in Table, 1. There was gradual increase in the body weight of the fetuses about 70-90 days (12 cm to 20 cm) CRL about (70-90 days) with steadily increases from 25cm CRL to term. There was an increase in the absolute weight of the adrenal gland in fetuses at 12 cm CRL of intra uterine development towards birth. The absolute weight of the adrenal glands increased steadily throughout gestation and increased greatly during the last 10-20 days of gestation. This finding is similar to those reported in fetal lamb (18), and monkeys (19). There were variations in the growth rate of the fetal adrenal gland when expressed per 100 gm of body weight.

Fetal adrenal cortex: At about 70 days of intrauterine life (12 cm CRL) the differentiation of cortical cell gives rises to two histological distinct zones, an outer glomerulosa cells surrounded by capsule, and an inner zones of fasciculate and reticular (Fig. 1) arranged in bull like clusters (5-7 cells per 'cluster). The cells within the inner zone are composed of large rounded or oval cells with dark stained nuclei between. At about 100 days (25cm CRL) of intrauterine life, the blood vessels are clearly seen' inside the capsule among the zona glomerulosa clusters cells (about 7-10 cells per cluster). The cells of



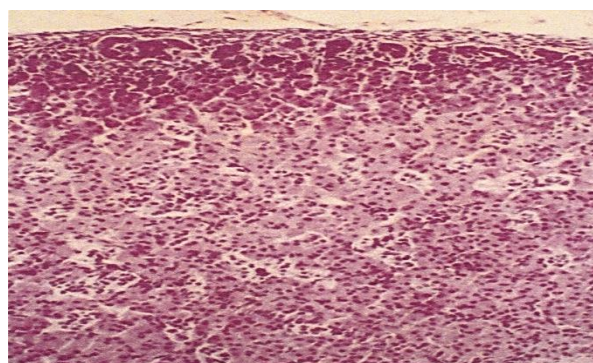
Figure, 1: Fetal goat adrenal gland ,CRL/12cm= 70 day ;Section show thin capsul with zona gloulerlosa , there is no demarcation between the zona fasculata and zona reticulars (M. H. X80).

One day old: the three zones are clearly seen. The zona glomerulosa cell clusters now have 10-15 cell/cluster. The cells of the zona

the inner zone increased in number with dark stained nuclei and pale cytoplasm (Fig. 2).

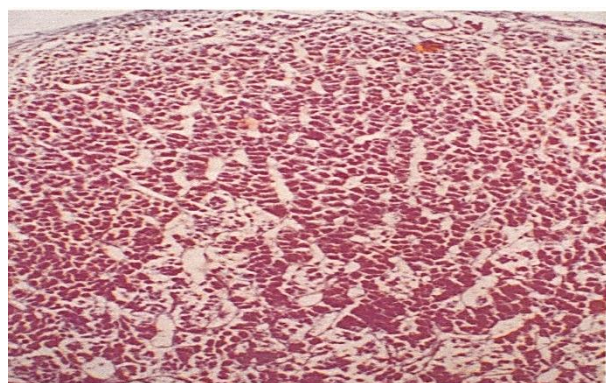
These cells are arranged in cords about 20-25 cells along. About 100 days of gestation (25cm CRL). The results of adrenal histology of the present study confirm those of Alexandra (20 and 21) who found the adrenals cortex of fetal lambs before 100 days of gestation consisted of two zones. During this period the cell number in the zona fasciculate increases.

The adrenal gland grows rapidly due to the increasing number of lipid rich cells in the zona- fasciculate- At about 120 days(35 cm CRL), the linnere zona (fasciculate and reticular) was about 40 cells thick-and more tightly packed with the zona glomerulosa (Fig. 3). The three zones can clearly at about 120-140 days (35-40 cm/CRL). The fasciculate zone cells are arranged in columns extending from zona glomerulesa. to the reticular is about 30-40 cells long, while the cells of the inner zonereticule are relatively do hold sparse cytoplasm with dark nuclei and are arranged in cord about 15-20 cells long(Fig. 4) . In fetal sheep an increase of about 5 to 6 folds occur in the size of adrenal cortex in the test 10 days of intrauterine life (22), Durand (23) also reported that the greatest increases in ovine fetal adrenal cell size and number occurred between 135 and 145 days of gestation.

