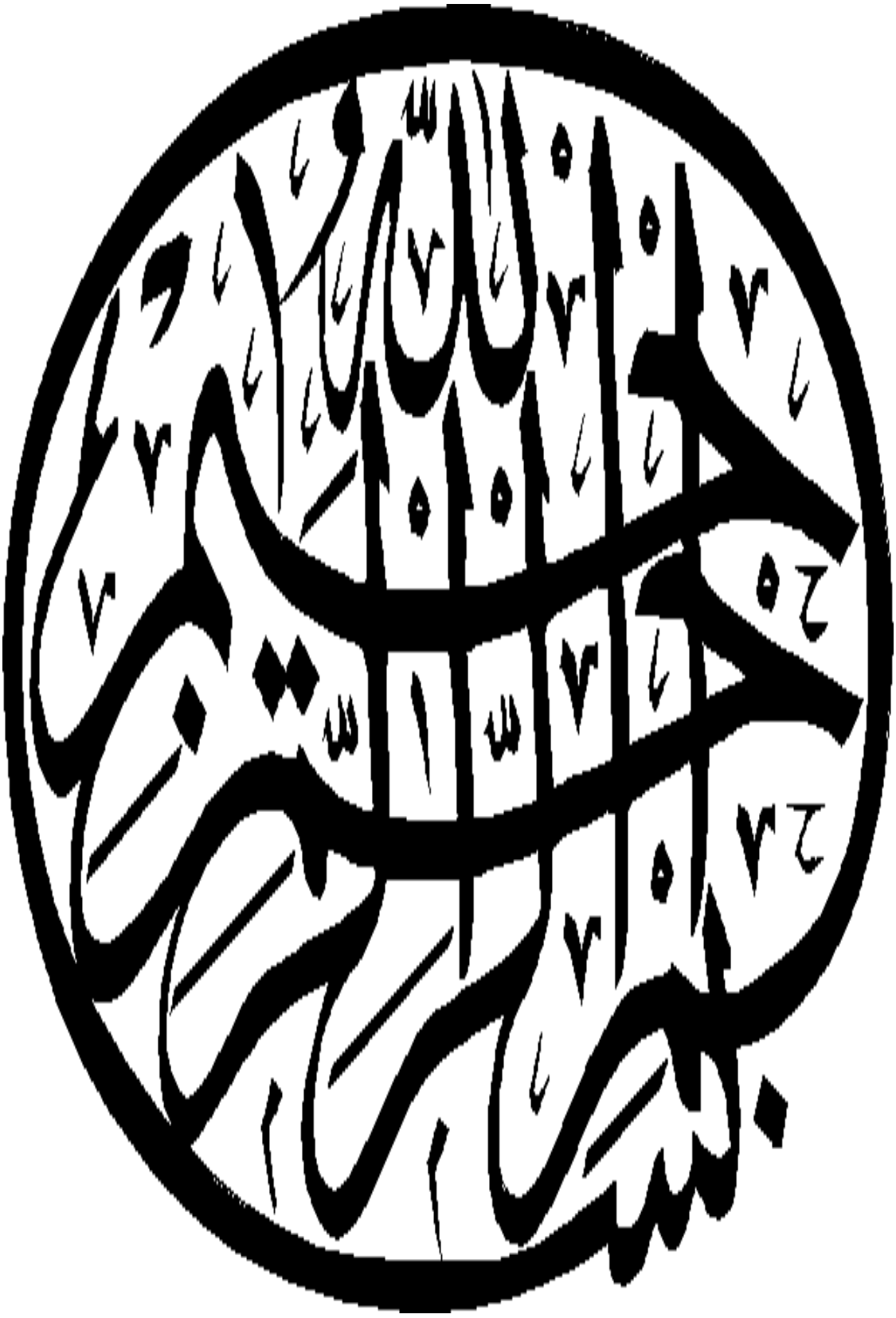

بسم الله الرحمن الرحيم

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صدق الله العظيم

البقرة الآية ٣٢

ZAGAZIG UNIVERSITY. BENHA BRANCH
FACULTY OF VETERINARY MEDICINE (MOSHTOHOR)
DEPARTMENT OF OBSTETRICS, GYNAECOLOGY; AND A.I.

ULTRASOUND STUDIES ON DIAGNOSIS OF REPRODUCTIVE STATUS IN ARABIAN MARES

THESIS

Presented to Faculty of Veterinary Medicine
(Moshtohor)-Zagazig University (Benha branch)

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قسم التوليد و التناسل و التلقيح الإصطناعى

دراسات عن استخدام الموجات فوق الصوتية فى تشخيص الحالة التناسلية للأفراس العربية

رسالة مقدمة الى
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مقدمه من
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VITA

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- The auther completed his military service as a reserve-officer in the Main Vet. Hospital of Vet. Services. Dept., of Egyptian Armed Forces from Jan., 1-1990 to Jun.,30-1992.

-The outher occupied the 3rd. horse breeding specialist in El-Zahraa stud for Arabian horse breeding E.A.O. from Nov.,15- 1992 tell now.

تاريخ حياة الباحث

- الباحث من مواليد القاهرة ١٩٦٦/١٠/٣٠.
- أتم الباحث دراسته الإبتدائية بمدرسة الفاتح الإبتدائية عام ١٩٧٨.
- أتم الدراسة الإعدادية بمدرسة د.طه حسين الاعداية عام ١٩٨١.
- أتم الدراسة الثانوية بمدرسة الظاهر الثانوية عام ١٩٨٤.
- حصل الباحث على بكالوريوس العلوم الطبية البيطرية من كلية الطب البيطرى بمشتهر - جامعة الزقازيق فرع بنها عام ١٩٨٩.
- أتم الباحث الخدمة العسكرية كضابط إحتياط بالمستشفى البيطرى الرئيسى التابع لإدارة الخدمات البيطرية للقوات المسلحة المصرية خلال المدة من ١٩٩٠/١/١ حتى ١٩٩٢/٦/٣٠.
- قام الباحث بالتسجيل للحصول على درجة الماجستير فى دور سبتمبر ١٩٩٠.
- يشغل الباحث وظيفة أخصائى تربية خيول ثالث بمحطة الزهراء لتربية الخيول العربية الاصيلة التابعة للهيئة الزراعية المصرية و ذلك من ١٩٩٢/١١/١٥ و حتى الان.

DEDICATION

TO MY FATHER, MY MOTHER AND MY BROTHERS
FOR THEIR LOVE, DEVOTION AND PATIENCE

CONTENTS

INTRODUCTION

INTRODUCTION

Long time, ago, horses were known to be very important mean of locomotion allover the world. They were widely used for draught, riding, race, military and show purposes. Today and due to the great progress in the process of life motorization, the use the horse for draught or travelling purposes is greatly diminished.

In Egypt ,the horses form a large compartment of animal wealth.There are about 40,656 horses distributed as follow: 24,356 in lower Egypt; 4,592 in middle Egypt and 11,708 in upper Egypt ***(Ministry of Agriculture,1994)*** .

Breeding of Arabian horses is supposed to be pleasurable hobby. Exportation of Arabian horses plays an important part of the national economics due to their high price. Of all breeds, the Arabian horses are considered not only the oldest breed, but also the most beautiful one. So, heavy attempts are taken out in Egypt, for maintaining the Arabian horse in pure form.

Preservation of Authentic Arab Horse at El-Zahraa:

The broadmares and StudStallions of the Egyption stud, El-Zahraa, are the descendants of the best horses in existence in ancient Arabia. Their forebears were imported to Egypt by Mohamed Ali the Great (1769-1849) and Abbas pasha I (1813-1854), and afterwards bred by Ali Pasha Sherif, who died in 1897. In addition, members of the Royal family and lady Blunt of England maintained stud near Cairo and obtained their horses both from Ali Pasha Sherif and from the Arabian desert.

The horses of the Egyptian National stud, El-Zahraa, are living tokens of the fascinating history of the Arabs. At El-Zahraa, breeding is carried on in natural surroundings and under the same conditions which produced the original horse. This means, among other things, the horses are not well-fed; round quartered and perfectly groomed animals seen in the stables of American or European horse- fanciers. As Abbas Pasha I once said to the German Baron "Could scarcely retain the quality and characteristics for their purity and distinguished type, and they have been imported periodically by many countries interested in improving the breeding of their Arab horses".

The foundation Mares of El-Zahraa:

It is good to group the mares and stallions of a stud according to family strains of the root mares, and each bloodline produces a different set of qualities.

According to ancient tradition, the horse-breeding tribes throughout Arabia identified a horse by its tail female heritage. The different strains were perpetuated through the mares, the foals taking their dam's strain name regardless of what the sires was. Long ago there was a lack of written stud records, so, the horses pedigree were conveyed from old to young by word of mouth. Thus this strain system was very useful, as the mare was always well known to every one in the tribe, the sire less so.

Nowadays strains do not have the some significance as they did in the desert. There is, however, a use for strains, as the focus attention no (Female) families, for there is no doubt that the mare have a strong influence on their

offspring. The system of family strains is also used for identification in the Egyption studbook ***(Erwin 1982).***

El-Zahraa's Root strains:

1- Kohailan Rodan

2- Saklawi Gidran.

3- Dahman Shahwan.

4- Hadban Enzahi.

5- Obeyan Sharrak.

Problems of equine reproduction needs more investigations to reach the accurate diagnosis to improve the reproductive capacity of this species. Seasonality of reproduction and relatively low conception rates were examples. Infertility problems were recorded in mares mainly due to pathological ultrations in the uterus as endometrial cysts, embryonic death, foetal death, and endometritis. Anovulatory luteinized follicles, follicular and ovarian haematomae, ovarian cyst and ovarian tumers were the most causes of pathological ultrations of the ovary.

The changes in the consistency of the uterus during the cycle and the possible presence of abnormal contents might be diagnosed by manual rectal examination. However, this method was subjective and interpretation was often quite difficult. The finer details of the uterine wall and the possible contents could not always be determined in this way.

For these reasons, it was very important to find-out some methods for diagnosis the physiological, as well as the pathological ultrations in the female reproductive tract .

The differentiation between pregnancy and other forms of pseudopregnancy could only be made with the help of echographic examination to ensure the presence or absence of foetal vesicle.

The aim of the present work was to clarify whatever hormonal profile and trace elements determination in the mare's blood during estrus and early pregnancy (which was confirmed by the use of ultrasound) could be used to diagnose (accurately) estrus and normal pregnancy. Moreover, cyclic changes in the ovaries (ovarian activity) could be detected by the use of ultrasound and whatever it could be used to diagnose the stage of estrous cycle.

The present study is an attenpt to investigate the following aspects:

1- Determination of estrous cycle length as well as the lengths of its phases.

2-Accurate diagnosis of estrous phases either by determining hormonal profiles, trace elements concentrations or by observing the cyclic ovarian changes using ultrasonography.

3- Early pregnancy diagnosis using ultrasonographic method.

REVIEW OF LITERATURE

I . REVIEW OF LITERATURE

1.1. Anatomy of mare genital organs:

1.1.1. The ovaries:

The ovaries unlike the testis, remain in the abdominal cavity. They perform both endocrine, and exocrine functions ***(Hafez, 1987 and McDonald, 1989).***

They are roughly bean-shape, although their actual shape and size vary with the breed, age, follicular content, and season. Anestrous ovary ranges from 4 X 2 X 2 cm to 8 X 4 X 4 cm, and about 70 to 80 gm weight ***(Sisson, 1975; Rossdale, 1981 and Edward ,1988).***

For description, each ovary has two smooth and rounded surfaces (medial and lateral), two borders; convex mesovarial one is enclosed in a part of broad ligament (mesovarium), and through this border the nerves and blood vessels reach the ovary. The free border is marked by a notch leads to a narrow depression (The ovulation fossa). It also has two extremities, both are rounded , and one of them (the cranial one) is related to the fimbriated end of the uterine tube and termed tubal extremity, while the caudal one is connected with the uterine horn by the ovarian ligament ***(Kainner, 1993).***

The ovaries are situated in the sublumber region, usually ventral to the 4th and 5th lumber vertebrae, and they are attached by the cranial part of the broad ligament (mesovarium). This situation is largely variable because of the passive movement which occurred due to lying of the ovaries on the top of the intestinal mass ***(Sisson, 1975).*** He added that, the distance from ovaries to the tips of the uterine horns varied according to the stage of gestation. During pregnancy, they are pulled cranio-ventrally. Moreover, the average distance from the ovaries to the vulvar orifice is about 50-55 cm in medium - sized non-pregnant mare.

1.1.2. Uterine tubes (Oviducts or Fallopian tubes):

The uterine tubes act as the excretory ducts of the ovaries. Each one consists of an expansive infundibulum covering the ovulation fossa, a highly tortuous ampulla about 6 mm diameter, and less tortuous isthmus 3mm diameter ***(Ono, Satoh, and Fujimoto ,1969; Sisson, 1975 and Kainner,1993).*** They added that, the length of equine oviduct is about 20 - 30 cm, and the ampulla comprising about the half of this length , moreover, the isthmus terminates at a small uterine ostium on a papilla within the end of the uterine horn.

Each oviduct is enclosed in a peritoneal fold derived from the lateral layer of the broad ligament (mesosalpinx) which covers the lateral aspect of the ovary **(Ginther, 1979)**. He also reported that, the mesosalpinx forms with the ovary and the broad ligament the ovarian bursa.

1.1.3 .The Uterus:

The uterus is a hollow muscular organ which is continuous with the uterine tubes cranially, and opens into the vagina caudally. It is situated chiefly in the abdominal cavity, but extends for a short distance into the pelvic cavity where it is attached to the sublumber region and the lateral walls of the pelvic cavity by the broad ligament. Both uterine horns are diverged sharply from the body and connected (to each other) caudally by a small intercornual ligament, and located entirely within the abdominal cavity, lie on or intermingled with the intestinal coils. Their length is ranged from 20 - 25 cm ***(Dyce, Sack and Wensing, 1987).***

Lieux (1970) and Sisson (1975) reported that, the uterine body is situated partly in the abdominal and partly in the pelvic cavities between the rectum and the bladder. Its average length is about 18 - 20 cm. They added that, the most constricted caudal part of the uterus (cervix) which joins the uterus with the vagina is a thick muscular sphinctor, 5 - 7.5 cm length, and 3.5 -4 cm diameter with a projected part into the vaginal cavity (portio vaginalis.)

1.2. Reproductive physiology in the mare:

1.2..1- Breeding Season:

The mare is a seasonal polyestrous animal and its breeding season is thought to respond to the influence of light. Ovulation percentage is lowest during the shortest daylight months, and is higher during long daylight months. Moreover, with ideal husbandry, many mares did not show winter

anestrous by using artificial light which encourage continuous cycling or early onset of breeding season ***(Ginther, 1979).*** Also, ***Oxender, Noden and Hafs (1977)*** reported that 16 hr photoperiod started in early December for anestrous mares induced normal estrous cycles within 2 months. Moreover ***McDonald (1989)*** illustrated that, in the northen hemisphere, after December 21, the pituitary - gonadal axis responded to the increased daylight length and follicular growth began, and this effect of the light is the same in the southern hemisphere but the period during the calendar year was reversed.

Other factors influnced breeding season than photoperiod, such as nutrition and climate, principally temperature, since in temperate climate zones, the mares undergo cyclic sexual activity during the spring and summer ***(Ginther ,1974).***

1.2..2. Estrous cycle:

It is the repetitive sequence of events that prepares the mare for conception. It may be conventionally divided into estrus (Follicular phase) and diestrus (Luteal phase) ***(Deals and Hughes, 1993).***

The estrus is that period during which the mare is sexually receptive to the stallion, the genital tract is prepared to accept and transport spermatozoa, and ovulation occurred approximately 24-48 hr before the end of sexual receptivity ***(Nishikawa, 1959; Asdell, 1964; Witherspoon and Talbot, 1970; and Hughes, Stabenfeldt and Evans,1972).*** Moreover, ***Stabenfeldt, Hughes and Evans (1972)*** reported that, the estrus phase is the interval between the

beginning of the onset of corpus luteum regression (1st day of significant progestins decline) and the day of ovulation.

During estrus, the dominant follicle secretes estrogen that induced sexual behaviour which is characterized by excitability, frequent urination, mounting other females, whinning or squealed, the tail-head raised, assuming a urinary stance with frequent exposure of clitoris by frequent winking of the vulva ***(McDonald, 1989 and Fraser ,1992).***

The response of the mare during teasing at luteal phase is by laying the ears back, biting , squealinge, kiking, and squatting ***(Back, Pickett, Voss and Seidel ,1974).***

Stabenfeldt et al. (1972) reported that, the average durations of estrous cycle, follicular phase, and luteal phase were 18.5, 7.7, and 12.5 days respectively. On the other hand ***Sharp and Black (1973); Back et al. (1974) and Hunt ,Lein and Foote (1978)*** recorded average durations of (20.0 - 22.0; 6.6 - 8.9 and 13.9 - 16.0 days) for estrous cycle, follicular phase, and luteal phase respectively.

1.2.2.1. Hormonal changes during estrous cycle:

Reproductive hormones from hypothalamus, pituitary, ovary, and uterus control the dynamic changes in the genital tract and sexual behaviour through complex interactions. These include Gonadotrophin releasing hormone (GnRH), Follicular stimulating hormone (FSH), Lutenizing hormone (LH),

Estrogen, Progesterone, Inhibin, and Prostaglandin ($PGF_{2\alpha}$) ***(Soliman, 1978 and Deals and Hughes ,1993).***

Progesterone is one of the key hormones controlling reproductive functions in the mare. Its secretion from the corpus luteum should be terminated to allow the reproductive cycle to be repeated ***(Squires ,1993).*** The plasma progesterone levels at 2-3 days interval can reflect the stage of estrous cycle in the mare accurately ***(Ammons, Therlfall and Kline, 1989)***..

