Improvement of camel reproduction with the help ultrasonography

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Abstract

The study was carried out on fourteen dromedary camels (Maghrabi b .These females were divided into 4 groups and examined weekly ultrasonography per rectum. The 1st group (4 females) was treated with Fe for induction of puberty and activation of their ovaries and developme follicles more than 0.8 cm in diameter (0.77± 0.02) before the breeding se The 2nd group (3 females) was used to monitor estrus and early pregr diagnosis and CL of pregnancy by ultrasonography. The 3rd group (3 fem were subjected to ultrasonographic scanning which revealed that the invo of the uterus and cervix was very rapid 20 – 25 days postpartum and ov scanning up to 70 days revealed no follicles ≥1.0 cm in diameter.In the 4th g (4 females) the use of 1000 IU of eCG (Folligon) in the non breeding se (May- June 2008) developed follicles (1 – 1.7) cm in diameter.

Keywords: Dromedary camel, ovarian activity, follicular wave, Pregn Postpartum, Ultrasound.

Introduction

One of the main problems for improvement of one-humped camel producti the low reproductive efficiency of this species. Moreover, seasonal breeding maturity and long intercalving periods are important factors (Wilson, 1989 Djellouli and Saint-Martin, 1992). The female camels are bred in one sea calve in the subsequent breeding season and remain sexually quiescent unt following breeding season leading to long inter-calving periods and signi economic losses. This is beside the lack of use of assisted reprodu techniques such as embryo transfer, artificial insemination and monitorir ultrasonography in this species (Skidmore, 2005). In fact, the reprodu without doubt is more closely to production, this means; there is no produ without reproduction.

Ultrasonography is a valuable aid to the examination of the female reprodu tract.The technique is useful to determine ovarian follicular development stage of the follicular cycle, the size of the pre-ovulatory follicles, the occurr of ovulation and the morphological development of the corpus luteum. It helps in the interpretation of ovarian abnormalities such as very large follicl cysts. Ultrasonographic examination of the uterus enables early and acc diagnosis of pregnancy and morphological evaluation of the development c conceptus, foetal viability and pathological conditions such as intrauterine accumulation. Ultrasonography is also fundamental to embryo tra programmes as the technique enables the monitoring of the multiple folli developments, superovulation and the suitability of recipients and ca intended for artificial insemination Mckinnon et al.(1988); Skidmore et al.(1 (Tinson and Mckinnon, 1992) and Musa et al. (1992). Ultrasonogr examination of the genital tract was done per rectum, pervagina or external

the abdominal wall. In practice, examination per rectum was the most widely practiced in camelidae followed by the use of the abdominal technique when palpation was not possible (small llamas and alpacas).Vaginal ultrasonography is used in research for echo-guided follicular aspiration or site-specific treatments (aspiration of uterine cysts, intrafollicular injection and aspiration of embryos (Tibary and Anouassi, 1997). Earlier studies on the follicular wave pattern in dromedaries were based on postmortem examination and on serial palpations of the ovaries per rectum in small number of camels. However, more recent studies used real time ultrasonography to monitor day-to-day ovarian follicular changes much more accurately Skidmore et al. (1996).

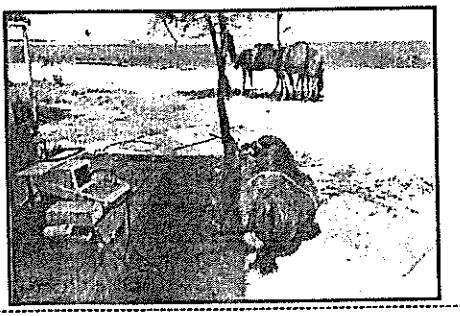
The present work is a clinical trial using real- time transrectal ultrasonography for:

1) Examination of reproductive tract of female dromedary camels.
2) Observation and recording follicular wave pattern in the she- camels.
3) Early pregnancy diagnosis in she-camels.
4) Detection of resumption of postpartum ovarian activity.
5) Follow-up of stimulation of ovarian activity by hormonal treatment (GnRH eCG).