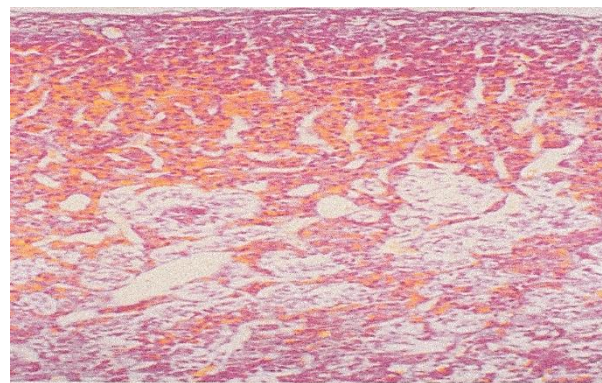


Figure, 2: Adrenal cortex of fetal goat, CRL= 25cm,100day : Zona glomerlosa cells are small and stain darker . There is clear line of demarcation between zona glomerlosa and fasciculate (M. H. X80).

fasciculate are usually vacuolated and form a large about 40 cells thick while the inner, zona reticular, is about 20 cells thick..



Figure, 3: Adrenal cortex of fetal goat CRL/cm = 35,120 day ; The cortex surrounded with capsule contain blood vessels. Zona glomerulosa cells are small , deeply stained and arranged in bull like cluster . The zone s of fascicularis and reticulate appears with large nucleus. (M. H. X80).



Figure,4: Fetal goat adrenal cortex CRL=40cm/140 day ; The capsule Of adrenal gland consist of dense C.T. Blood vessels are seen . Zone fasciculation cells and reticularis are increased in number and size , yellow in color. Medulla is found in the middle of section. (M.H. X84.4).

Table, 1: Fetal Crown Rump Length and weight. Absolute and relative weights of the Adrenal and pituitary gland in fetal Iraqi goat.

Number of Fetal Kids	Fetal Weight, g.	CRL, cm	Fetal adrenal Gland weight mg/100mg body weight	%
25	54.6±6	12	21.0±4	38.5
20	103.6±10	15	31,9±6	30.8
15	213.2±27	18	56.6±11	26.5
15	273,5±24	21	66.9±9	24.5
15	437.6±23	24	86.3±20	19.7
15	590±29	27	120.3±7	20.4
15	943.3±51	30	140.2±33	14.8
15	1150.7±71	34	166.3±36	14.5
15	1470.1±117	37	183,3±14	12.5
15	1360.1±150	40	179.5±13	9.7
10	2675.2±205	45	248.1±17	9.3

From histological observation the present study indicated that the adrenal cortex grow faster about day 120(37 cm/CRL) to term and the majority of this terminal growth take place in the zone fasciculate.

The FAG sponges did not fall off; no doe exhibited estrus while being treated with progesterone, indicating that the 20 mg intra vaginal FAG was adequate to suppress estrus activity. Romano (24) 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 present protocol in inducing estrus. Ahmed (25) reported estrous onset was 53 hrs in goats. Luteinizing Hormone (LH) surge was recorded at the hours of 41 – 51 after progesterone removal (26). Cameron (27) reported an ovulation occurred between 36 – 48 hrs.

The cause of the variation in the interval of the onset of estrus after FGA treatment in present work could be, due to the individual variation in FGA absorption and elimination rate. And this agrees with the suggestion that, it is possible that temporal relationships between estrus manifestation, LH surge and ovulation can be affected (28). The results of

the present study revealed that the estrous synchronization protocols may be 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 (fig-5 and 6) out-breeding season. In present work, injection of 500 IU PMSG could lead to the presence of 1-3 follicles of different sizes on each goat ovary and to increase estrogen secretion and estrus emergence in 100% during non-breeding season. Estrogen peak closely correlated with maximum size of pre-ovulatory follicle (28).