Progesterone hormone, as most steroid hormones, is derived from a common precursor molecule, cholesterol. Cholesterol is synthesized from acetate in the liver and is released into the blood as lipid droplet, or can be obtained by de-novo synthesis in the smooth endoplasmic reticulum of steroid-secreting cells. The 1st step in the synthesis of progesterone is a side-chain hydrolysis to yield a 21-carbon containing compound, pregnenolone. The side-chain hydrolysis reaction needs side- chain cleavage enzyme present in the mitochonderia ***(Norris, 1985).*** The granulosa cells of the ovarian follicles, are essential for steroidogenesis since it contained 3b-hydroxy - Δ^5 - steriod dehydrogenase enzyme (3b-HSD) which converts the pregnenolone to progesterone ***(Meinecke, Gips and Tillmann, 1987).***

The preovulatory surge of gonadotrophins induced a change in the follicular compartments, resulting in releasing the ovum and transformation of the follicle into a corpus luteum. Progesterone, in follicular fluid, has been suggested as having a role in follicular rupture at ovulation ***(Tsafrui,Abisogun***

and Reigh, 1987). After ovulation, granulosa cells became luteinized and conversion of progesterone to 17α- hydroxy progesterone was retarded and progesterone accumulated with some conversion to 20α - dihydroxy progesterone. The ketone group at carbon 3 and the double bond between carbon 4 and 5 were believed to be necessary for biologic activity of progesterone ***(Kenney, Condon, Ganjan and Channing , 1979).***

LH is the major pituitary lutotropin controlling progesterone secretion in most domestic species. Unique to the mare, LH remained quite high for several days after ovulation and this elevated concentrations is thought to be important for the development of corpus luteum ***(Noden, Oxender and Hafs, 1975).***

The role of gonadal steroids in the regulation of gonadotrophin secretion had been extensively studied in many species. Progesterone is one of the major components of negative and positive feedback systems controlling the release of several pituitary hormones including Prolactin, FSH, and LH ***(Fink, 1988).***

The mean plasma progesterone level during follicular phase was 0.4 ng/ml ***(Hunt et al. ,1978)***, while during luteal phase was 3.88 ng/ml ***(Ginther, Foley, Gaverick and Plotka ,1980).*** In contrast,***Smith, Bassett and Williams (1970); Stabenfeldt, Hughes and Evans (1971); Plotka, Witherspoon and Goetsch (1971) and Sharp and Black (1973)*** reported that , the mean average plasma progesterone levels during follicular and luteal phases were 0.53 - 0.63

ng/ml and 6.8 - 10.9 ng/ml respectively. Moreover ***Sharp and Black (1973)*** added that, the progesterone level during follicular phase may be below the detectable limit (< 0.22 ng/ml). It ranged from 0.4 - 0.9 at day of ovulation ***(Smith et al., 1970 and Kooistra and Ginther, 1976)***. Moreover ***Plotka, Foley, Witherspoon, Scholler and Goetsch (1975)*** reported that, the progesterone levels were 60 - 100 pg/ml, 140 pg/ml, and 346 pg/ml 24hr before ovulation, 10 hr, and 26hr postovulation respectively. The mean plasma progesterone concentrations increased to a level more than 1ng/ml 24 hr postovulation, and reached the 1st peak (7.7 ng/ml) on day 6 postovulation during luteal phase. The progesterone level remains elevated for several days and reached the 2nd peak (7.8 ng/ml) on day 10 postovulation ***(Smith et al., 1970; Oxender et al., 1977 and Ginther et al., 1980)*** .They also found that, the progesterone level decreased upto 0.97 ng/ml at the onset of new estrus on day 15 after ovulation. The life span of cyclic corpus luteum is 12 - 13 days;

the interval between regression of corpus luteum and onset of estrus is 3 days ***(Hughes et al., 1972; Stabenfeldt et al., 1972; Sharp and Black, 1973 and Oxender et al. ,1977)***.

1.2.2.2. Changes in trace elements in relation to estrous cycle:

Trace elements are essential to maintain the optimal health conditions of animals in the most critical periods; not only reproductive life of female animals, but also most life functions ***(Morrow, 1980).*** In addition to their vital role as co-enzymes, trace elements as components of body fluids (electrolytes); can serve to bind, transport, and release of oxygen. Also, they

can be structural components of non-enzymatic macromolecules ***(Schwarz, 1979)***. Various trace elements can influence reproductive performance of ruminants. Reproductive failure may be induced by deficiency of a single or combined trace elements which may act as co-factors or activators of enzymes or stabilizers of secondary molecular structures ***(Vallee and Wacker, 1976 and Hidiroglou ,1979)***.

Copper is an important and essential trace mineral for different biological functions ***(Sorenson, 1987)***. Also, Copper plays an important role in erythrobiosis because its deficiency leads to anaemia through reduction of ceruloplasmin which has a role in haemoglobin synthesis ***(Zidar, Shadduck, Zeigler and Windelstein, 1977; Jones and Suttle, 1983 and Vruwink, Keen Gershwin and Hurley ,1987)***.

Hypocuprosis in cattle leads to infertility in the form of subestrus, irregular estrous cycle, inactive ovaries and retained placenta ***(Loosli, Chairman, Huffman, Petersenfi and Phillips, 1964)***. Moreover, Copper deficiency may affect reproductive behaviour since, an old female goat when kept on low Copper diet for an extended period showed nymphomania which may be due to changes in gonadal steroid metabolism ***(Hidiroglou, 1979)***. Also,***Gerloff and Morrow (1986)*** recorded that , the mechanism of impaired fertility due to Copper deficiency was unknown or it might be due to anaemia and unthriftiness.

Blackmore and Brobst (1981) reported that, the normal Copper level in the serum of the American thoroughbred horse was 24-35 μmol/liter, and that of English thoroughbred horse was 8-18 umol/liter, while that of Quarter horse was 5-31 umol/liter. Moreover, ***Edger (1992)*** recorded that, serum Copper concentrations were 13-36, 124-233, 138-200, and 104-177 μg/100ml for foals at birth, foals at 1 month age, foals at 6 months age , and mature horses respectively.

Abdel -Ghaffar, Shawki, El-Dawy and Barakat (1993) recorded that , mean plasma Copper level of non-pregnant she-camel was 82.80 μg/100ml, while ***Hidiroglou (1979) and Farrage and Hassan (1983)*** reported that, the Copper levels in dairy cows were 2.1 μg/liter, and 120- 210 μg/dl respectively. Moreover, ***Leech, Howrth, Thornton and Lewis (1982) and Davis and Mertz (1987)*** recorded that, the normal level of serum Copper in non-pregnant cattle ranged between 60-80 μg/dl, and that, the level of Copper less than 50 μg/dl was considered a case of hypocuprosis.

The mean plasma Copper levels during follicular phase, at heat, at time of ovulation and during luteal phase for non-pregnant buffaloes were 204.97,

189.99, 189.76 and 195.35 μg/dl respectively ***(Desai, Thakkar, Dharshana and Janakiraman,1978 &1982).*** Moreover, ***Chandolia and Verma (1987)*** reported that , the Copper levels of non- pregnant buffalo-hifers were 109.62, 111.67, and 107.50 μg/dl for anoestrous hifers, and during estrus and diestrus phases respectively. In the same respect, ***Radwan, Waffaa, Mona, Anwar and***

Ismail (1991) recorded, 100.26 and 64.72 μg/dl for Copper plasma concentrations during follicular and luteal phases respectively for non-pregnant buffaloes.

Iron is one of the important essential trace elements, since more than 90% of the Iron in the body is combined with proteins, such as haemoglobin in R.B.Cs., transferrin in the plasma which bind two atoms of ferric ions, ferretin, and haemosedrin. It is also a component of many metaloenzymes ***(McDonald, Edward and Greenhalgh, 1979).*** Generally, Iron is required in relatively large quantities, and there is no evidence of Iron deficiency ever in grazing sheep or cattle except as a result of disease or parasitic infestation, and consequently reproduction is secondarly affected due to Iron deficiency ***(Hidiroglou, 1979).***

The mean plasma levels of Iron for thoroughbred horses, Quarter horses, Arabian horses, Standardbred horses, and Shet- land horses were 28.0, 28.0, 23.0, 30.0 and 19.0 μmol/liter respectively ***(Blackmore and Brobst, 1981)*** While, the mean plasma Iron levels of camel at ages of 2-3 years, 3-4 years, and 5-6 years were 92.78, 114.04 and 131.00 μg/100ml respectively ***(Shekhawat, Bhatia and Ghosal, 1987).*** Moreover, ***Shehata and Zaghloul (1990)*** cited that, the mean plasma levels of Iron in non-pregnant she-camel at the different ovarian stages; non-follicular, mature follicular, and luteal stage were 0.61, 0.14, and 0.16 μg/100ml respectevely. While, ***Abdel-Ghaffar et al. (1993)*** reported that, the mean plasma level of Iron of non-pregnant she-camel was 210.55 μg/100ml. In the same respect, ***Fouad, Awad, Abel-Ghaffar,***

Georgy and Bishara (1972) and Blum and Zuber (1975) reported that, the mean serum Iron levels for normal dairy cattle were 144.4 and 146 μg/dl respectively. While, ***Haroun and Hussein (1975)***; ***El-Belbasi (1980) and Abdel-Maksoud (1991)*** recorded that, the mean serum Iron levels in normal non-pregnant cows were 216.0, 208.0, and 208.13 μg/dl respectively.In surti-beffaloes, ***Desai et al. (1978 & 1982)*** reported that, theIiron levels during follicular stage, at heat, at time of ovulation, and luteal phase were 879.25, 787.10, 837.51 and 882.7 μg/100ml respectively. Also, ***Chandolia and Vevma (1987)*** recorded that, the iron levels of anoestrous buffalo-hifers, and during estrus and diestrus phases were 305.40, 311.20 and 316.80 μg/dl respectively. Moreover, ***Shehata and Zaghloul (1990)*** reported that, the mean plasma Iron level in non-pregnant buffaloes was 200 μg/dl.

Zinc is an essential element for normal growth and health. It is closely associated with many enzymes as a co-factor and it has a role as activator in bone metabolism by enhancing the synthesis of DNA in the diaphseal cells to increase cell proliferation and enhancing the effect of 1,25-dihyroxy-cholicalicfarol ***(Underwood, 1977; Vallee, 1983 and Yamaguchi and Inamoto, 1986).***

Zinc deficiency causes various malformations, and has deleterious effects on sexual functions. In different species, Zinc deficiency causes more pronounced impairment of fertility in males than in females, it mostly appears to be specific to the final stages of sperm maturation ***(Hidiroglou, 1979)***.

Moreover, he added that , the fertility was improved in ewes and cows that received zinc supplementation in diets.

The mean plasma Zinc concentration depends on age and breed of equids, and it is 30 - 80% higher in newborn and one-week old than yearling and mature horses ***(Nadia, Frank and David, 1986)***. They added also that, it was 22% higher in Draft-cross horses than other equids. At the same aspect, ***Edger (1992)*** reported that, the mean average plasma Zinc concentrations of different ages were 75.0 - 130.0,, 70.0 - 100.0, and 80.0 - 220.0 μg/dl for foals at birth , foals at 1-6 months age, and mature horses respectively. ***Abdel-Ghaffar et al. (1993)*** reported that, the mean plasma Zinc level in non-pregnant she-camel was 189.99μ/100 ml. While, ***Hidiroglou (1979) and Fahmy, Amer and Abdel-Aziz (1979)*** reported that, the plasma Zinc levels were 3 mg/liter for adult cows, and 78.57 -98.80 μg% in adult buffaloes at 15 months age. Moreover, ***El-Azab, Sharawy, Labib and Doidar (1985) and Chandolia and Verma (1987)*** reported that, the mean plasma Zinc concentrations for anestroum hifer, during estrus, and during diestrus phase were 120.75, 128.80, and 121.67 μg/dl respectively. Also ***Radwan et al (1991)*** reported that, the Zinc levels in buffaloes during follicular and luteal phases were 74.8 and 100.86 μg/ml respectively. They added that, the low level of Zinc during follicular phase was due to failure in binding with protein.

1.2..3. Pregnancy:

1.2.3.1. Hormonal changes during pregnancy:

Secretion of progesterone from the corpus luteum is essential for maintenance of normal pregnancy in all demostic species ***(Squires, 1993).*** There was a significant differences between mean concentrations of plasma progesterone for pregnant and non-pregnant mares from the beginning of luteal function till day 44 of gestation ***(Ginther, 1979).*** Continued high levels of progesterone are essential for pregnancy maintenance. The primary corpus luteum which formed at the time of conception is the sole source of progesterone ***(Holtan, Nett and Estergreen, 1975)*** . They added that, the secreted progesterone form the primary corpus luteum resulted in an increase in it is level from day 0 to day 8 of gestation (day 0 = day of ovulation), followed by a gradual decrease until day 28 to day 30 .

During the time of high concentration of progesterone, LH levels are depressed. In contrast progesterone has little effect on FSH secretion. This may lead to considerable follicular activity in mares when exposed to elevated progesterone levels for extended period ***(Garcia and Ginther ,1976).***

Kooistra and Ginther (1976) recorded a partial regression in the primary corpus luteum during the time of progesterone decreased till day 30. A dramatic increase in progesterone level was reported by the same authers between days 30 and 40 of gestation. ***Allen (1969) and Squires and Ginther (1975)*** reported that, the rise in progesterone secretion was coincident with the

formation of endometrial cups and secretion of equine chrionic gonadotrophin (eCG).

The increased levels of progesterone after day 40 of gestation is due to development of secondary corpora lutea. Many of secondary corpora lutea are physically identical to the primary corpus luteum. These secondary corpora lutea are mushroom-shape with a tracts toward the ovulation fossa and are considered to be formed from ovulatory follicles and the formation of these secondary corpora lutea more likely during days 40 to 70 than after day 70 ***(Ginther, 1979)***. The number of secondary corpora lutea increased up to approximately day 140 and their total number varied among mares. Some mares having no secondary corpora lutea and others forming 30-40 secondary corpora lutea. The primary corpus luteum is maintained functional until days 160 - 180 of gestation, and its regression at the same time with the secondary corpora lutea and completely regressed by days 180 - 220 of pregnancy ***(Squires, Douglas, Steffenhagen and Ginther 1974$_a$. and Squires, Wentworth and Ginther, 1974$_b$).***

Uterine milk secreted from the uterine glands is apparently important in nursing the early conceptus. The ability of the endometrium to provide adequate amounts of appropriate secretions depended to a large extent on progesterone. The total protein content of uterine secretion throughout the estrous cycle of mare decreased abruptly as progesterone declined. In contrast, with luteal maintenance and continued progesterone production, protein content of uterine secretions remained elevated ***(Zavy ,1979)***.

The progesterone concentrations during early pregnancy in the mare ranged from 4.22 - 5.70 ng/ml ***(Hunt et al., 1978; Ginther et al., 1980 and Irvine, Sutton, Turner and Mennick, 1990).*** In contrast, ***Kooistra and Ginther (1971); Ginther (1979) and Tamanini, Gaiani and Bono (1981)*** reported that, the plasma progesterone concentrations ranged from 6 - 13 ng/ml during early pregnancy.

The levels of progesterone on pregnant mares declined from a peak of 12 ng/ml on days 8 -10 to a level of 6 ng/ ml by day 18 - 20 , and remained low and rose (more than 12 ng/ml) in association with the development of secondary corpora lutea after day 35 ***(Ginther, 1979; Muesi, Falkay and Kuesoral 1989 and Schwab, Evans and Potter, 1990).***

1.2.3.2. Trace elements concentrations in relation to pregnancy:

The concentrations of trace elements and metabolites in the blood varied with age, stage of pregnancy, and milk yield ***(Rowland, Manston, Pocok and Dew ,1975 and Hidiroglou, 1979).*** Moreover, ***Goble (1989); Jones (1989); Amy (1992) and Joseph (1992)*** reported that, deficiency in trace minerals was one of the factors associated with osteochondrosis in young foals, since mare's milk is almost devoided of trace minerals, foals might rely on liver stores acquired during gestation. They added that, the prevention of the disease should be started during last trimaster of gestation, the mare should be fed a balanced ration including Copper, Zinc, and Manganese that provided the foal

with adequate liver stores of trace elements until it could consume adequate supplement.

Copper deficiency, particularly during early development, has been shown to negatively affect the immune system in sheep, cattle, and human ***(Zidar et al., 1977 and Vruwink et al., 1987).*** Hypocuprosis in cattle is associated with female reproductive disorders, the most common symptoms are prenatal mortality, particularly early embryonic loss, and sometime high incidence of retention of placenta ***(Hidiroglou, 1979)*** . He added that, in sheep and goats, low Copper content in the diet prevented implantation and induced embryonic loss and fetal death. While in pregnant ewes, abortion and sometimes stillbirth might occur.Moreover, plasma Copper levels in ewe falls during pregnancy and rises after parturation, where in cows, increased during pregnancy and reached the highest level at 5th month of pregnancy.

Ullery, Struthers, Hedricks and Brent (1966) and Ullery, Ely and Covert (1974) recorded that, the levels of Copper in the mare's milk during 4 months of lactation were 16.0, 10.0 and 4.0 μmol/ liter at parturation, 1-8 days and 9 - 120 days postpartum.

During different stages of pregnancy in she-camel, ***Abdel-Ghaffar et al. (1993)*** emphasized that, the plasma Copper levels were high significantly elevated during 1st (110.73 μg/100ml), 2nd (135.60μg/100ml), 3nd (164.23 μg/100ml), and 4th. (17.8.14 μg/100ml) stages of gestation. While, in cows and buffaloes, ***El-Azab et al. (1985); Pathak, Patel and Janakiraman (1986)***

and Wafaa, Radwan, Anwar and Ismail (1991) claimed that, the serum Copper levels at different stages of pregnancy ranged from 66.7-183.3 μg/100ml, with 3 peak-levels at 2nd, 4th and 5th months of pregnancy. While ***Shehata and Zaghloul (1991)*** cited that, the Copper plasma level during 2nd trimaster of pregnant buffaloes was 115.5 μg/dl.

Iron present in maternal serum is mainly bounded to transferrin and ferritin. During pregnancy, a large amount of ferritin is loaded on placental villous tissues and incorporated into the placenta by piocytosis in the trophoplast ***(Yamaguchi and Inamoto, 1986).*** The amount of serum ferritin in maternal blood is small and its Iron content is also small ***(Walter, Miller and Warwood, 1973).*** They added also that, serum ferritin had been considered to reflect the amount of Iron stored in the body, and that, its concentration characteristically decreased with the advancement of pregnancy. Moreover, the concentration of maternal haemoglobin was also greatly reduced in the late stage of pregnancy. Similarly, ***Church and Pond (1988)*** observed a considerably species variability existed in the efficiency of transfer iron to foetus across the placenta. They also noticed that, Iron transported to the foetus by an active process and its concentration in the foetal circulation exceeded that detected in maternal plasma. They also add that, transferrin did not cross the placenta, so, Iron dissociated from transferrin an the maternal side of the placenta, and reassociated with transferrin on the foetal side. With the more progress of gestation, Iron was transferred to the foetus and decreased in the maternal plasma. Furthermore, ***Orten and Neuhaus (1982)*** reported that, the amount of Iron which passed from the placental barriers to

the foetus during pregnancy augmented the foetus for about 6 months after parturition. They attributed that for the Iron deficiency of the milk.