Materials and Methods

Animals:

The present study was carried out on fourteen female dromedary camels (Camelus dromedarius) of Maghrabi breed, raised in the farm of the faculty of veterinary medicine at Sadat City. Animals were housed in an open-shed and fed concentrate ration, Barseem, hay and water was provided ad-libitum. The study was conducted before, during and after the breeding season (October 2007- December 2008) and these animals were examined weekly using ultrasonography and were divided into 4 experiments. Ultrasonographic examination was done following the technique described for dromedary camels by (Tinson and Mckinnon, 1992) and (Tibary and Anouassi, 1996) by a real time B and M-mode linear array ultrasound scanner (Scanner 480 – Vet – Scan, Pie Medical Co.) which was used in this study. The scanner was provided with a transrectal linear transducer (5 and 7.5 MHZ) for endo-rectal scanning and a thermal paper video-printer (up-895 CE, Sony) used for printing frozen images.The examined camels were sedated in the standing position by intramuscular injection of 2% Xylazine in a dose of 0.125 mg /kg B.w (20 mg Xylazine Hcl, Xylaject, ADWIA) (Abou-El-Ella and Housainy, 2006). The camels were restrained in sternal recumbency posture after sedation with all four legs correctly fastened with ropes and a kinch rope tied up around her hind legs as in photograph (1).



Photograph (1): Female cam
undergo transrectal
ultrasonography

Experiment 1: Ultrasonographic scanning of she-camels reproductive in non breeding state:

This experiment included 4 females (3 young female dromedaries 3-4 y old) and one mature non-lactating female (10 years old with long post pa anestrum of 2 years) to monitor follicular dynamics using real ultrasonography. Ultrasonographic examination of three young females reve that their ovaries were inactive (quiescent), small one or two follicles (less 0.3cm diameter) in one or both ovaries and the ovaries of those heifers hard to palpate by hand or rectal palpation. The whole size of the ovary small and appears by ultrasound elliptical in shape. The uterus and c appeared normal in diameter and echotexture. Ultrasonographic examinati the old mature female dromedary which has long post partum anestrum years revealed that its ovaries have no structures.This group was given treatments of (Fertagyl, Gonadorelin 0.1 mg /ml, Intervet) 20 days apa induction of puberty in young camels and to activate their ovaries. T animals were examined weekly by ultrasound for monitoring the chang ovaries and follicular wave dynamics.

Experiment 2: Ultrasonographic scanning of reproductive tract breeding:

This experiment included 3 females (2 young female dromedaries 4-5 yea and one mature non lactating female 8 years old) to monitor oestrus animal early pregnancy diagnosis. All females were examined weekly at the begi of the breeding season (January 2008) and were bred whenever a dom follicle (size between 10- 22 mm) was found in either of the ovaries an uterus showed maximum tone and oedema was the criteria used to decid mating of a female camel with a virile stud. Pregnancy diagnosis was do visualize the corpus luteum of pregnancy and amniotic vesicle, embryc heart beats until 2 months of pregnancy.

Experiment 3: Ultrasonographic scanning of reproductive tract d postpartum period:

This experiment included 3 postpartum lactating females (2 primiparou years and one pleuriparous female 10 years old) to monitor the involution uterus and cervix and resumption of ovarian activity during post partum p The three post partum lactating females with suckling calves (two primip and one pleuriparous) calved on March, April 2008 normally without interference, expulsion of foetal membranes rapidly after parturition and females discharged a normal lochia. Those post parturient females scanned weekly after parturition through the first 90 days post pa Ultrasound of ovarian follicles and diameter of both uterine horns and were taken at each examination. Ovarian activity was evaluated by follicle and uterine involution was measured in term of reduction in uterine dian Moreover, the size of the uterus, its location and tone were also used indicator for the uterine involution.

Experiment 4: Ultrasonographic scanning of reproductive tract during breeding season:

This expriment included 4 females (2 post parturient old females and 2 y heifers 4- 5 years) to study the response of the dromedaries to equine cho gonadotrophine (eCG) during the non breeding season.The four females scanned with ultrasonography at the non breeding season (May and

2008).Each animal was treated with 1000 IU equine chorionic gonadotropin (eCG) hormone (Folligon, Intervet, Holland) then all animals were examined weekly starting 4 days after treatment for one month to monitor ovarian activity in the non breeding season.