Laprosopical examination to the goats ovaries reported ,that the number of the different size follicles were 20 and 6 corpus luteum for the right ovaries and the left ovaries 15 follicles and 6 corpus luteum.(figs,5 and7).

The overall pregnancy rate recorded in this study was 81.22% while, Fonseca (29) reported that 81% pregnancy rate in goat, and superior to that reported by Baril (30) which found the pregnancy rate was 59% in goats treated for 11 days with FGA intra vaginal sponges. Iraqi goats have very good responses to the protocol applied during non-breeding seasons by increasing goat's fertility rate.

The results of hormonal measurement following induced estrus, laprosopical AI and during pregnancy are shown in table-2.

Table, 2: Serum cortisol profiles and their temporal interrelationship –during progesterone treatment, induce estrous ,laprosopical insemination and pregnancy in Iraqi goat.(M+S.E.).

Treatment	Cortisol nmol/L
During treatment	79.889±5.412 b
Post treatment	76.778±4.672 b
1 st month pregnant	64.800±5.722 c
2 nd month pregnant	63.11±5.43 c
3 rd month pregnant	73.889±9.432 b
4 th month pregnant	94.00±10.09 a
5 th month pregnant	97.11±6.24 a
Over all	78.93±2.96

According to the evaluation of cortisol concentration in collected samples (Table 1),The mean cortisol concentration in first and second sampling were significantly

($P<0.01$) higher than those at 1st and 2nd month of pregnancy. Also the mean concentration of the cortisol hormone in the 3rd,4thand 5thmonths of gestation significantly ($P<0.01$) higher than those of the periods.

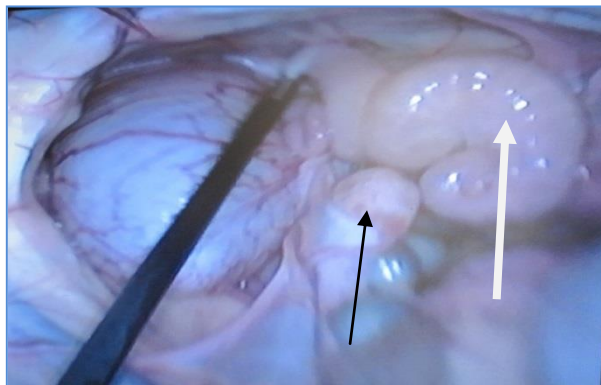
High level of cortisol, in first samples(table-2) could be due to stress – induce by the presences of intravaginal sponges for 10days, which cause inhibition of tonic gonadotropin secretion below levels required to inhibit ovulation, acute stress mechanism could interfere with ovulation by inhibition of the preovulatory gonadotropin surge (31).Breen (3) reported that plasma cortisol interferes the follicular phase by suppressing the development of high frequency LH phase, which compromises the preovulatory estradiol rises and LH – FSH surges. Cortisol act centrally to suppress pulsatile GnRH secretion in follicular phase in cow (32) and ewes (3).

The maternal cortisol concentration (table-2) during pregnancy revealed high values than that reported by Romano (24) who found that maternal cortisol concentration for normal delivering goat was 35.5 nmol/l at peripartum, higher than cortisol level (24.7 ± 4.36 nmol/L)reported by Manalu and Sumaryadi (33). The present results agreed with suggestion made by Edwards (34) that the influence of maternal nutrient restriction has markedly different effect on cortisol between early and mid-gestation compared with late gestation, maybe due to the much higher fetal glucose demands in late gestation trimester (35).In goat, Hennessy (36) reported that during late pregnancy the maternal cortisol levels increased due to placenta transfer from the fetus to the mother.

Suganaya and Gomathy (1) found that cortisol started to increase 15 days prior to kidding and reach a maximum on the day of kidding, this increase is attributed to stress – induced by the fetus in initiating parturition (37). Also Madibela and Segwagwe (38) reported that birth weights in goats with control body condition score had similar level of serum cortisol, and they suggested that cortisol may not have contributed of reproductive wastage or kid viability.Figure8. Ultrasonography of

pregnancy diagnosis showed the fetus at about 45day post insemination.