Ullrey et al. (1966 &1974) recognized that, the mean iron levels in the Arabian-mare's milk at different stages of lactation were 24, 17, and 12 μmol/liter at partum, 1 - 8, and 9 - 120 days post partum respectively.

Abdel-Ghaffar et al. (1993) observed a progressive significant reduction in iron serum level during the 1st (191.74 μg/100 ml) , 2nd (180.33 μg/100 ml), 3nd (151.77 μg /100 ml) and 4th (139.53 μg/100 ml) stages of gestation in she-camel. Moreover, ***Shehata and Zaghloul (1991)*** reported that, the mean plasma iron concentrations in the buffaloes was 176.0 μg/dl during the 2nd trimaster.

Zinc has an important role in conception and continuation of normal pregnancy, since Zinc deficiency leads to low conception rate, poor foetal growth, under weight, malformation, and dead foetus ***(Gerloff and Morrow, 1986 and Orma, Eassa, Dowidar and El- Sherbiny, 1992).***

Zinc in plasma of cows decreased during parturition, and this changes in Zinc concentration is related to the dramatic increase in prostaglandin associated with parturition which bins Zinc and facilitate its transport to the foetus ***(Hidiroglou, 1979).***

The mean Zinc levels in the English thoroughbred mare's milk during different stages of lactation were 98.0, 52.0, and 36.0 μmol/liter at partum, 1-8, and 9-120 days postpartum respectively ***(Ullrey et al., 1966 &1974)***. While in she-camel, ***Abdel-Ghaffar et al. (1993)*** recorded a significant reduction in the plasma Zinc levels with the advancement of gestation. They reported that, the mean plasma Zinc concentrations during 1st, 2nd and 3rd. stages of pregnancy were 173.68, 153.63 and 140.17 μg/100 ml respectively. They also observed a marked significant increase during the late stage of gestation (148.18 μg/100 ml).

1.3. Basic principles of Ultrasound:

Gray-scale diagnostic ultrasonography is the most profound technological advance in the field of large-animal research and clinical reproduction since the introduction of transrectal palpation and radioimmuno-assay of circulating hormones ***(Ginther, 1986)***. However, ultrasonography is a complex and expensive technology in which operator and scanner must interact to produce a useful and pleasing image.

A range of different techniques has been developed for scanning the ultrasound beam through the patient. These different systems each have their own advantages and limitations which may influence their desirability for obstetric and gynaecological scanning. Mechanical and electronic scanners were developed. The majority of mechanical systems comprised a single ultrasound crystals. Three to four alternative crystals were used. The advantages of mechanical sector scanners are their small area of contact with

relatively wide field of view deep ***(Dewbury, Meire and Coserove, 1993).*** All electronic scanning systems comprised transducers in which the active piezo-electric crystal is divided into small elements. Their advantage were the wide field of view near the examined surface. Transabdominal, transvaginal and transrectal scanning were developed with different frequencies of sound waves. Diagnostic ultrasound used frequencies of 1 to 10 MHZ ***(Zagzebsi , 1983)***. A 5.0 MHZ transducer was used for imagining equine and bovine genitalia.

1.3.1. Ultrasonographic examination of mare's reproductive organs:

1.3.1.1.Examination of ovaries:-

Structures in the equine ovary are excellent for ultrasonic imaging,because they are large and readily accessible by the transrectal route. Follicles appear as roughly spherical, well-circumscribed black (non-echogenic) structures on the ultrasonic image ***(Adams, 1992)***. He added that, determination of the size and shape of the follicles provides the clinician with considerable diagnostic information regarding the stage of the estrous cycle.

Transrectal ultrasonography has made possible detailed studies of the dynamics of follicular populations in mares. Results supported the hypothesis that, follicular growth occurs in waves and that most mares have only one wave per estrous cycle. Growth of a cohort of follicles larger than 15 mm. in diameter is evident by days 8 to 10 (day 0 = day of previous ovulation) and is maximum by about day 15. By individual follicle mapping , it has been shown that, the dominant follicle destined to ovulate and is detectable as a number of

the cohort by day 6, and within a few days it becomes the largest follicle ***(Ginther, 1988).*** Results have shown that, significant changes occurred in follicle diameter, shape, and thickness of the follicular wall as ovulation approaches. Follicle diameter can be measured more accurately by ultrasound than palpation, to estimate follicle diameter, measurements are taken of the largest cross sectional image. In non-spherical follicles, the average of two measurements made perpendicular to each other is used. The dominant follicle increases in diameter at a rate of approximately 3mm per day until the day before ovulation, at which time, the mean diameter is approximately 45mm. Non of the follicles ovulated before reaching 35mm ***(Mckinnon and Squires, 1991).***

The ability to detect accurate time of ovulation has profound applications.Various characteristics can be used to predict time of ovulation by using ultrasonography. Softening of the follicle occurred 24hr before ovulation, this is frequently associated with a change in follicular shape from spherical to an irregular shape, this may be due to disruption of ovarian stroma as the follicle protrudes toward the ovulation fossa in preparation for ovulation ***(Townson and Ginther, 1989).***

Ovulation can usually be diagnosed by ultrasonic detection of an ovulation site on the day of occurrence and subsequently can be confirmed by visualization of corpus luteum. The ovulation site appears on ultrasonic image as an area of increased echogenicity in the ovary representing the apposition of the collapsed follicular wall ***(Adams, 1992).***

Corpus luteum can be identified for an average of 17 days starting on the day of ovulation using a 5-MHZ transducer. Two distinct luteal morphologies have been described on ultrasonic image. Approximately 50% of corpora lutea develop a central fluid-filled non-echogenic area, where as the remaining 50% are uniformly echogenic (luteinized) ***(Mckinnon and Squires, 1991)***.

1.3..2. Ultrasonic-early pregnancy diagnosis:-

The bovine embryo in the day 17 of pregnancy an elongated, non echogenic embryonic vesicle was visualized. On day 23, the embryonic vesicle increased in size. On day 30, the embryo proper was seen within the embryonic vesicle. On day 35, the embryo and umbilical cord imaged in an approximately frontal plane. On day 36, amnion and chorioallantoic membrane were detected. The chorionic vesicle extended into the caranial and caudal portions of the curved uterine horn. On day 48, the head, front limbs, rear limbs, umbilical cord and tail were seen. Descrete, non-echogenic areas were first visible within the uterus between days 12 and 14 when they were approximately 22 mm in diameter. The presence of an embryo within the embryonic vesicle was detected as hyper-echogenic area with rhythmic pulsations (heartbeat). The embryonic vesicle gradually increased in length from the day of first observation until day 26 when it extended past the curvature of the pregnant horn and began to encroach into the centrolateral horn. By day 32, the vesicle extended to the tip of the centrolateral horn. The embryo proper was first visible between days 26 and 29 when the mean length

was 10 mm.The embryo increased in length an average of 1.1 mm per day. A heart beat was detectable in the embryo on the first day observed. Transrectal scanning could be done in any species or individual large enough to accommodate rectal palpation ***(Pierson and Ginther ,1984).***

In the mare, ultrasound is useful for visualizing the functional structures of the genitalia. The 5 MHZ transducer gives good resolution ***(Ginther and Pierson, 1984)***. Both the absence of signs of estrus and the manual determination of an increased tonus on the uterus might indicate pregnancy. These same findings, however, might also be present in case of early embryonic death followed by persisting corpus luteum. The differentiation between pregnancy and these forms of pseudopregnancy could only be made with help of echographic examination aimed at detecting the presence or absence of foetal death. Pregnancy might be diagnosed at an early stage by rectal examination ***(Bain, 1967 and Van Niekerk, 1965).*** By day 21, the tonus on the uterus was slightly increased and the location of the foetal vesicle in the uterus was as large as a walnut. At 5-6 weeks the diagnosis might be made with more certainty. The tonus was more pronounced and the location of the foetal vesicle was by now as large as an orange. The cervix was firm, long and thin. Using a scanner a much more reliable pregnancy diagnosis might be made at a much earlier stage ***(Fontijne and Hennis, 1989).*** The developing blastocyst could first be recognized using ultrasonography 9-10 days postovulation ***(Ginther, 1984$_a$.).*** After only 11 days post ovulation, it might already be possible to detect the foetal vesicle. With a 3 MHZ transducer, pregnancy could first be detected at about 13-15 days. The early equine

conceptus was highly mobile within the uterine lumen ***(Ginther, 1983a)***. It moved between the 2 uterine horns and uterine body. The transuterine movement occurred at intervals of less than 4hr. Mobility began to decrease by day 15 ***(Ginther, 1983b)***. Evidence of embryonic heat activity remained a most reliable parameter for the viability of the embryo. Shortly prior to death, bradycardia with a heart rate of 100 beats per minute or less could sometimes be determined by ultrasound. For comparison, and intact embryo should have a heart rate of at least 150 beats per minute, ***(Leidl and Kähn, 1989).***

The yolk sac was spherical before day 17, whereas after day 17 it often was irregular. The vesicle had a growth plateau between days 17 and 24; then growth resumed at a slightly slower rate ***(Ginther, 1984a&b)***. Fixation of the early conceptus on days 16-17 apparently was due to increased uterine tone and thickening of the uterine wall, as well as rapid expansion of the conceptus. Fixation generally occurred in the caudal portion of the uterine horn near the bifurcation with greater frequency in the right horn in median and barren mares ***(Ginther, 1983a&b).*** The ultrasound image of young yolk sac (10-14 days postovulation) appeared as a small (1.5 cm in diameter) water filled balloon placed in the uterus ***(Ginther and Pierson, 1983)***. The embryo within the vesicle was first detected ultrasonically at days 20-25 and was observed on the ventral aspect of the vesicle. A heart beat was often detected around day 22 ***(Allen and Goddard, 1984).*** After the yolk sac had degenerated on day 40, the umbilical cord elongated from the dorsal pole, allowing the foetus to gravitate back to the ventral floor, where it was seen in dorsal recumbency from day 50 and later ***(Mckinnon and Squires, 1991)***. A 5.0 MHZ transducer

was used in 57 ponies and 19 horses. The embryonic vesicle was first detected on days 9 to 11, when it reached 3 to 5 mm in diameter. sixteen mares, later shown to the pregnant, also were examined on days 7 and 8, but no vesicles were found. The vesicle was first detected by day 11 in 98% of the mares. The few vesicles that were not detected until after day 11 were smaller than the average for that day. There was no difference between ponies and horses in the day of first detection or in the day of detection.It was emphasized that detecting the embryonic vesicle this early required at least a 5.0 MHZ transducer and a high quality scanner ***(Ginther, 1986)***.

MATERIALS AND METHODS

2.MATERIALS AND METHODS

2.1. Animals:

The present work was carried out at El-ZAHRAA stud for Arabian horses (E.A.O) * in Cairo.72 pure Arabian (Egyptian strain) mares aged 6-12 years were observed for the beginning of estrus signs. A teaser stallion was used daily for detection of estrus mares,(Fig 4,5,7 & 8).

The mares were divided randomly into:

Group I: Comprised 60 mares used to observe the length of different stages of estrous cycle and examined ultrasonically for the cyclic changes of ovaries and early pregnancy diagnosis .

Group II: Comprised 12 mares where blood samples were collected daily during estrus phase till the end of estrus sings, thereafter every third day till pregnancy diagnosis ultrasonically.

2.2. Sampling:

Blood samples were daily withdrown from the Jugular vein at the beginning of estrus until the end of estrus signs. Thereafter every 3 days until the day of pregnancy detection by using ultrasonographic method or beginning of new estrous cycle for non-pregnant mares.

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* (E.A.O) = Egyptian Agricultural Organization.

The blood samples were drown into clean, dry and sterile centrifuge tubes, allowed to coagulate at room temperature, then centrifuged for 5 minutes at 3000 r.p.m. Clean and clear(non-haemolyzed) sera were aspirated carefully by Pasteur pipettes and transferred into dry and sterile labelled glass vials with rubber stoppers. The separated sera were kept at -20 °C till subjected to estimate concentrations of Copper, Zinc, Iron, Manganese, and hormonal assay of Progesterone.

The serum samples were divided according to the period of sampling to:
Samples of esterus phase of non-pregnant mares (26 samples) .
Samples of luteal phase of non-pregnant mares (48 samples) .
Samples of esterus phase of pregnant mares (26 samples) .
Samples of early pregnancy stage of pragnant mares (44 samples) .

2.3. Ultrasonographic examination of the mare genitalia:

2.3.1. Principles of ultrasonic imaging:

Ultrasonography uses high-frequency sound waves produced by electrical stimulation of piezo-electric crystals. As the ultrasonic waves passe through tissue, a portion of the waves are reflected back to the transducer and converted to electrical impules to create an image on a screen Fig.(2) . The transducer is both a transmitter and receiver of ultrasonic-waves. The magnitude of reflected ultrasound waves is directly proportional to the density differences at the interfaces of 2 tissues. As ultrasound waves passe deeper into the body, they are attenuated. In general, the greater the impedance to the propagation of ultrasound waves, the greater the strength of the echo produced. Fluids (follicular fluid, yolk sac fluid, .. etc) are excellent medium

for transmission of ultrasound waves and provided little impedance to ultrasound waves until the signal encountered an interface with an adjacent tissue of different density. Both air and gas are poor propagators of ultrasound signals and resulted in severe attenuation, for this reason close contact between the transducer and examined tissue is essential. Very dense tissue, such as bone reflected the majority of the beam, and the image on the screen appears white, other tissues are seen in various shades of gray depending upon their ability to reflect ultrasound waves , i.e. their echogenicity.

2.3.2. Instruments:

2.3.2.1.Scanner: Modern ultrasonographic instruments used for examination of the mares reproductive tract are B-mode* , real-time scanners.

2.3.2.2. Transducers: The 3 major types of real- time transducers used for examination are: Linear array, Convex array and Sector transducers .The type of transducer depends on the number and arrangement of piezo- electric crystals .The frequencies used for examination are 3.5, 5.0 and 7.5 MHZ. The routes of examination are transrectal, tranvaginal and transabdominal.

2.3.2.3 Image recording: For recording images, freezing** of the image must be done and then one of the following methods of recording can be used :

A. Radiographic film

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* B-Mode = Brightness Modality Fig (1,C).
* Freezing of the image = Stopping the image's movement on the screen using freez-switch of the sceanner.

B. Polaroid film

C. Thermal printers

D.Video recorders(not need freezing of the image)

E. Disks (not need freezing of the image)

2.3.3. Procedure of transrectal ultrasonic examination of the mare:

The procedure of transrectal ultrasonographic examination is similar to that of rectal palpation Fig.(1 A&B). No additional restraint is required and the same precautions that applied to palpation per-rectum are applicable. After evacuation of fecal material from the rectum, the transducer is intreduced, care should be taken to ensure good contact between the transducer and rectal wall. The transducer also should be protected by the examiner's hand to avoid excessive trauma to the rectal wall and ultrasonic-gel should be used to lubricate the hand and transducer and to avoid air between the rectal wall and transducer. The trausducer is moved to scan the reproductive tract in this pattern: blodder, uterine body, right uterine horn, right ovary, right uterine horn, uterine body, left uterine horn, left ovary, left uterine horn, uterine body, and cervix, Fig (1A).

2.3.4. Ultrasound diagnosis of the present work:

The mares of group I were examined ultrasonically to detect the cyclic changes in ovarian follicles and early pregnancy diagnosis .

The mares of group Π were examined to diagnose pregnancy, about 25 days after the last day of estrus signs.

Pie-Medical scanner model **150 -VET.** With B., M.* , M.-B.** and B.-B.*** modes; with Multi- angle# , sector, 5.0-7.5 MHZ transrectal transducer and MITSUBISHI, thermal video- printer for recording images were used Fig (10).

2.4. Hormonal assay:

2.4.1. Radioimmuno- assay of serum progesterone:

Quantitative measurements of progesterone in serum was carried out using the SPECTRIA PROGESTERONE (^{125}I) coated tube RIA KITS provided by Orion Diagnostica - Finland

2.4.1.1. Principles of the test:

The coated A-count procedure was a solid- phase RIA, where in ^{125}I-labeled progesterone competed for a fixed time with progesterone in the sample for antibody sites. The antibody being immobilized to the wall of polyproplene tube, decanting the supernatant terminated the competition and to isolate the antibody bound fractional the radiolabeled progesterone. Counting the tube in a gamma counter, then yield a number, which converted by way of calibration curve to a measure of the progesterone present in the sample ***(Kubasik, 1984).***

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* M. mode = Motion mode (time motion or TM mode) ,used primarily in echocardiographic studies Fig (1,D).

** M.-B. = Mixed images, (B. and M. , modes)were appeared at the same time on the screen Fig (1,F)

*** B. -B. mode = Double images were appeared at the same time on the screen Fig (1,E).

Multi-angle = Different angles used for scanning .

2.4.1.2. Materils supplied:

*** Coated tubes:**

Vacuum packed. Geometrically optimized test tubes coated with polyclonal progesterone antiserum.

*** Progesterone (^{125}I) Reagent:**

(^{125}I) labelled progesterone.A concentrated solution in buffer containing a red colour additive. Total radioactivity was less than 200 KBq.

*** Progesterone Standards:**

Ready to use preparations of human serum containing, 0, 0.8,2.3, 4.0, 10, 20, 40, and 100 nmol/l progesterone respectively.

*** Tracer diluent:**

A ready to use PBS-buffer solution for tracer dilution

2.4.1.3. Procedure of the assay:

- The samples were transferred from the deep-freez (-20 ^{o}C) to the refrigerator a day before assay for gradual thawing.
- The coated tubes were labelled serially typically as samples.
- 50μ1. of each standard, control and samples were pipetted into the coated tubes.
- 500 μ1 of pre-diluted progesterone (^{125}I) with tracer diluent to all tubes.
- All tubes were mixed on a vortex mixer, then covered with a plastic film and left at room temperature for 2hr.
- Each tubes was decanted and the head of the tubes were firmly tapped against absorbent paper.
- 1.0 ml of ice-cold water was pipetted into each tube for quickly wash.

- The ice- cold water decanted immediately and the heads of each tube firmly tapped against absorbent paper.
- Then the tubes taken for counting using a gamma counter for 1 minute per tube.