Results

Experiment 1: ultrasound scanning of ovaries of the three young female dromedaries (3 – 4 years old) revealed that their ovaries were inactive and difficult to locate but the echotexture of the ovary was very characteristic and more echogenic than the surrounding tissues and appeared elliptical or oval on the ultrasonograms (Image 1). The uterus appeared homogenous light gray in echotexture during the luteal phase or in the non breeding season (Image 2). The cervix of the dromedary camel appeared of moderate hyperechogenicity and 2 or 3 transverse cervical echogenic bands were observed and were prominent during the luteal phase or in the non breeding season (Image 3). This group of she-camels was treated with (Fertagyl) and these animals responded slowly to the first treatment and develop two or three follicles not more than 0.4 cm in diameter in two animals and the other two animals were quiescent with smooth ovaries and no signs of oestrus appeared after the first treatment with Fertagyl. After the second treatment with (Fertagyl) the ovaries of three animals developed follicles more than 0.8 cm and these follicles did not enlarge in size to become dominant follicles and remained for ten days then regressed again (Image 4). Females with active ovaries had continued follicular activity on both ovaries with overlapping follicular waves characterized by the transition of the follicles thorough 4 stages of development: quiescence (Image 5), follicular recruitment (Image 6), follicular growth and dominance (Image 7) and follicular regression or degeneration(Image 8,9,10,11,12,13).

Experiment 2: Two camels showed oestrus signs at the beginning of the breeding season and the ultrasonographic scanning of those females before breeding revealed the presence of large non echoic, black, thin walled, roughly circular follicles (1.51 cm & 1.38 cm diameter) in the left ovary (Image 14). Ovulation did not occur in the present study when the follicle was $\leq$9 mm in diameter, but ovulation increased when the follicle was between (1.0 – 1.5 cm) in diameter in addition to turgid uterus with high oedema, it decreased in ovulation when the follicle continued to grow $\geq$2.0 cm in diameter. There was no any significant difference in the follicular activity between the left and right ovary. The conceptus was recognized at day 18 post mating and the embryonic vesicle appeared as a star-shaped non echogenic "black hole " type of fluid accumulation in the lumen of the left uterine horn (Image 15). By 22 days the amniotic vesicle became oval measuring about 2 cm and blunt in one end and broader in the other end at which the embryo rests and became visible. The embryo became visible as a small echogenic 'blob' within the fluid fixed at one pole of the vesicle (Image 16). By 34 days the diameter of the vesicle increased more rapidly due to accumulation of foetal fluid measuring 2.8 cm and the embryo measured about 1.1cm length (Image 17). By day 45 the foetal fluid increased and embryonic vesicle measured about 7 cm in diameter and the foetus became more visible and rested on the floor of the pregnant horn (Image 18). By 55 to 60 days the head, neck, abdomen and individual limb buds of the foetus could be easily identified measuring 5.12 cm in length and the foetal fluid increased to an extent that the foetus could no longer be viewed easily as it

generally lies beyond the penetration range of a (5MHz) transrectal
(Image 19). The CL was distinctly spherical with clear borders and
hypoechogenic relative to the surrounding tissue and the corpus lutet
pregnancy was observed to have two forms, the 1st is compact (Image 20
the 2nd is dense and homogenous in echotexture (Image 21).

Experiment 3: Ultrasonographic scanning of the uterus in the first week
parturition revealed enlarged uterus containing non echoic black fluid (Ic
(Image22) and the cervix become large (Image 23). At 25 days post partu
uterus and cervix were completely involuted and they regain their n
size(Image 24) and at 45 days postpartum follicles (0.5- 0.7) cm in dia
were detected (image25). After 80 days postpartum only one animal shov
follicle ≥2 cm in diameter and became anovulatory follicle (Image 26) an
animal showed uterine cyst inside the left horn which appeared as large,
thick wall, well defined circumference, non echoic, black ≥4.55 cm in dia
(Image 27).

Experiment 4:). After treatment with 1000 IU eCG (Folligon, Intervet, Holl
the two young animals which were having follicles ≤1.0 cm diameter respo
to this treatment and developed follicles of 1.38 cm and 1.12 cm in diame
the first week(image 28). The follicle enlarged in the second week (Image
After the third week of treatment, no characteristic change occurred an
follicles remained in the ovary (image30). Also the young animals with
dominant follicles showed oestrus signs and the scanning of those two an
after one month revealed no pregnancy. There was a significant diffe
($P<0.05$) before and after treatment by Folligon (eCG).