In conclusion: Levels of serum cortisol hormone were influenced by hormonal treatment, and pregnancy. Estrous could be



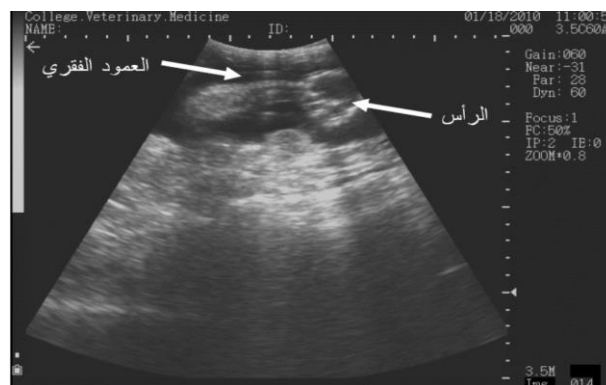
Figure, 5: Laparoscopic diagnosis (white arrow) of early pregnant goat uterus. Ovary (black arrow) contended small follicles and C.L.



Figure, 6: Laparoscopic artificial insemination in goat uterus .



Figure, 7: laparoscopic diagnosis of ovary contained large C.L.



Figure,8: Ultrasonographic image of goat uterus at 40 days of pregnancy.

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تطور قشرة الغدة الكظرية للأجنة مع مستوى هرمون الكورتيزول في مصل المعز العراقي بعد توحيد الشبق والتلقيح بالناظور خارج موسم التناسل

علي فاضل علوان

فرع الجراحة والولادة – كلية الطب البيطري - جامعة بغداد – العراق

الخلاصة

تضمن الجزء الاول من الدراسة جمع 160 جنين وحسب الطول التاجي العصصي تم تقدير عمر الجنين لغرض دراسة تطور الغدة الكظرية في أجنة المعز. أظهرت النتائج بدء الغدة الكظرية في التطور النسيجي قرب النصف الثاني من فترة الحمل , وحصلت زيادة سريعة في وزن الغدة عندما يكون طول الجنين التاجي العصصي 24 سم . بينما الزيادة في وزن الغدة الكظرية عندما يكون الطول التاج العصصي للجنين 12 سم فصاعداً . بينت الدراسة النسيجية بأن قشرة الغدة الكظرية تتطور بسرعة عندما يكون fasciculate طول الجنين 27 سم الى نهاية الحمل ومعظم هذا النمو يحدث نتيجة للزيادة العددية والحجمية لخلايا الطبقة fasciculate للقشرة الكظرية بصورة خاصة . شمالالجزء الثاني في لدراسة تقدير مستوى هرمون الكورتيزول في مصل المعز خلال العلاج بهرمون البروجسترون، والشبق المستحدث والتلقيح بالناظور وفحص الحمل بالسونار. استحدثت الشبق بواسطة اسفنجات مهبلية تحوي على 20 mg fluorogestone لمدة 13 يوم . قبل سحب الاسفنجات بـ 24 ساعة تم حقن 500 وحدة دولية من مصل الفرس الحامل بالعضلة . أظهرت 16 معزة الشبق خلال 24- 36 ساعة وبمعدل 46.9 ± 4.9 بعد سحب الاسفنجات تم فحص الحمل بالسونار ايام 30،60،90 . نسبة الحمل بلغت 13 معزة (81.22%) 3 معزات كانت تحمل توأم (23.01). معدل مستوى الكورتيزول في التجربة كان بمعدل 78.93 ± 2.96 نانومول /لتر. تستنتج الدراسة الحالية ان محور النخامية – الكظرية يكون فعال عندما يكون طول التاج العصصي للجنين 21 سم وما يقارب 90 يوم. وان المعز المحلي العراقي يستجيب لتوحيد الشبق خارج موسم التناسل واستعمال الناظور لا يؤثر على خصوبة الحيوان مستقبلاً.

كلمات مفتاحية : الغدة الكظرية – هرمون الكورتيزول – التلقيح بالناظور