2.4.1.4. Calculation of results:

Net counts =Average counts per minute(CPM)minus average NSB CPM

The binding of each tube is determined as a percent of maximum binding (MB), with the NSB- corrected of the A tubes (calibrator zero) taken as 100%.

$$\text{percent-bound} = \frac{\text{Net counts}}{\text{Net MB counts}} \times 100 \qquad \text{percent-bound} = \frac{\text{Net counts}}{\text{Net MB counts}} \times 100$$

Percent bound is plotted on the vertical axis against concentration on the horizontal axis for each of the calibrators by using the logic - long graph paper provided with the kits. Straight line is drawn approximating the path of these six points progesterone concentrations of the samples are estimated from the line by interpolation.

2.5. Trace elements assay:

The determination of serum zinc, copper, iron, and manganese were carried out using 5 pg Atomic absorption spectrophotometer (Pye Unicum) as described by ***Bauer (1982)***.

The gases used were oxyacetylene and compressed air for Copper, Zinc, Iron and Manganese. The wave lengths were 424.8, 213.9, 124, and 279.5 for Copper, Zinc, Iron, and Manganese, respectively.

2.5.1. Principles of atomic absorption:

In conventional flame atomic absorption spectrophotometry the sample in a solution state were sprayed by a pneumatic nebulizer into an air-acetylene oxidizing flame, in it the solutions droplets were firstly dried. The resulting solid particles thermally decomposed to give molecules in the gaseous phase, which finally dissociated into free atoms. These processes were in part of equilibrium reactions dependent upon the tempreture of the flame and must occurre within few milliseconds which are required to pass through the flame. Also small proportion of the resulting atoms were then excited to higher energy level. The decay of the excited atoms caused radiation of light characteristics of the element concerned. This light was utilized in analysis by the flame emission spectrophotometer. The majority of the atoms in the flame remained in the ground state of energy level and these were the atoms which measured in atomic absorption spectrophotometer. A hollow cathod lamp produced a coloured light similar in the spectral characteristic to the element to be determined. This coloured light was passed through the flame and undergo some absorption by the ground state atoms of the respective element found in the flame. The light was then absorped by photocells that could be transfer the light energy into an electrical current which gave a reading on a digital screen denoting the concentration of the element in part per million (ppm) in the given sample.

2.5.2. Preparation of the serum samples:

2.5.2.1. Serum Iron:

*** Reagent used:**

- Trichloroactic acid (TCA) 20% (W/V).
- Deionized water.

*** Preparation:**

1 ml of the serum plus 1 ml of 20% TCA were mixed in plastic tube 12 X 75 mm. The tubes were incubated in a water bath for 15 min. at 90°C, then the tubes were cooled and centrifuged. Clear supernatant (protein free fluid) solution was used for iron estimation.

2.5.2.2. Serum Copper and Zinc:

*** preparation:**

Dilution of the serum samples was carried out using deionized water, the dilution was 1:1 for copper, and 1:4 for zinc .

2.5.2.3. Serum Manganese:

Estimation of manganese in serum was carried out after dilution of serum samples using deionized water at a rate of 1:10, 1:5 , 1:3 and undiluted serum, but the sensitivity of the atomic absorption was not able to detect the concentration of manganese in the serum samples (i.e. manganese level was below the detectable level).

2.5.3. Calculation:

The atomic absorption reading X 100 X dilution rate =.....mg/dl.

2.6. Statistical analysis:

Data obtained were statistically analyzed to illustrate the results of this study using methods of ***Spiegel (1987 &1988).***

n.b. SEM = Stander error mean .

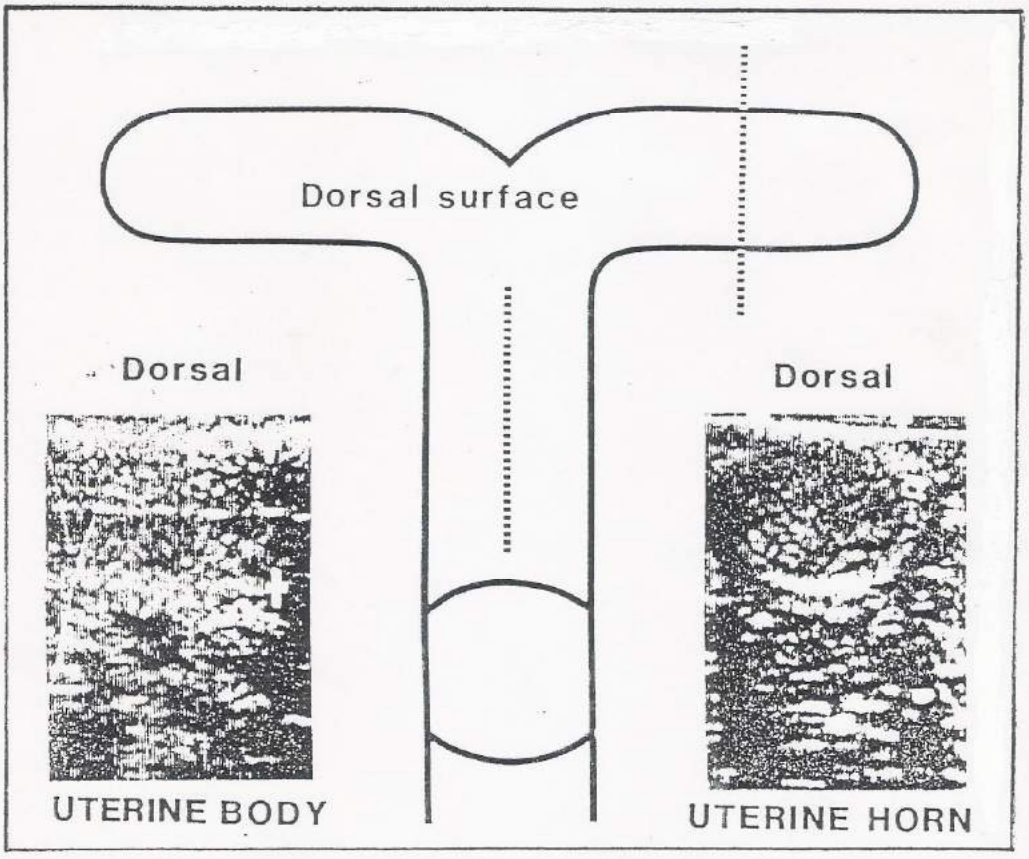


Fig.(1,A): Diagrammatic presentation of the relationships between the orientation of transducer and the uterus of a mare. The transducer is represented by the broken lines. The image of the uterine body is longitudinal and the uterine horn is in cross-section.
After(Pierson, Kastelic and Ginther, 1988).

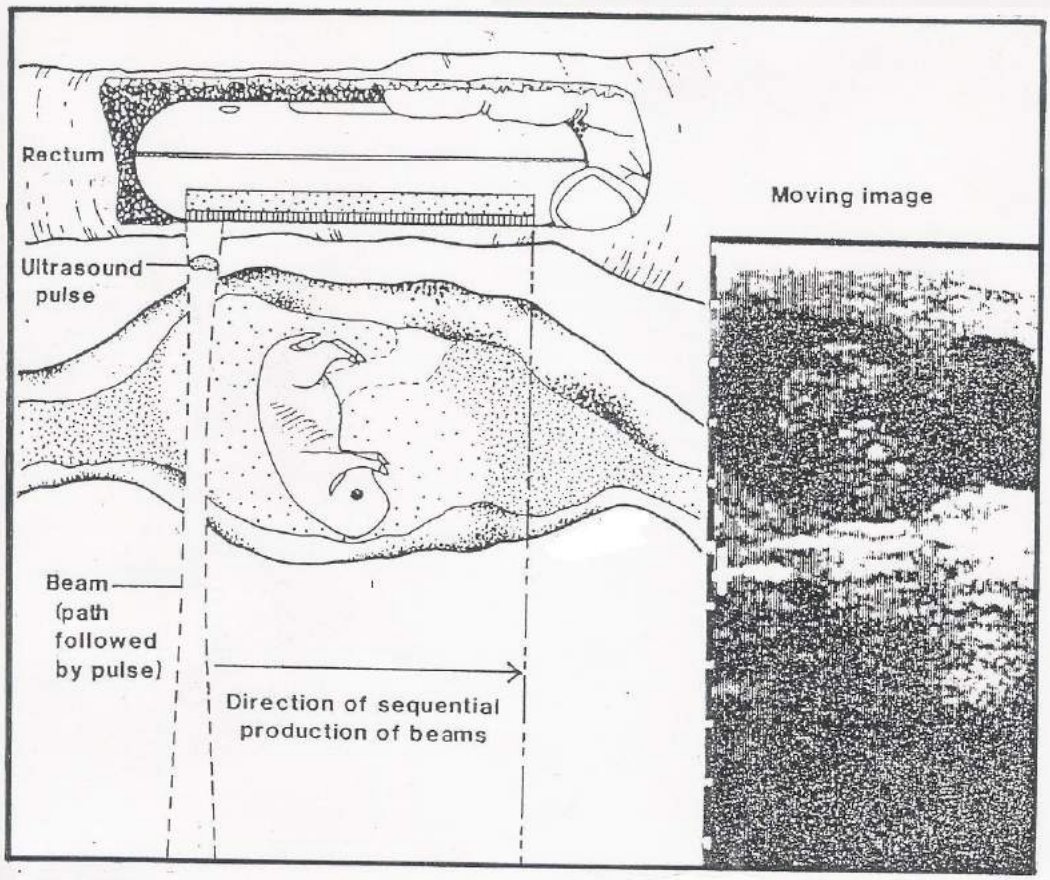


Fig.(1,B): *Relationship between intrarectal placement of the transducer and the development of an ultrasound image of a 48 day bovine fetus. An ultrasound pulse is generated by a sequentially fired set of piezoelectric crystals in the transducer. The resulting pulse follows the confines of an imaginary beam and echoes are returned to the crystals. After the completion of echo-gathering data, a second cluster of crystals further down the array is fired producing a second pulse and beam. This sequential, segmental firing of crystals moves along the array, completing 30 passes per second. The processed echo signals result in the displayed image which represents a two-dimensional slice through the conceptus. Slowly moving the transducer produces sequential slices, which may be mentally assembled so that three-dimensional aspects of the fetus may be appreciated.*

After(Pierson, Kastelic and Ginther, 1988).

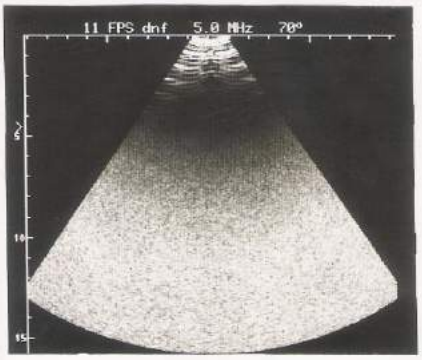


Fig.(1,C): An image of B.- Mode

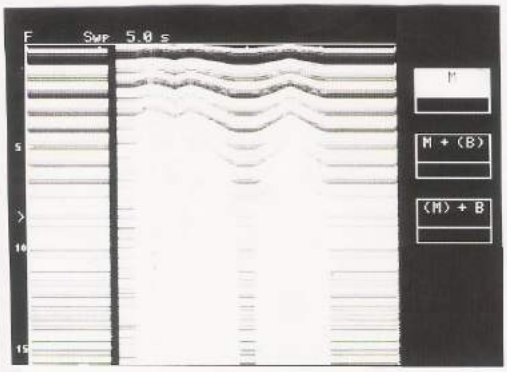


Fig.(1,D): An image of M.- Mode

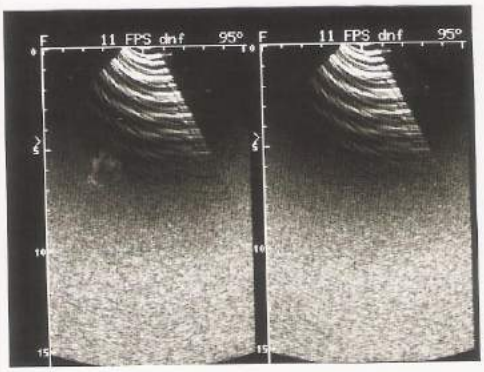


Fig.(1,E): An image of B.- B. Mode

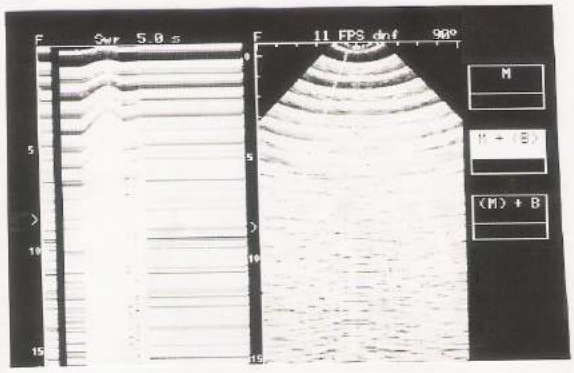


Fig.(1,F): An image of M.- B. Mode

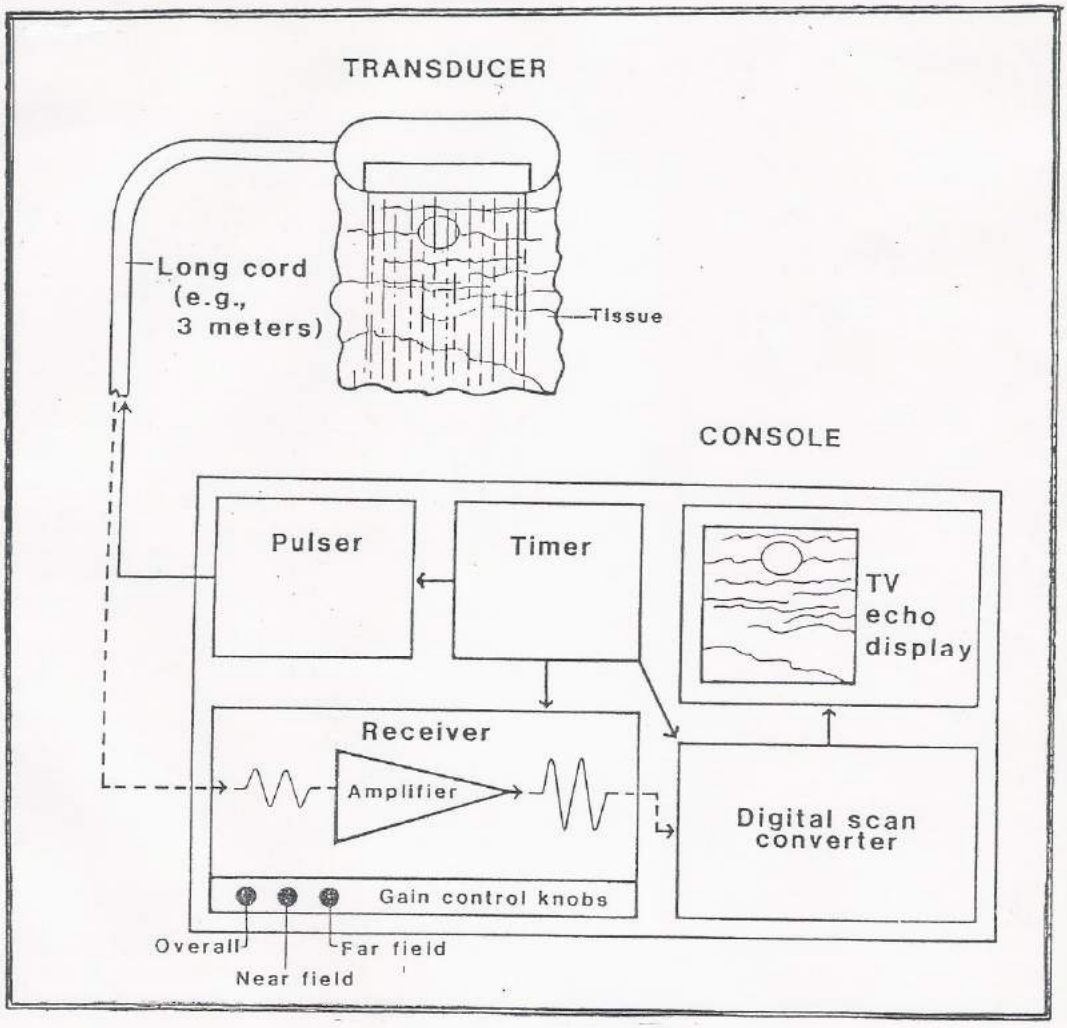


Fig.(2): Components of an ultrasound scanner.
After (Pierson, Kastelic and Ginther,1988)

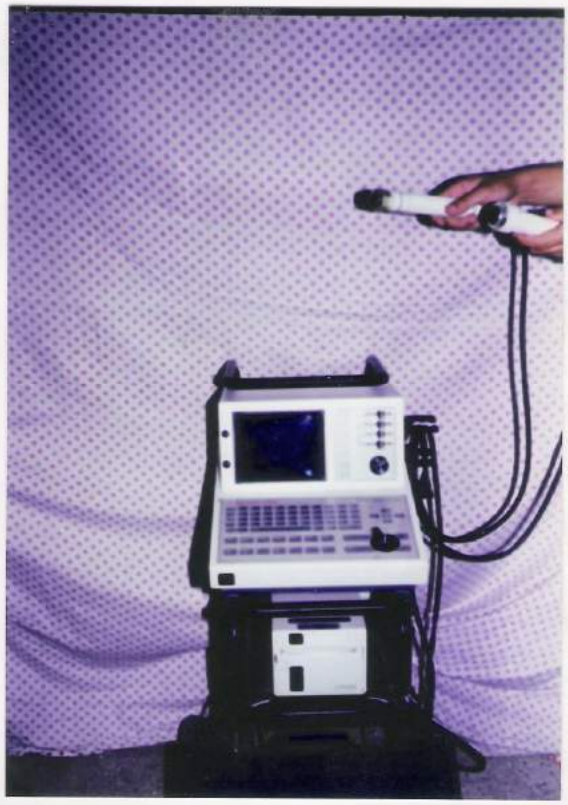


Fig. (3): PIE- Medical 150 Scanner with MITSOBISHI thermal video - printer and 5.0/7.5 MHZ transrectal and 3.5 transabdominal transeducers.

RESULTS

3.RESULTS

3.1. Estrous cycle in the Arabian mares:

3.1.1. Clinical signs of estrus:

During estrus, the mare was excitable,whinnied or squealed. Seeked other mares or stallions, assumed a urinary stance, urinated frequently. Mucoid urine was ejected in small quantities which may be splashed at the animals heels Fig.(4). Following this, the animal maintained the straddling stance for a time. The tail-head was often raised and hind limbs abducted and extended Fig.(5). The heels of one or other hind hoof were commonly seen to be tilted- up of the ground, so that only the toe of that hoof remained touching the ground. While this stance was maintained, the mare exhibited flashing (winking) of the clitoris Fig (6).