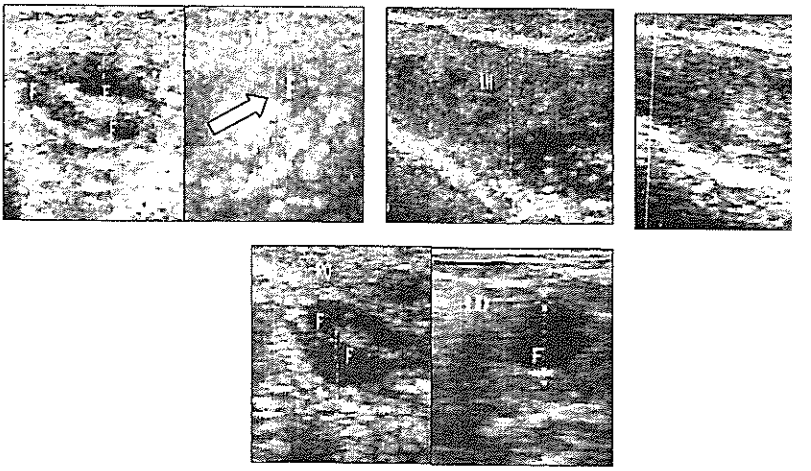


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Image 1: Ultrasonograms of inactive ovaries of heifers.(Notice that
ovaries are small and elliptical)

Image 2: Ultrasonograms of the uterus of dromedary heifers (homogenous light gray).

Image 3: Ultrasonograms of the cervix of the dromedary heifers (mod
hyperechogenicity).

Image 4: Ultrasonograms of ovaries of dromedary heifers after second treatmen
Fertagyl (Gonadorelin). Note the increasing in the diameter of follicles.



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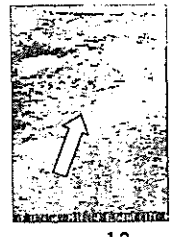
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Image 5: Ultrasonograms of ovaries showing phase of quiescence.
Image 6: Ultrasonograms of ovaries showing phase of follicular growth.
Image 7: Ultrasonograms of ovary and uterus showing phase of follicular maturation.
Image 8: Ultrasonograms of follicular regression. Note Thin walled large follicle containing clear fluid.
Image 9: Ultrasonograms of follicular regression. Note Thick walled large follicle containing clear fluid.
Image 10: Ultrasonograms of follicular regression. Note Thick walled large follicle with some floating debris within its cavity.
Image 11: Ultrasonograms of follicular regression. Note Thick walled large follicle and criss-crossing fibrin strands within the cavity.
Image 12: Ultrasonograms of follicular regression. Note luteinized like structure.
Image 13: Ultrasonograms of the uterus at the follicular regression.

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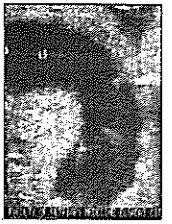

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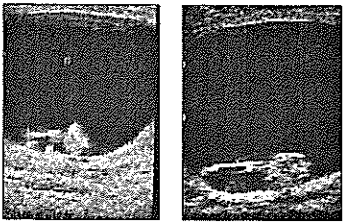

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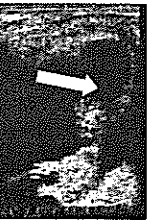

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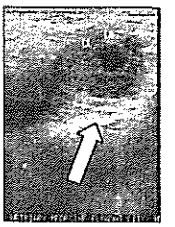

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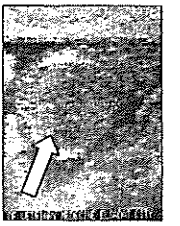