Particular interest was shown toward a presented stallion Fig (7). In the presence of the stallion and in response to his nudging the mare will orientate her hind-quarter towards him and adopt a stationnary stance with the tail raised Fig. (8).

During the luteal phase (Fig.9 & 10) , the mare showed a specific behaviour, resembling kiking, squealing and biting toward the male.

3.1.2. The average length of estrous cycle in Arabian mares:

Table (1) showed, the average length of the follicular phase (5.5 $\pm$ 1.25 days), and luteal phase (12.58 $\pm$ 1.35 days). The whole cycle averaged (18.08 $\pm$ 1.07 days).

3.2.. Progesterone profile in the serum of Arabian mares:

3.2.1. Progesterone profile in the serum of the non-pregnant Arabian mares:

With respect to the individual variation among mares, Table (2) and Graph. (1), indicated that, the highest progesterone concentrations (4.00±0.646, 3.821±1.641, 5.20±1.201, 3.90 ±0.504, 5.921±1.237, and 4.841±1.215 ng/ml) were observed during luteal phase for mares number I, II, III,IV, VI, and VII respectively. These values were significantly higher than that detected during the same phase (2.879±0.596 ng/ml) for mare number V. The lowest concentrations were determined for the whole mares during follicular phase (ranged from 0.301 ± 0.153 to 0.851 ± 0.3 ng/ml).

3.2.2.Progesterone profile in the serum of the (early) pregnant Arabian mares:

With respect to the individual variation among mares, table (3) and Graph. (2) showed a significant increase in progesterone level during early stage of pregnancy for all mares than these values of estrus phase. The highest levels of progesterone were recorded for mare number XI (6.587 ± 1.820 ng/ml) during early pregnancy stage. The lowest value observed for mare number XII (0.032±0.004 ng/ml) during estrus phase. Moreover, the progesterone levels of mare number X during estrus phase were under the detectable level by RIA, and calculated as zero during calculation the mean ± SEM.

3.3. Trace elements levels in the serum of Arabian mares:

3.3.1.Trace elements' levels in the serum of non-pregnant Arabian mares:

3.3.1.1. Copper levels:

There was a non-Significant difference in Copper levels during both follicular and luteal phases among various individual mares under the present

study (Table 4 and Graph. 3). The Copper levels ranged from 1.420 ± 0.100 to 2.020 ± 0.33 μg/ml.

3.3.1.2.Iron levels:

Regarding the individual variation among mares, table (5) and Graph. (4) indicated that, the highest concentrations of Iron detected during follicular phase (3.31±0.401, 3.519±0.42, 3.179±0.178 and 3.168±0.689 μg/ml) for mares number Π,IV,VΠ, and VI respectively; and during luteal phase (3.61±0.19 and 3.97±0.103 μg/ml) for mares number I and IV respectively, which were significantly higher than that observed during follicular phase (2.98± 0.58 μg/ml) for mare number V and during luteal phase (3.151±0.211, 3.15±0.282. 2.7 ±0.14, 3.244±0.246 and 3.008 ± 0.125 μg/ml) for mares number Π,IΠ,VI, and VΠ, respectively. The lowest levels (2.45 ± 0.4 and 2.381±0.408 μg/ml) were obtained during follicular phase for mares number I and IΠ respectively.

3.3.1.3.Zinc levels:

Regarding the individual variation among mares, table (6) and Graph. (5), indicated that, the maximum Zinc levels were detected during follicular phase (0.998±0.208, 0.622±0.15, 0.833±0.14, 0.928±0.141, and 0.650±0.079 μg/ml) for mares number I, Π, IΠ, V, and VI respectively; and during luteal phase (0.641 ± 0.105, 0.781±0.166, 0.701±0.045, and 0.665+0.043 μg/ml) for mares number I, V, VI, and VΠ respectively, which were significantly higher than that observed during follicular phase (0.563±0.061, and o.527±0.066 μg/ml) for mares number IV and VΠ respectively, and during luteal phase (0.461±0.107 and 0.491±0.062 μg/ml) for mares number IΠ and IV

respectively. The minimum Zinc concentration was recorded during luteal phase (0.388±0.044 μg/ml) for mare number Π.

3.3.2.Trace element's levels in the serum of (early) pregnant Arabian mares:

3.3.2.1.Copper levels:

As shown in table (7) and Graph. (6), Copper levels decreased non-sigificantly during early stage of pregnancy in copmairing with those observed during estrus phase for all examined mares except mare number VIΠ Copper levels increased non-significantly during early stage of pregnancy. The maximal levels of Copper were recorded for mare number VIΠ during early stage of pregnancy (1.869±0.457 μg/ml) and mare number XI (1.704 ± 0.270 μg/ml) during estrus phase. While the minimal levels were observed during early stage of pregnancy (1.335 ±0.490 and 1.210 ± 0.360 μg/ml) for mares numbers IX and X respectively.

3.3.2.2.Iron levels:

With respect to the individual variation among mares, Table (8) and Graph. (7) showed that, the Iron levels decreased non-significantly during early stage of pregnancy compared with the levels observed during estrus phase in all pregnant mares (in this study) except in the mares number VIΠ and X, there was a non- significant inceras in Iron levels during early stage of pregnancy than those recorded during estrus phase.

The highest values of Iron recorded for mare number XI during estrus and early stage of pregnancy (4.393 ± 0.780 and 3.679 ± 1.79 μg/ml). The minimal values were recorded for mares number VIΠ and X (2.207 ± 0.861

and 2.381±0.820 μg/ml) respectively during estrus phase, and for mare number IX (2.728 ± 0.42μg/ml) during early stage of pregnancy.

3.3.2.3. Zinc levels:

Regarding the individual variation among mares, Table (9) and Graph. (8) showed that, the highest concentrations of Zinc were for mare number VIII (0.975±0.377 and 0.868±0.364 μg/ml) during estrus phase and early stage of pregnancy respectively; mare number X (0.840 ± 0.290 μg/ml) during estrus phase; and mare number XI (0.024±0.490 μg/ml) during the same phase. While, the lowest concentrations were recorded during early stage of pregnancy (0.452 ± 0.14 and 0.469 ±0.16 μg/ml) for mares numbers IX and X respectively. There was a non-significant decrease in Zinc levels during early stage of pregnancy than estrus phase for all mares except in mare number XII, Zinc levels increased non-significantly in the same stage.

3.4. The mean + SEM of the whole parameters in the serum of Arabian mares:

3.4.1. The mean + SEM of the whole parameters in the non- pregnant mares:

As shown in Table (10) and Graph. (9), the concentrations of Progesterone (4.366 ± 0.346 μg/ml), Copper (1.609 ± 0.097 μg/ml) and Iron (3.262 ± 3.262 μg/ml) during luteal phase were significantly higher than that observed during follicular phase (0.548 ± 0.062 μg/ml, 1.517 ±0.037, and 3.0 ± 0.163 μg/ml) for the same items respectively. In contrast, Zinc concentration was significantly higher during follicular phase (0.73 ±0.069 μg/ml) than luteal phase (0.59 ±0.054 μg/ml) of the estrous cycle.

3.4.2.The Mean + SEM of the whole parameters in the (early) pregnant mares:

As shown in Table (11) and Graph. (10), the Progesterone levels were significantly higher during early stage of pregnancy (4.145 ± 2.11 μg/ml) than that recorded during estrus phase (0.390 ±0.800 μg/ml). The measured concentrations of Iron were non-significantly higher during early stage of pregnancy (3.247 ± 1.03 μg/ml) Than during estrus phase (3.049 ±1.27 μg/ml). In contrast, the levels of Copper and Zinc were higher during estrus phase (1.528±0.26 and 0.782 ±0.37 μg/ml for Copper and Zinc respectively) than those observed during early stage of pregnancy (1.477 ±0.49 and 0.611±0.24 μg/ml) for Copper and Zinc respectively.

3.5 . Ultrasonographic investigations:

3.5.1. The follicular diameters during different stages of estrous cycle:

As shown in Table (12),the greatest follicular diameter (4.85 cm) recorded at the 4th. day of estrus phase Fig.(11, B & C). It was significantly higher than that detected (2.57, 3.69, and 4.04 cm) at 1st , 2nd and 3rd day of estrus phase respectively Fig. (11, A & 14) while the largest diameter recorded during late diestrus was 2.13 cm Fig (12, C). The smallest follicular diameter (1.01 cm) was detected during early diestrus Fig. (12, A & B). Moreover, there were 7 anestrum mares, their ovaries contained follicles with 1.51 cm diameter or less, was significantly decreased than all the above mentioned values Fig. (13).

The CL is visible as strongly echogenic circumscribed mass of tissue Fig.(15).

3.5.2. Diagnosis of early pregnancy :

The developing blastocyst could first be recognized 10-12 days post ovulation (Fig. 16). The image appeared as black circumscribed (non-echogenic) structure resulted from the enclosed collection of yolk sac fluid with gray mottled area revealing the uterine horn.

The early equine conceptus was highly mobile within the uterine lumen, and mobility decreased by day 15, so the conceptus could only be seen after day 17 as irregular and inconsistent shape (Fig. 17 & 18).

The allantoic sac was well visible and separated from yolk sac by an echogenic line, and the embryo could be detected clearly between the two sacs (Fig. 19).

With the advancement of gestation, the allantoic sac increased in size, and concurrent contraction of the yolk sac (Fig.20).

After the yolk sac had degenerated on day 40, the umbilical cord elongated from the dorsal pole allowing the foetus to gravitate back to the ventral floor (Fig. 21).

Fig. (4): The female in estrus in front of the stallion during teasing.

Fig. (5): The female in estrus raising the tail-head and stand with splayed limbs.

Fig. (6): Frequant exposure of clitoris (winking) is a common sign of estrus behaviour.

Fig. (7) : The female during estrus accepts stallion to smill and bite in the external genitalia.

Fig. (8): The most prominant signs of estrus: splayed legs, raising the tail-head, drippling mucoid cervical secretion and standing close to the stallion.

Fig. (9): The mare (out of estrus phase), kiking and sometimes bitting the stallion.

Fig. (10): The female standing on the hind limbs (this is a reaction against the stallion in anestrus period).

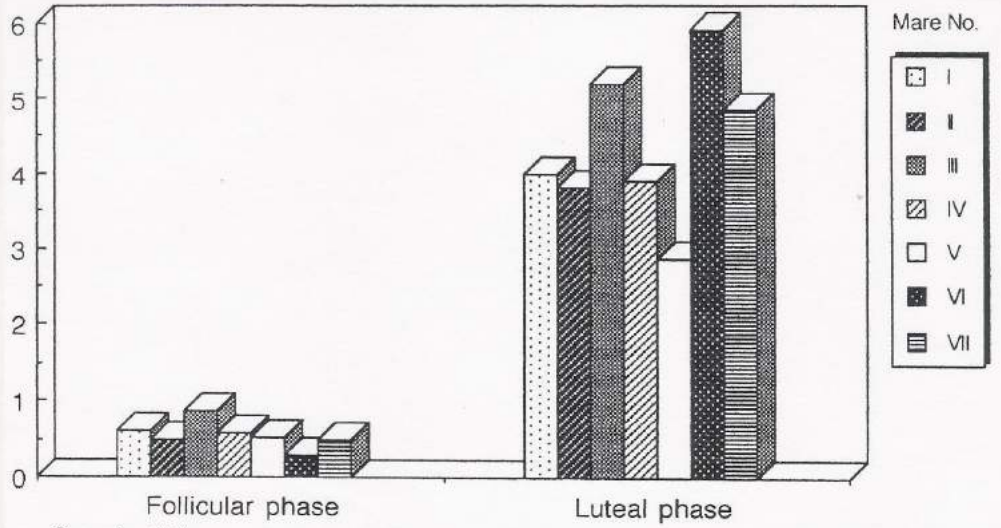
Table (1): The average length (days) of follicular phase luteal phase, and the whole estrous cycle of Arabian mares (Mean ± SEM). (n=60).

Phases of Estrous cycle	Length (days) (Mean ± SEM)
Follicular phase	5.5 ± 1.25
Luteal phase	12.58 ± 1.35
Whole cycle	18.08 ± 1.07

Table (2): Serum profile of Progesterone (ng/ml) during follicular and luteal phases for non-pregnant mares (Mean ± SEM). (Where n.= number of samples for each phase).

Phase / Mare No.	Follicular phase n.		Luteal phase n.	
I	6	0.600 ± 0.320^{C}	6	4.001 ± 0.646^{ab}
II	5	0.501 ± 0.460^{C}	6	3.821 ± 1.641^{ab}
III	5	0.851 ± 0.300^{C}	5	5.200 ± 1.201^{ab}
IV	5	0.588 ± 0.168^{C}	6	3.900 ± 0.504^{ab}
V	4	0.514 ± 0.167^{C}	10	2.879 ± 0.596^{b}
VI	5	0.301 ± 0.153^{C}	8	5.921 ± 1.237^{a}
VII	6	0.482 ± 0.169^{C}	7	4.841 ± 1.215^{ab}
Total	36	0.548 ± 0.06	48	4.366 ± 0.346

Means with different alphabetica superscripts are significantly different from each other at level ($P<0.05$).



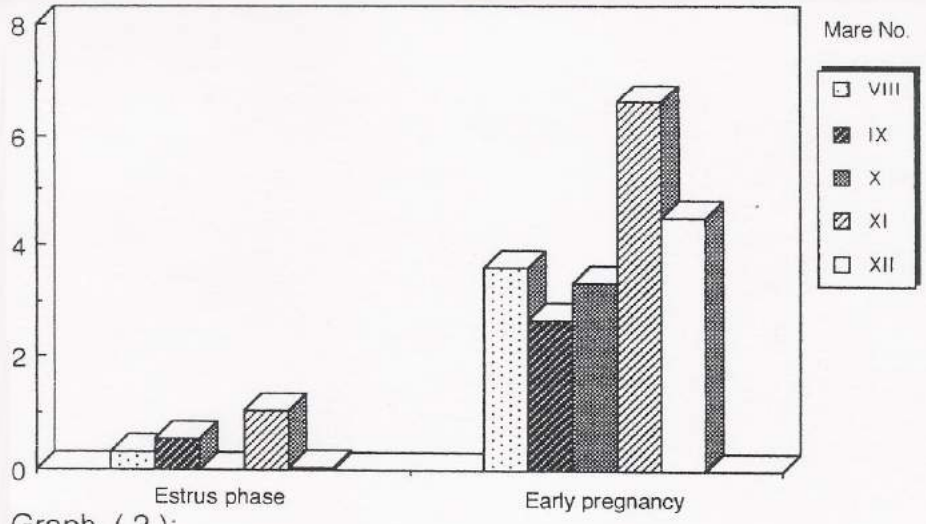
Graph. (1):
Serum progesterone levels (ng/mL) during follicular and luteal phases for non-pregnant Arabian mares.

Table (3): Serum profile of Progesterone (ng/ml) during estrus and early pregnancy for pregnant mares (Mean ± SEM). (Where n.= number of samples for each phase).

Phase / Mare No.	Estrus phase		Early pregnancy	
	n.		n.	
VIII	5	0.297 ± 0.145^{Cd}	7	3.625 ± 1.185^{ab}
IX	5	0.556 ± 0.556^{C}	8	2.673 ± 1.770^{b}
X	5	undetectable^{e}	10	3.346 ± 1.440^{ab}
XI	7	1.064 ± 0.850^{bC}	8	6.587 ± 1.820^{a}
XII	4	0.032 ± 0.004^{d}	11	4.492 ± 2.110^{ab}
Total	26	0.390 ± 0.080	44	4.145 ± 2.11

Means with different alphabetica superscripts are significantly different from each other at level ($P<0.05$).

N.b. The Mare No. X during estrus phase calculated as Zero.



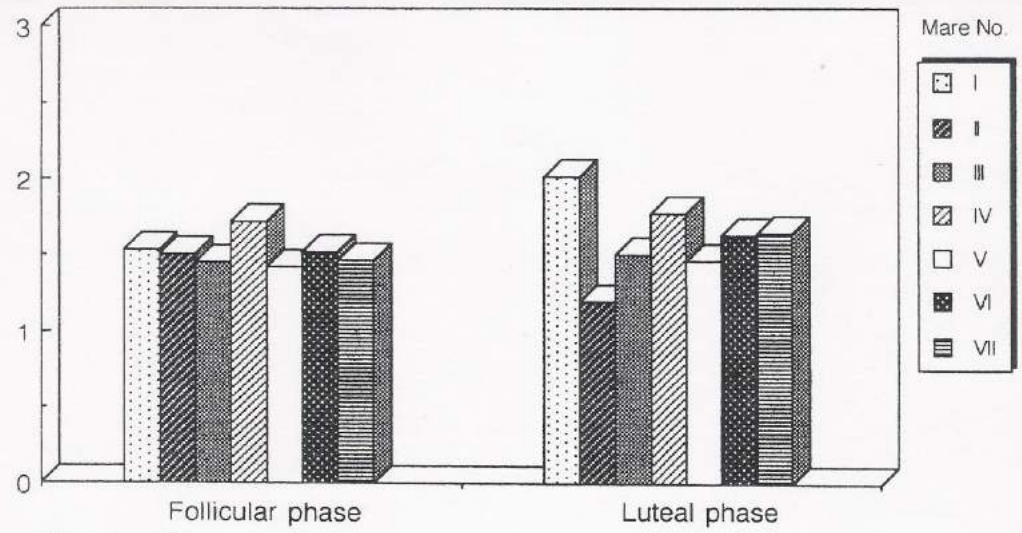
Graph. (2):

Serum profile of progesterone (ng/mL) during estrus and early pregnancy for pregnant Arabian mares.

Table (4): Serum Copper levels (μg/ml) during follicular and luteal phases for non-pregnant mares (Mean ± SEM). (Where n.= number of samples for each phase).

Phase / Mare No.	Follicular phase n.		Luteal phase n.	
I	6	1.540 ± 0.140[a]	6	2.020 ± 0.330[a]
II	5	1.500 ± 0.160[a]	6	1.200 ± 0.280[a]
III	5	1.455 ± 0.117[a]	5	1.511 ± 0.152[a]
IV	5	1.722 ± 0.118[a]	6	1.771 ± 0.120[a]
V	4	1.420 ± 0.100[a]	10	1.471 ± 0.290[a]
VI	5	1.522 ± 0.205[a]	8	1.635 ± 0.172[a]
VII	6	1.462 ± 0.191[a]	7	1.654 ± 0.123[a]
Total	36	1.517 ± 0.037	48	1.609 ± 0.097

Means with the same alphabetica superscripts are non-significantly different from each other at level (P>0.05).

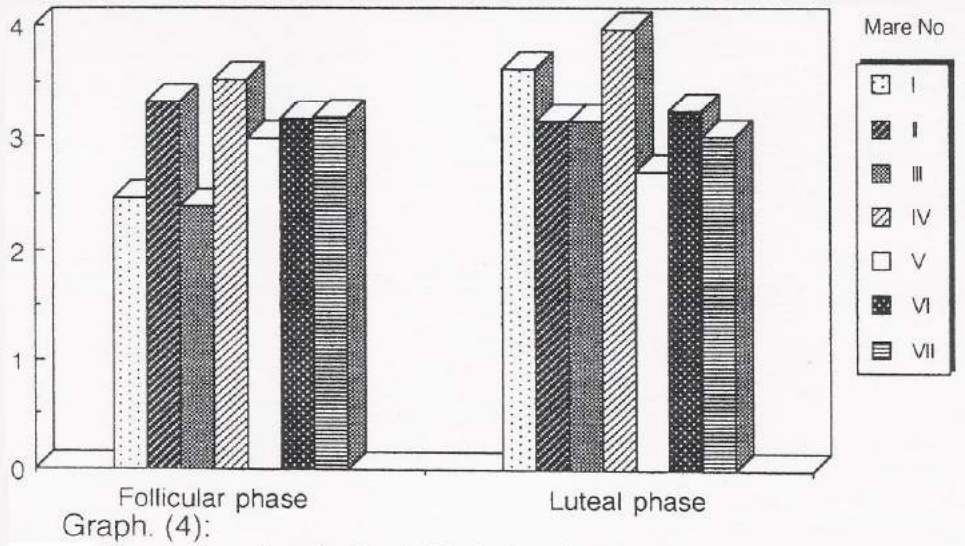


Graph. (3):
Serum copper levels (μg/mL) during follicular and luteal phases for non-pregnant Arabian mares.

Table (5): Serum Iron levels (μg/ml) during follicular and luteal phases for non-pregnant mares (Mean ± SEM). (Where n.= number of samples for each phase)

Phase / Mare No.	Follicular phase		Luteal phase	
	n.		n.	
I	6	2.450 ± 0.400^{c}	6	3.610 ± 0.190^{ab}
II	5	3.310 ± 0.401^{abc}	6	3.151 ± 0.211^{bc}
III	5	2.381 ± 0.408^{c}	5	3.150 ± 0.282^{bc}
IV	5	3.519 ± 0.420^{abc}	6	3.970 ± 0.103^{a}
V	4	2.980 ± 0.580^{bc}	10	2.700 ± 0.140^{bc}
VI	5	3.168 ± 0.689^{abc}	8	3.244 ± 0.246^{b}
VII	6	3.179 ± 0.178^{bc}	7	3.008 ± 0.125^{b}
Total	36	3.000 ± 0.163	48	3.262 ± 0.156

Means with different alphabetica superscripts are significantly different from each other at level ($P<0.05$).

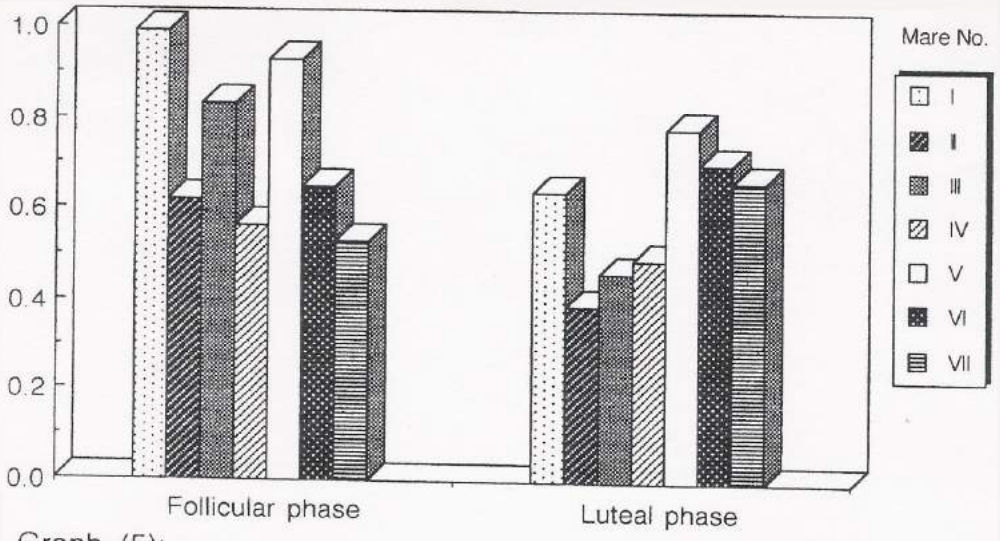


Graph. (4):
Serum iron levels (μg/mL) during follicular and luteal phases for non-pregnant Arabian mares.

Table (6): Serum Zinc level (μg/ml) during follicular and luteal phases for non-pregnant mares (Mean ± SEM). (Where n.= number of samples for each phase).

Phase / Mare No.	Follicular phase		Luteal phase	
	n.		n.	
I	6	0.988 ± 0.208^{a}	6	0.641 ± 0.105^{a}
II	5	0.622 ± 0.150^{ab}	6	0.388 ± 0.044^{c}
III	5	0.833 ± 0.140^{ab}	5	0.461 ± 0.107^{bc}
IV	5	0.563 ± 0.061^{b}	6	0.491 ± 0.062^{bc}
V	4	0.928 ± 0.141^{a}	10	0.781 ± 0.166^{ab}
VI	5	0.650 ± 0.079^{a}	8	0.701 ± 0.045^{ab}
VII	6	0.527 ± 0.066^{b}	7	0.665 ± 0.043^{ab}
Total	36	0.730 ± 0.069	48	0.590 ± 0.054

Means with different alphabetica superscripts are significantly different from each other at level ($P<0.05$).

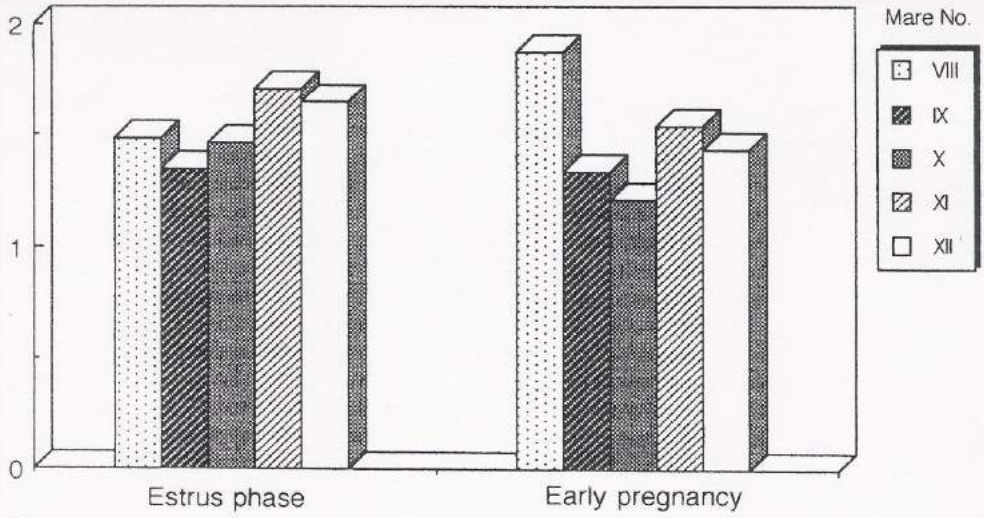


Graph. (5):
Serum zinc levels (μg/mL) during follicular and luteal phases for non-pregnant Arabian mares.

Table (7): Serum Copper levels (μg/ml) during estrus and early pregnancy for pregnant mares (Mean ± SEM). (Where n.= number of samples for each phase).

Phase / Mare No.	Estrus phase n.		Early pregnancy n.	
VIII	5	1.486 ± 0.357[a]	7	1.869 ± 0.457[a]
IX	5	1.344 ± 0.142[a]	8	1.335 ± 0.490[a]
X	5	1.459 ± 0.260[a]	10	1.210 ± 0.360[a]
XI	7	1.704 ± 0.270[a]	8	1.541 ± 0.360[a]
XII	4	1.647 ± 0.090[a]	11	1.432 ± 0.570[a]
Total	26	1.528 ± 0.260	44	1.477 ± 0.490

Means with the same alphabetica superscripts are non-significantly different from each other at level (P>0.05).

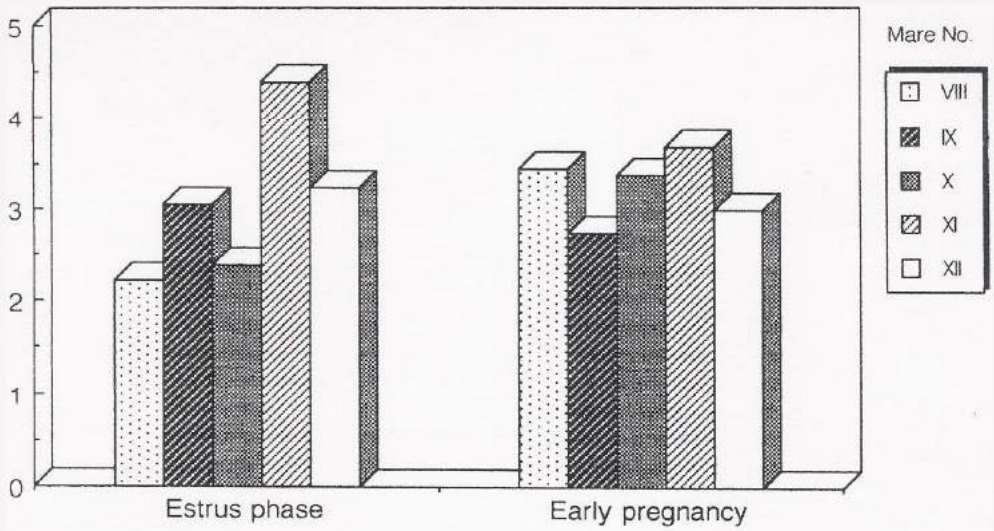


Graph. (6):
Serum copper levels (μg/mL) during estrus and early pregnancy for pregnant Arabian mares.

Table (8): Serum Iron levels (μg/ml) during estrus and early pregnancy for pregnant mares (Mean ± SEM). (Where n.= number of samples for each phase).

Phase / Mare No.	Estrus phase		Early pregnancy	
	n.		n.	
VIII	5	2.207 ± 0.861[a]	7	3.448 ± 0.599[a]
XI	5	3.042 ± 1.575[a]	8	2.728 ± 0.420[a]
X	5	3.381 ± 0.820[a]	10	3.373 ± 1.020[a]
XI	7	4.393 ± 0.780[a]	8	3.679 ± 1.790[a]
XII	4	3.224 ± 0.990[a]	11	3.006 ± 0.400[a]
Total	26	3.049 ± 1.270	44	3.247 ± 1.030

Means with the same alphabetica superscripts are non-significantly different from each other at level (P>0.05).

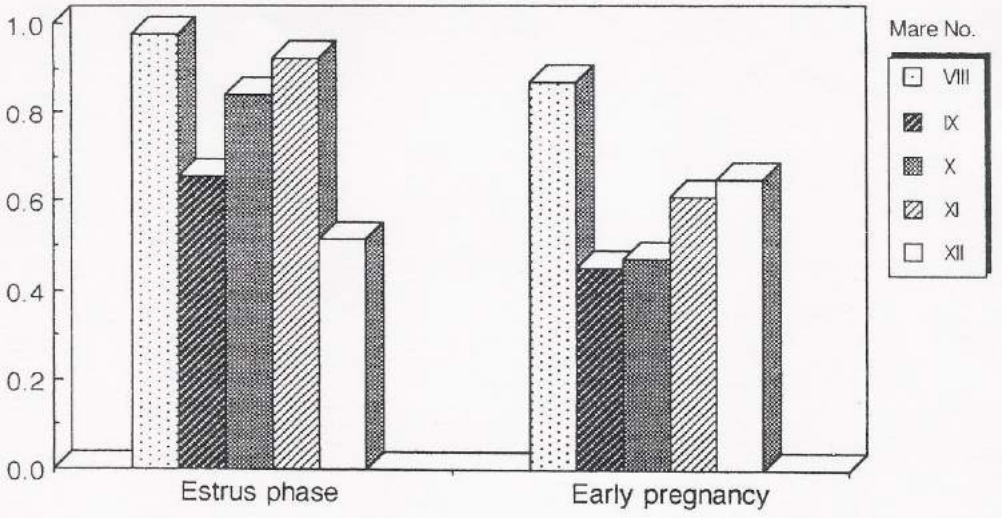


Graph. (7):
Seum iron levels (μg/ml) during estrus and early pregnancy for pregnant Arabian mares.

Table (9): Serum Zinc levels (μg/ml) during estrus and early pregnancy for pregnant mares (Mean ± SEM). (Where n.= number of samples for each phase).

Phase / Mare No.	Estrus phase n.		Early pregnancy n.	
VIII	5	0.975 ± 0.377[a]	7	0.868 ± 0.364[a]
IX	5	0.657 ± 0.236[a]	8	0.452 ± 0.140[a]
X	5	0.840 ± 0.290[a]	10	0.469 ± 0.160[a]
XI	7	0.924 ± 0.490[a]	8	0.613 ± 0.190[a]
XII	4	0.514 ± 0.090[a]	11	0.651 ± 0.090[a]
Total	26	0.782 ± 0.370	44	0.611 ± 0.240

Means with the same alphabetica superscripts are non-significantly different from each other at level (P>0.05).

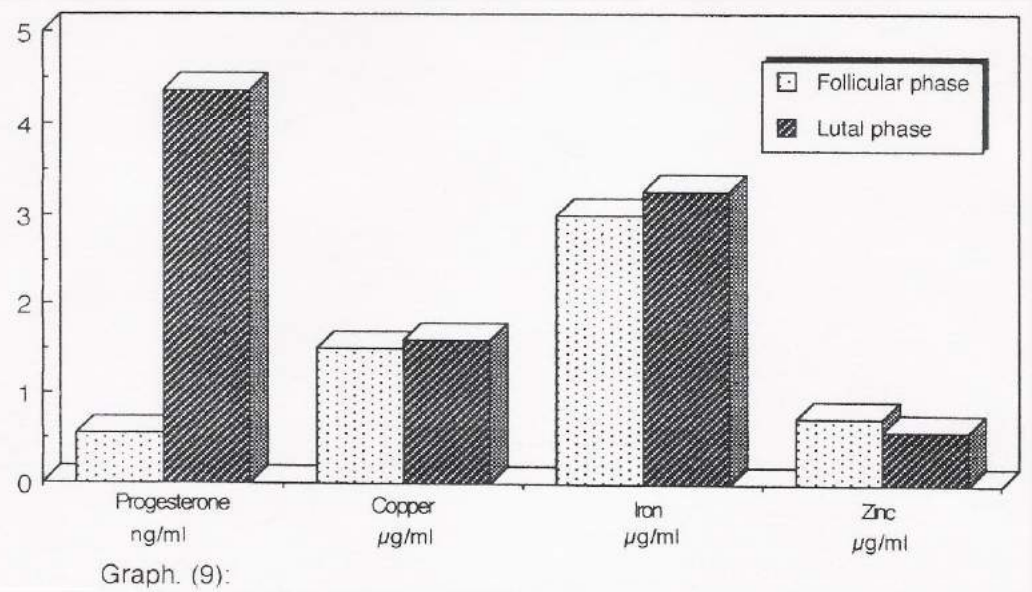


Graph. (8):
Seum zinc levels during estrus and early pregnancy for pregnant Arabian mares.

Table (10) Serum profiles of Progesterone; and Copper , Iron, and Zinc levels during follicular and luteal phases for non-pregnant mares (Mean ± SEM). (Where n.= number of samples for each phase).

Phase / Parameter	Follicular phase (n.=36)	Luteal phase (n.=48)
Progesterone(ng/ml)	0.548 ± 0.062^{b}	4.366 ± 0.346^{a}
Copper(μg/ml)	1.517 ± 0.037^{b}	1.609 ± 0.097^{a}
Iron(μg/ml)	3.000 ± 0.163^{b}	3.262 ± 0.156^{a}
Zinc(μg/ml)	0.730 ± 0.069^{a}	0.590 ± 0.054^{b}

Means with different alphabetica superscripts in the same category are significantly different from each other at level ($P<0.05$)

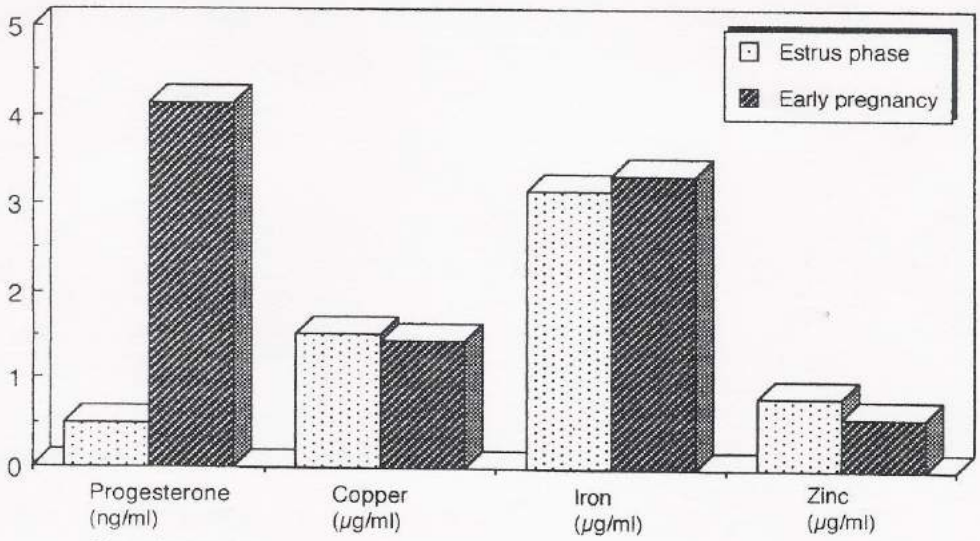


Graph. (9):
Serum profiles of Progesterone; and Copper , Iron and Zinc levels during follicular and luteal phases for non-pregnant Arabian mares.

Table (11) : Serum profiles of Progesterone; and Copper, Iron, and Zinc levels during estrus phase and early pregnancy for pregnant mares (Mean ± SEM). (Where n.= number of samples for each phase)

Phase / Parametar	Estrus phase (n.= 26)	Early pregnancy (n.= 44)
Progesterone(ng/ml)	0.390 ± 0.080 ^{b}	4.145 ± 2.110 ^{ab}
Copper(μg/ml)	1.528 ± 0.260 ^{a}	1.477 ± 0.490 ^{a}
Iron(μg/ml)	3.049 ± 1.270 ^{ab}	3.247 ± 1.030 ^{a}
Zinc(μg/ml)	0.782 ± 0.370 ^{b}	0.611 ± 0.240 ^{b}

Means with different alphabetica superscripts in the category are significantly different from each other at level ($P<0.05$)



Graph. (10):
Seum profiles of Progesterone; and Copper, Iron and Zinc levels during estrus phase and early pregnancy for pregnant Arabian mares.

Table (12): The follicular diameter (Mean ± SEM) for estrus and diestrus phases. (Where n.= number of mares examined for each phase).

Phases of Estrous cycle	n.	Follicullar diameter (cm)
Estrus phase :		
1st day of estrus	22	2.57 ± 0.06^{d}
2nd day of estrus	20	3.69 ± 0.06^{c}
3rd day of estrus	4	4.04 ± 0.22^{b}
4th day of estrus	7	4.85 ± 0.21^{a}
Diestrus phase:		
Early diestrus	3	1.01 ± 0.04^{g}
Late diestrus	4	2.13 ± 0.02^{e}

Means with different alphabetica superscripts are significantly different from each other at level ($P<0.05$)

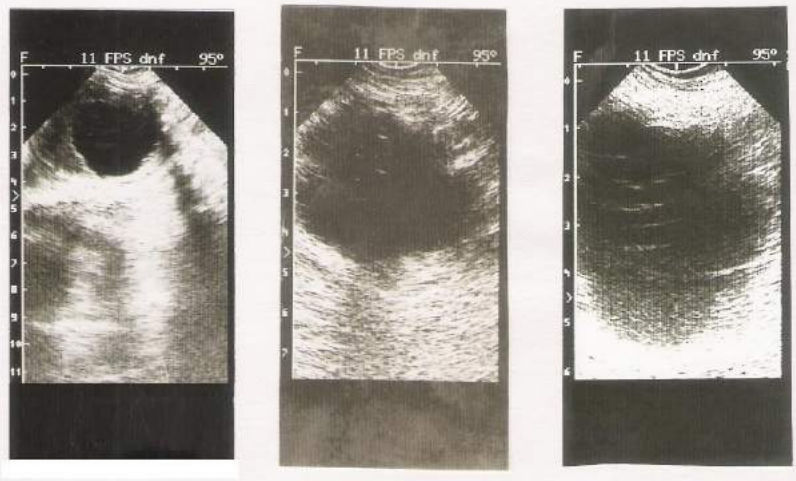


Fig.(11) :
Ultrasound image of ovary snowing :
A; Mature follicle (32mm) B; and C; Pre-ovulatory follicles (42.5, 43.5mm).
Note that, the non-spherical shape of B.

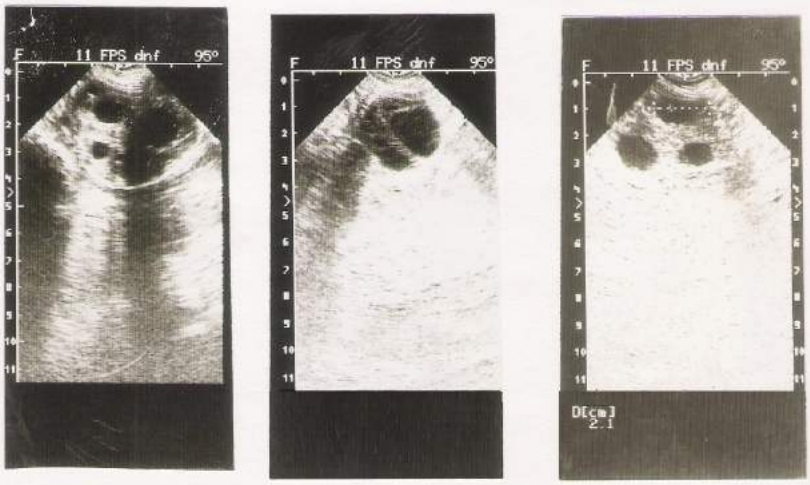


Fig.(12):
Ultrasound images of ovary showing follicles at diestrus: A; and B; Early diestrus and C; late diestrus.

Fig.(13) :
Ultrasound image of ovary showing a small follicle (Anoestrous)

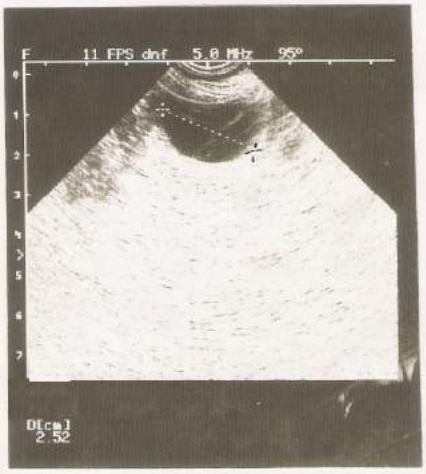


Fig.(14):
Ultrasound image showing ovarian fallicle at early estrus.

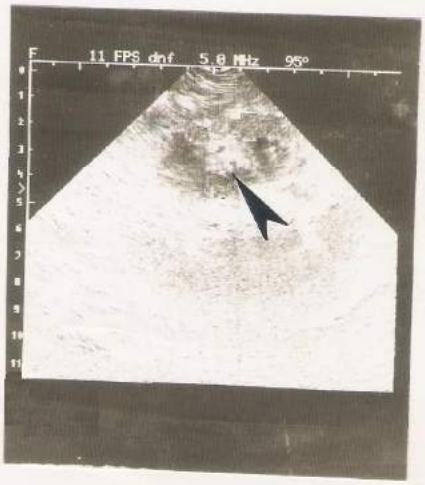


Fig.(15):
Ultrasound image of corpus luteum :A mature CL does not contain a central cavity. The structure is ultrasonically homogenous

Fig (16):
Ultrasound image of embryonic vesicle (10-15 days) The vesicle appears black (nonechogenic) structure in the center of the cross-sectional view. The grey mottled area (black arrow) reveals the uterine horn. The vesicle is spherical and produce a black circumscribed image resulting from the enclosed collection of yolk sac fluid.

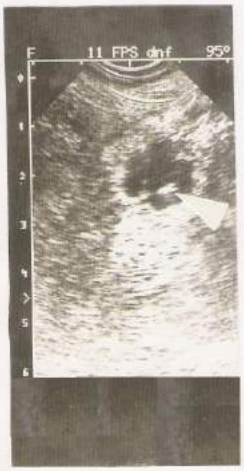
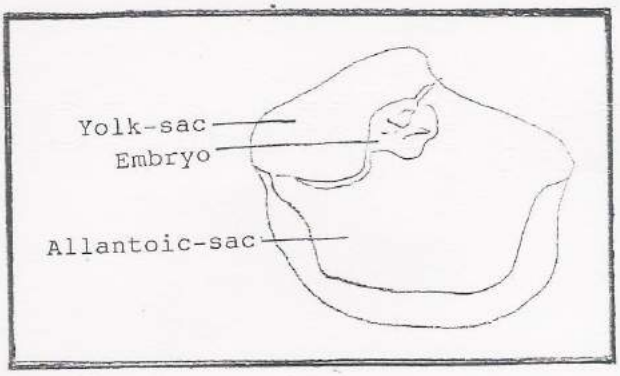


Fig (17):
A 18 days conceptus is seen in a cross-section as irregular and inconsistent shape.

Fig (18):
The embryo can only be visible on day 20. The allantoic sac is visible as a light echogenic line beneath the embryo.



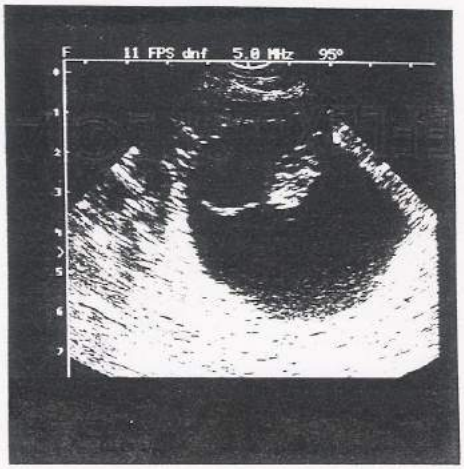
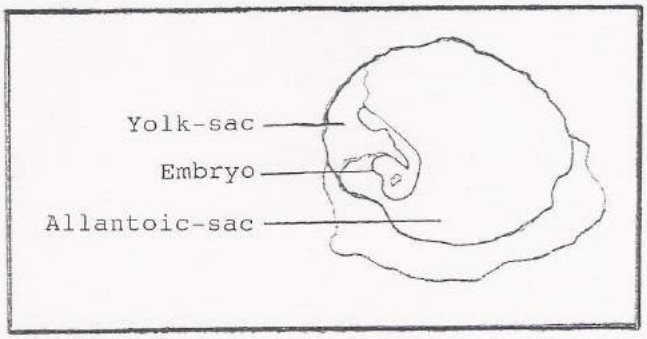


Fig.(19):
A 24 days embryo. The allantoic sac is well visible and separated by an echogenic line. Heart beats can be detected at this stage.



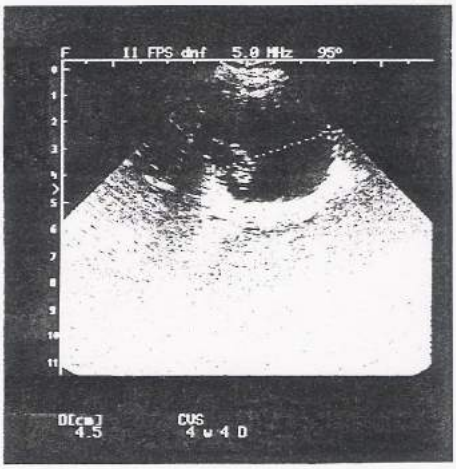
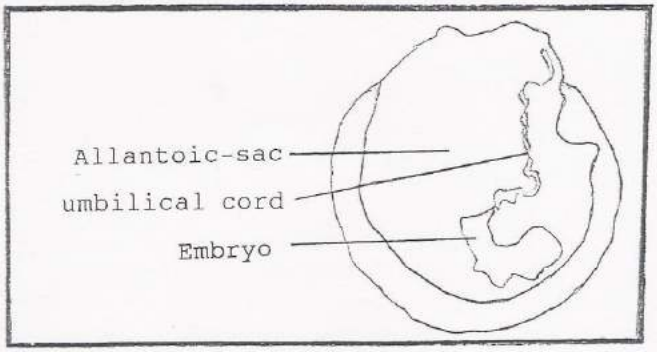


Fig.(20):
A 28-33 days embryo. The presence of echogenic line separatig the two sacs, allantoic and yolk sac. The latter is relatively smaller than before.



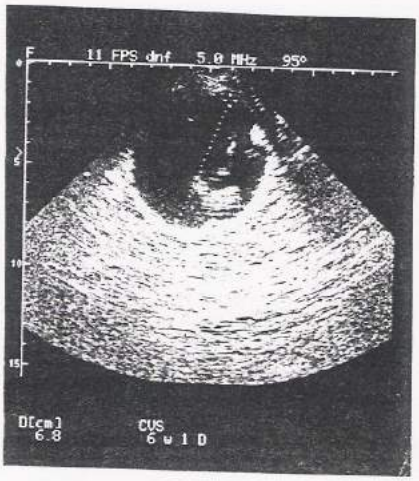


Fig.(21):
A 40-45 days embryo. The embryo proper is seen. Note that the umbilical cord remains attached to the dorsal aspect of the vesicle. The foetus comes to rest on the bottom of the vesicle.

DISCUSSION

4. DISCUSSION

The most common method for detection of estrus was teasing with a male which was reliable but time consuming ***(Aitken, 1927)***. In the current work, estrus was detected using a teasting stallion. Estrus was reported to occur if the mare stood firmly, with the tail up while being mounted. One of the following signs, at least, was reported as an indicator for estrus: Winking of vulva, urination and/or raising the tail. Similar signs were taken as a guide for estrus detection detection by ***Ginther, Whitmore, and Squires (1972), Back et al. (1974). and Fraser (1992)***.. During diestrus, the teased mare would respond by laying the ear back, biting, squealing, kicking and striking. This behaviour was typically recorded in the present work. ***Aitken (1927)*** attributed the variability in these signs to the mare itself, cycles and years.

There was considerable information, primarily from clinical and slaughterhouse materials, on the estrous cycle and ovulation in mares ***(Asdell, 1964)***. The signs of estrus which could be taken as a guide for estrus detection were in accordance with that described by ***Ginther et al. (1972)***. . For this purpose, the stallion was allowed to approach and tease the mare from behind.

In accordance with ***Hughes et al. (1972)***, the length of the follicular phase of estrus cycle was recorded in this study to be 5.5 days. ***Ginther et al. (1972) and Stabenfeldt et al. (1972)*** gave the length of follicular phase as 7.7 days, ***Hunt et al. (1978)*** as 8.9 day .While, ***Sharp and Black (1973) and Back et al. (1974)*** recorded it as 6.6 and 6.8 days respectively. The average length of luteal phase was recorded in the presnt work (12.58 days) was nearly

similar to that reported by ***Stabenfedt et al. (1972)*** as 12.4 days, and ***Hunt et al. (1978)*** as 13.9 days. Longer length of luteal phase was given by ***Sharp and Black (1973), Back et al. (1974) and Hunt et al. (1978)*** as 16.0 , 21.5 and 22.8 days respectively. For the whole estrous cycle the current results were nearly parallel to that obtained by ***Stabenfeldt et al. (1972)*** as 18.5 days. While ***Hughest et al. (1972), Sharp and Black (1973), Back et al. (1974) and Hunt et al. (1978)*** gave the whole length of estrous to be 20.5, 22.6, 21.5 and 22.8 days respectively.

Variations in estrous length in the mare were attributed to the differences in techniques of estrus determination, in definitions of estrus and types of mares studied.

As pregesterone was used to assess ovarian activity in mares better than observation of estrous patterns ***(Hunt et al., 1978)*** . The mean serum progesterone level during follicular phase in the present study was 0.548 ng/ml, this value was nearly resembling those recorded by ***SHarp and Black (1973)***, 0.58 ng/ml; ***Oxender et al. (1977)***, 0.5 ng/ml and ***Ginther et al.(1980)***, 0.53 ng/ml. Different plasma progesterone levels were recorded by other authers as, ***Smith et al. (1970)***, 0.64 ng/ml; ***Plotka et al. (1971)***, 0.61 ng/ml; ***Stubenfeldt et al. (1971)***, 0.65 ng/ml and ***Hunt et al. (1978)***, 0.4, ng/ml. The lowest values of progesterone concentration were recorded for anestrus mares as well as those in estrus ***(Burkhardt ,1974 and Osborne, 1966)*** . This could be attributed to the absence of luteal tissue in this phase and non-breeding season ***(Oxender et al., 1977).***

The mean serum progesterone level during luteal phase in this study was 4.366 ng/ml, this value was nearly similar to those determined by ***Ginther et al. (1980)***, 3.88 ng/ml. On the other hand, higher values were given by ***Short (1959); Smith et al. (1970); Plotka et al. (1971); Stabenfeldt et al. (1971); Sharp and Black (1973); Oxender et al. (1977) and Hunt et al. (1978)*** engaged 6.8 - 10.9 ng/ml. ***Oxander et al. (1977),*** recorded that progesterone concentration was minmal during estrus (< 1 ng/ml) increasing after ovulation to cocentrations approximating those during diestrus. In addition, the profile of serum progesterone appeared to be a reliable indicator of ovarian luteal activity.

The progesterone concentration in the serum at early stage of pergnancy of Arabian mares was 4.145 $\pm$ 2.11 ng/ml. This value was nearly similar to that recorded by ***Hunt et al. (1978)***, 5.4 ng/ml and ***Ginther et al. (1980)***, 4.22 + 0.09 ng/ml. Where ***Kooistra et al. (1976)*** reported 9.7,6.7, 8.2 and 14.4 ng/ml for progestrone levels at days 24, 32, 40, and 48 postovulation respecrively. Regardless, some pregnant mares would have low progesterone measurements and some diestrus mares (with a retained CL) would have high progesterone measurements similar to those in pregnant mares, ***(Evans and Irvine, 1975)***. However, the increase in progesterone levels during early pregnancy was attributed to the formation of primary CL which was maintained beyond day 15 until at least days 140 through 180 and probably longer ***(Squires and Ginther, 1975)***. Secondary CL meight form from follicles that were stimulated by 10- days cyclic pulses of FSH and either ovulation and/or luteinization meight be initiated by eCG (***Squires and Ginther,1975, Evans and Irvine, 1975 and Allen ,1980).***

The estimated concentrations of trace elements in the present work showed that, copper concentrations during follicular phases were 1.517 ± 0.037 and 1.528 ± 0.26 μg/ml for non- pregnant, and early pregnant Arabian mares respectively. Where ***Chandolia and Verma (1987) and Sossa (1994)*** reported 103.5 - 109.62 and 69.87 μg /100 ml for anestrum , during estrus and first postpartum observed estrus for buffaloes, respectively. ***Hidiroglou (1976)*** recorded 2.1 mg/liter for adult cows; ***Desai et al. (1978 & 1982) and Pathak et al. (1986)*** recorded 204.79, 170-189.99 and 189.76 μg/100ml during follicular phase, fertile heat and at time of ovulation respectively for Surti-buffaloes. Moreover, ***Shehata and Zaghloul (1990); and Radwan et al.(1991)*** reported 102.0 and 100.26 μg/100 ml respectively in Egyptian buffaloes.

Nadia et al. (1986) clamid that, the difference in plasma copper concentration in mare between one week and one month after parturition (Foaling heat) might be associated with hormonal variations.

During luteal phase, and early stage of pregnancy in Arabian mares, copper levels were recorded as 1.609 ± 0.097 and 1.477 ± 0.49 μg/ml respectively. The recorded mean value during luteal phase was nearly similar to that recorded by ***Chandolia and Verma (1987)*** 107.50 μg/100ml during luteal phase of buffalo- hifer. While, the result differed from these recorded by ***Desai et al. (1978 & 1982) and Radwan et al. (1991)*** who recorded 195.35 and 64.72 μg/100 ml for Surti and Egyptian buffaloes respectively during luteal phase. Also , ***El-Azab et al. (1985); Shehata and Zaghloul (1990) and***

Wafaa et al. (1991) reported 66.7 , 115.5 , and 70-85 μg/dl during 1st month, 2nd trimaster, and 1st month of pregnancy in Egyptian buffaloes respectively.

Regarding iron concentrations, the present work reported 3.0±0.163 and 3.049±1.27 μg/ml during follicular phase of non-prgnant and early-pregnant Arabian mares, respectively and 3.262±0.156 and 3.247±1.03 μg/ml for luteal phase of non-pregnant, and early pregnant Arabian mares respectively. These values were nearly similar to these observed by ***Chandolia and Verma (1987)*** who recorded 305.4, 311.2, and 316.80 μg/100 ml for Surti-buffaloes during anestrus, estrus, and luteal phases respectively . Where these data differed from those recorded by ***Desai et al. (1978 & 1982)*** in Surti-buffaloes (879.25, 787.10, 837.51, and 882.70 μg/100 ml for follicular phase, fertile heat, time of ovulation, and luteal phase respectively). In she-camel ***Shekhawat et al. (1987); Shehata and Zoghloul (1990); and Abdel-Ghoffar et al. (1993)*** reported 92.78-131.0 μg/100ml; 0.16 mg/100 ml, and 191.74 μg/100 ml for iron concentration for 2-6 years she-camel; mature she-camel during non-follicular stage and during 1st trimaster of gestation respectively. ***Mohamed (1989)*** attributed the changes in iron values to the changes in estrogen levels.

Zinc concentrations at the present work were evaluated for Arabian mares during follicular phases as (0.73±0.069 and 0.782±0.37 μg/ml) of non-pregnant and early pregnant groups respectively; during luteal phase (0.590±0.54 μg/ml) of non-pregnant group, and during early stage of pregnancy (0.611±0.24 μg/ml) for pregnant group. These values were nearly similar to those reported by ***Fahmy et al. (1979)*** as 78.57- 98.8 μg% for adult

non-pregnant buffaloes; ***El-Azab et al. (1985)*** as 50 μg/100 ml for non-pregnant cows and buffaloes and ***Radwan et al. (1991)*** as 74.80 μg% in buffaloes. Where the results differed from these obtained by ***Hidiroglou (1979)*** (3.0 mg/liter in cows), ***Chandolia and Verma (1987)*** (120.75 and 128.80 μg/100 ml for anestrus and estrus Surtibuffaloes respectively) and ***Radwan et al. (1991)*** (86-100 μg/100 ml for Egyptian buffaloes during luteal phase). In pregnant cows and buffaloes ***El-Azab et al. (1985)*** reported 120, 117, and 114 μg/100 ml for zinc levels during 1st, 2nd and 3rd. month of prgnancy respectively for cows and buffaloes; ***Shehata and Zaghloul (1990)*** recorded 148.1 μg/100ml during 2nd trimaster of gestation for Egyptain buffaloes, and ***Wafaa et al. (1991)*** observed 118, 115 and 119 μg% during 1st, 2nd and 3rd month of pregnancy respectively for Egyption buffaloes. Moreover, ***Abdel-Ghaffar et al. (1993)*** detected 173.68, 153.63, and 140.14 μg/100 ml for 1st, 2nd, and 3rd. stage of pregnancy respectively for she - camel.

Orten and Neuhaus (1982) observed some reproductive dysfunctions and irregular estrous cycle due to defects in steroid synthesis, and attributed these dysfunctions to zinc dificiency. Moreover, ***Dylewski, lytton, and Bunce (1986)*** suggested that, the steroid recptors were zinc dependent metalaprotein or that zinc enhances the binding of steroid-receptors complex. In addition ***Giroux and Henkin (1972)*** demonestrated that, the decrease in plasma zinc concentrations following increased progesterone level during pregnancy could be related to the net effect of several factors including the possible tripping of zinc from its albumin binding sites with its subsequent loss from the circulation. Furthermore, ***David and Vincent (1983)*** reported that,

progesterone and estrogen contraception produced changes in trace elements in concomitant with the alteration in circulatinf plasma proteins, that due iron, copper, and zinc within the plasma are complexed to specific transport proteins.

N.B. *The results of the present work were discussed with the results of some authers who worked on other species differed from equines ,that due to the shortage in the works on equines ,specially for trace elements and their relation to reproduction.*

Utrasonography is useful for monitoring dynamic follicular and luteal changes of equine ovaries, because it permits rapid, non invasive access to the reproductive tract and creates a visual image. A 5-MHZ transducer has greater resolving power and is more suitable for evaluation of ovaries than 3.0 or 3.5 MHZ ones. Potential applications of ultrasonographic examination of the ovaries include estimating the stage of estrous cycle, assessing perovulatory follicles, determining ovulation, examining the CL and diagnosing ovarian irregularities and lesions.

Follicles, like other fluid-filled body structures, are nonechogenic and appear as black, roughly circumscribed structures. Sequential monitoring of the dynamic changes in follicles during estrous cycle has been made possible by ultrasonography.

In the present study, the ovarian activity was determined during the different stages of estrous cycle revealing that, during anestrus, inactive avaries were readily differentiated from functional ones. Small follicles 1.51 +

0.04 cm diameter or less occasionally meight be present. but absence of an ultrasonographically visible CL suggested an anestrus condition. This result was nearly similar to that observed by ***Mckinnon and Squires (1991).***

Determination of estrus phase could be done by estimating follicular diameter compression by adjacent follicles, luteal structures or ovarian stronma often caused irregularity in the follicular shape (Fig. 11B). Similar observations were recorded by ***Pierson and Ginther (1985).*** Diameter could be estimated by adjusting an irregularly shaped follicle to an equivalent circular form. In the present study, follicular diameters during estrus phase, and diestrus (early and late stages) were recorded. Data revealed an increase in the follicular diameters during estrus phase (2.57, 3.69, 4.04 and 4.85 cm) at 1st, 2nd, 3rd and 4th days of estrus phase respectively. These measurments were nearly similar to those oblained by ***Parker (1971) and Pierson and Ginther (1985).***

Various characteristics could be used to predict time of ovulation. Softening of the follicular wall occured 24 hr befor ovulation in about 70% of the mares ***(Parker, 1971)***. This was frequently associated with a change in follicular shape from spherical to an irregular shape ***(Pierson and Ginther, 1985).*** This meight be due to disruption of ovarian stroma as the follicle protruded toward the ovulation fossa in preparation of oocyte release ***Bergin and Shipley (1968); Mossman and Duke (1973); Whitmore, Wentworth and Ginther (1973); Ginther (1979); Guraya (1985); Lipner (1988) and Morioka (1989).*** Ultrasonography permited accurate detection of ovulation which commonly associated with wellcircumscribed corpora lutea that, though

smaller in size, fell similar to fluid-filled follicles. Such follicles collapsed and refil with vlood (corpus hemorrhagicum).

Ultrasonographic examination indetified multiple follicles, in the present study during early distrus, measured 1.01 + 0.04 cm diameter. Where ***Ginther and Pierson (1984)*** detected 2-5mm follicles during early diestrus.

Early pergnancy detection could first be done using ultrasonography 9-10 days postovulation ***(Ginther, 1984 a,b)***. Developing blastocyst could first be recogonized at this time using 5 MHZ transducer, where 3.5 MHZ transducer could be used to detect pregnancy at 13.15 days postovulation.

The early equine conceptus was highly mobile within the uterine lumen. It moved between the two uterine horns and uterine body. Transuterine movement occurred at intervals of less than 4 hr ***(Ginther 1983 a&b).*** The small yolk sacs (days 9-10) were found more frequently in the uterine body. Mobility began to decrease by day 15, after day 17 trasuterine migration no longer was detected.

The yolk sac vesicle was spherical before day 17, whereas after day 17 it, often, was irregular. The vesicle had a growth resumed at a slightly slower rate. Fixation of early conceptus on days 16-17 apparently was due to increased uterine tone and thinkening uterine wall as rapid expansion of the conceptus. Fixation generally occurred in the caudal portion of the uterine horn near the bifurcation.

The ultrasonic images of yolk sacs (10-14 days) often had a bright echagenic line at the dorsal and ventral poles. These were not associated with the embryonic disc or other structures of developing conceptus, but rather were due to specular reflection. A small water filled-balloon (1.5 cm diameter) vesicle placed in the uterus had identical ultrasound characteristics of yolk-sac (10-14 days).

The embryo within the vesicle was first detected ultrasonically at days 20-25. Heart beats were often detected around day 22.

After the yolk sac degenerated on day 40, the umbilical cord elongated from the dorsal pole allowing the fetus to gravitate back to the ventral, floor, where it was seen in dorsal recumbency from day 50 and later, in accordance with ***Ginther (1983 a,b and 1984).***

SUMMARY AND CONCLUSIONS

5. SUMMARY

This study was carried out at EL-ZAHRAA stud for Arabian horses (E.A.O), in Ain-Shams, Cairo. Using 72 pure Arabian (Egyptian strain) mares 6-12 years old. These mares were divided randomly into groups:

Group I: Comprised 60 mares, to observe the length of follicular, and luteal phases, and the whole estrous cycle in Arabian mares. This was done by using a teaser male to detect estrus mares. The length of follicular phase was 5.5 ± 1.25, luteal phase was 12.58+1.35,and the whole estrous cycle was 18.08 ±1.07 days. This group of mares were were investigated using ultrasonography to detect the ovarian cyclic changes and early pregnancy diagnosis . It was found that, anestrum mares had ovaries with follicles measuring 1.51±0.04 cm in diameter.During estrus phase, there were Graffian follicles measuring 2.57 ±0.06, 3.69±0.06, 4.04 ±0.22, and 4.85 ±0.21cm in diameter in the 1st, 2nd, 3rd and 4th days of estrus respectively. where during luteal phase, the ovarian follicles were 1.01±0.04 and 2.13±0.02 cm in diameter at early and late diestrus respectively.

Group II: Comprised 12 mares, where blood samples were collected daily from the beginning of estrus signs till the end of the estrus signs. Thereafter, the samples were collected every 3 days till pregnancy

diagnosis by using ultrasonography. Pregnancy was detected in 5 mares, while 7 mares were non-pregnant.

The serum samples were subjected to hormonal essay of Progesterone and estimation of Copper, Iron, and Zinc levels. We tried to estimate Manganese levels, but it was under the detectable levels.

The samples were classified according to the phase into:

1- Samples during estrus phase of non-pregnant mares (36 samples).
2- Samples during luteal phase of non-pregnant mares (48 samples).
3- Samples during follicular phase of pregnant mares (26 samples).
4- Samples during early stage of pregnancy (44 samples).

It was found that:

* There were significant increases in the progesterone during luteal phase (4.366±0.346 ng/ml) and early stage of pregnancy (4.144±2.11 ng/ml) than follicular phase (0.548±0.062 and 0.390 ±0.80 ng/ml) for non-pregnant, and early pregnant mares respectively.

* There were significant increase in Copper levels during luteal phase (1.609±0.097 μg/ml) than follicular phase (1.517 ±0.037 μg/ml) for non-pregnant mares. Where in early pregnant mares, the Copper levels increased non-significantly during estrus phase (1.528±0.26 μg/ml) compared with the levels observed during early stage of pregnancy (1.477±0.49 μg/ml).

* The levels of Iron increased significantly during luteal phase and non-significantly during early stage of pregnancy (3.262±0.156 and 3.247

±1.03 μg/ml) respectively, as compared with those recorded during follicular phases (3.00±0.163 and 3.049 ±1.27 μg/ml) for non pregnant and early pregnant mares respectively.

* Zinc levels were significantly higher during follicular phases for non-pregnant mares (0.730 ±0.060 μg/ml) and non- significantly higher for preagnant mares (during the same phase) (0.782±0.37 μg/ml) as compared with those of luteal phase and early stage of pregnancy (0.590±0.54 and 0.611 ±0.24 μg/ml) for non-pregnant and early pregnant mares respectively.

CONCLUSION

From this study, it could be concluded that, estimation of progesterone levels, trace elements concentrations, in addition to ultrasound observations of ovarian changes during estrus, and early pregnancy detection, could be an aid for diagnosis of reproductive status in the Arabian mares.

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ARABIC SUMMARY

الملخص العربى

أجريت هذه الدراسة بمحطة الزهراء لتربية الخيول العربية الاصيلة التابعة للهيئة الزراعية المصرية بمنطقة عين شمس-القاهرة.

و قد تمت هذه الدراسة على 72فرس عربية أصيلة (سلالة مصرية) تتراوح اعمارها بين 12-6 سنة.

و قد تم تقسيم الافراس بطريقة عشوائية الى مجموعتين:

* - المجموعة الاولى:

و تتكون من 60فرس، حيث تم متابعة دورة الشبق لهذه الافراس باستخدام طريقة التشميم بواسطة احد الطلائق القوية و ذلك لقياس اطوال مراحل دورة الشبق فى الافراس العربية، و قد وجد أن:

أ- فترة النشاط الاستروجينى (الشياع): كان طولها (1.25 ± 5.5) يوم.

ب- فترة النشاط البروجستيرونى للجسم الاصفر : كان طولها (1.35 ± 12.58) يوم.

جـ- طول دورة الشبق كاملة (1.07 ± 18.08) يوم .

ملحوظة:

تلاحظ وجود 7أفراس لم تظهر عليها علامات الشياع أثناء عملية التشميم ، و بالفحص بالموجات الفوق صوتية وجدت هذه الافراس فى حالة خمول للمبايض (خارج مرحلة الشياع) و وجد على المبيض حويصلات قطرها (0.04 cm ± 1.51) و لم يلاحظ وجود الجسم الاصفر.

كذلك استخدمت أفراس هذه المجموعة فى الفحص بالموجات الفوق صوتية لمتابعة التغيرات الدورية فى المبايض و التشخيص المبكر للحمل حيث وجد الآتى:-

أ- أفراس فى مرحلة النشاط الاستروجينى (الشياع) : ووجد على المبيض حويصلات قطرها كالآتى:

فى اليوم الاول: (0.06 cm ± 2.57)

" الثانى: (0.06 cm ± 3.69)

" الثالث: (0.22 cm ± 4.04)

" الرابع: (0.21 cm ± 4.85) و هو أقصى قطر تصل اليه الحويصلات قبل التبويض.

ب- أفراس فى فترةالنشاط البروجستيرونى للجسم الاصفر: حيث تم فحص الافراس مبكرا اثناء تلك الفترة، ووجد على المبيض حويصلات قطرها (0.04 cm ± 1.01) و هو أقل قطر للحويصلات سجل فى هذه الدراسة . و فى آخر هذه الفترة، وجد على المبيض حويصلات قطرها (0.02 cm ± 2.13) .

** - المجموعة الثانية:

و تتكون من 12فرس، حيث تم جمع عينات دم (لفصل سيرم) كل يوم من أول يوم لظهور علامات الشياع و اثناءها، حتى اختفاء علامات الشياع، و بعد ذلك جمعت العينات كل ثلاثة ايام الى آن يتم تشخيص الحمل مبكرا باستخدام الموجات الفوق صوتية.

و لقد سجلت5 أفراس عشار ، و 7 أفراس غير عشار ، حيث تم قياس مستوى هرمون البروجستيرون و بعض العناصر النادرة (نحاس- حديد- زنك) . و لقد حاولنا قياس تركيز عنصر المنجنيز ، و لكنه كان بتركيز أقل من الممكن قياسه.

و قد قسمت العينات حسب فترة اخذها الى:-

أ- عينات فترة النشاط الاستروجيني (الشياع) للأفراس الغير عشار (26 عينة).

ب- " " البروجستيروني للجسم الاصفر للأفراس الغير عشار (48 عينة).

جـ- " " الاستروجيني (الشياع) للأفراس العشار (26 عينة).

د- " الحمل المبكر للأفراس العشار (44 عينة).

تم تسجيل زيادة معنوية فى مستوى هرمون البروجستيرون أثناء فترة النشاط البروجستيرونى للجسم الاصفر للافراس الغير عشار (0.346 ± 4.366 ng/ml) و أثناء فترة الحمل المبكر للأفراس العشار (2.11 ± 4.145 ng/ml) عنه أثناء فترة الشياع لكل الافراس (0.062 ± 0.548 ng/ml) و (0.800 ± 0.390 ng/ml) للأفراس الغير عشار و الحوامل على لتوالى.

كذلك تم تسجيل زيادة معنوية فى تركيز عنصر النحاس أثناء فترة النشاط البروجستيرونى للجسم الاصفر للأفراس الغير عشار (0.097 ± 1.609 µg/ml) عنه أثناء فترة النشاط الاستروجيني (الشياع) (0.037±1.517 µg/ml) فى حين سجلت زيادة غير معنوية فى تركيز عنصر النحاس اثناء فترة النشاط الاستروجيني (الشياع) للأفراس الحوامل (0.26 ± 1.528 µg/ml) عنه فى فترة الحمل المبكر (0.49 ± 1.453 µg/ml).

تم تسجيل زيادة معنوية فى تركيز الحديد أثناء فترة النشاط البروجستيرونى للجسم الاصفر و غير معنوية اثناء فترة الحمل المبكر لافراس المجموعتين (0.156 ± 3.262 µg/ml) و (± 3.247 1.03 µg/ml) على التوالى و ذلك بالمقارنة لتركيز الحديد أثناء فترة النشاط الاستروجيني (الشياع) لافراس المجموعتين (0.163 ± 3.00 µg/ml) و (1.27 ± 3.049 µg/ml)

الغير عشار و الحوامل على التوالى.

و على العكس من العناصر السابقة، تم تسجيل زيادة معنوية فى تركيز عنصر الزنك اثناء فترةالنشاط الاستروجينى (الشياع) لافراس المجموعة الاولى (0.730 ± 0.060 µg/ml) و غير معنوية لافراس المجموعة الثانية (0.782 ± 0.37 µg/ml) ، مقارنا بالتركيزات (0.590 ± 0.054 µg/ml) و (0.611 ± 0.24 µg/ml) للأفراس الغير عشار و الحوامل على الترتيب.

الإستنتاج

من هذه الدراسة نستنتج أن:-

تقدير مستوى هرمون البروجستيرون و تركيز العناصر النادرة، بالإضافة إلى استخدام الموجات فوق الصوتية لملاحظة التغيرات فى المبيض أثناء دورة الشبق و كذلك التشخيص المبكر للحمل يمكن أن تكون من الوسائل المشخصة للتعرف على الحالة التناسلية فى الافراس العربية.