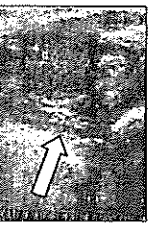

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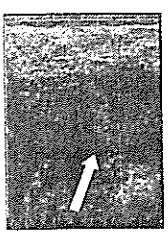
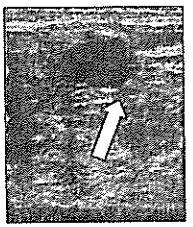
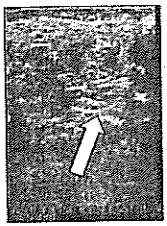
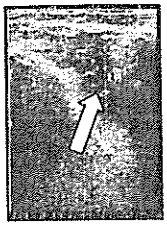
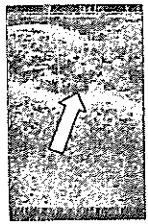
Image 14: Ultrasonograms of ovary with a dominant follicle and uterus of estrus camel.
Image 15: Ultrasonograms of pregnant she-camel at day 18 of gestation. Note st amniotic vesicle.
Image 16: Ultrasonograms of pregnant she-camel at day 22 of gestation.
Image 17: Ultrasonograms of pregnant she-camel at day 34 of gestation.
Image 18: Ultrasonograms of pregnant she-camel at day 45 of gestation.
Image 19: Ultrasonograms of pregnant she-camel at day 55-60 of gestation.
Image 19: Ultrasonograms of pregnant she-camel at day 55-60 of gestation.
Image 20: Ultrasonograms of the CL of Pregnancy. Note the 1st form as compac central hypoechogenic area.
Image 21: Ultrasonograms of the CL of Pregnancy. Note the 2nd form as large, dens homogenous.

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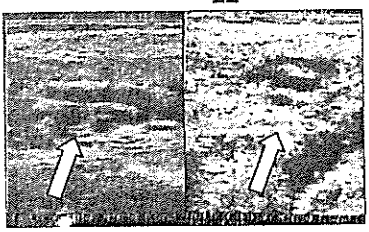
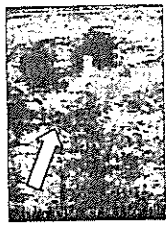
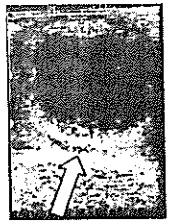


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Image 22: Ultrasonograms of she-camel at 7 days postpartum. Note the non echoic lochia and the large diameter of uterine vein.
Image 23: Ultrasonograms of she-camel at 7 days postpartum. Note the large diameter of cervix.
Image 24: Ultrasonograms of she-camel at 20 days postpartum .uterus and cervix.
Image 25: Ultrasonograms of she-camel at 45 days postpartum. Note ovaries carrying follicles.
Image 26: Ultrasonograms of she-camel at 80 days postpartum. Note large follicle 2.03 cm in diameter. **Image 27:** Ultrasonograms of she-camel at 80 days postpartum. Note presence of a uterine cyst inside the left horn as large, very thick walled, circumscribed, fluid filled structure and non echoic ≥4.55 cm diameter.

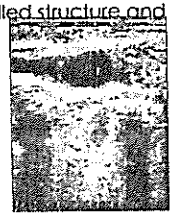
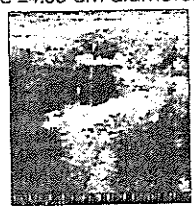
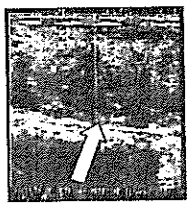


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Image 28: Ultrasonograms of ovary one week after eCG treatment. Follicle 1.38 cm diameter.
Image 29: Ultrasonograms of ovary and uterus two weeks after eCG treatment.
Image 30: Ultrasonograms of ovary three weeks after eCG treatment.

Discussion

It was possible to increase the reproductive efficiency of camels by good management, controlled breeding, strategic use of hormone treatment and the use of assisted reproduction techniques such as artificial insemination, ultrasonography, and embryo transfer (Skidmore, 2005). Ultrasonographic

examination revealed that inactive ovaries were more difficult to locate but echotexture of the ovary was very characteristic as it is more echogenic than surrounding tissue and appeared elliptical or oval on the ultrasonogra imaging. This is agreement with the findings of Tibary and Anouassi (1997) v Mckinnon and Squires (1991) recorded that, the inactive ovaries were re differentiated from the functioning ones. Induction of puberty before bree season at as early as 3-4 years of age has attempted by using two injection days apart of Fertagyl. The majority of females responded to this treatment developed follicles ≤1.0 cm in diameter. Similar results were obtained by Ni et al. (2008). The best time for breeding or induced ovulation by male whe parameters were present: the first, when the dominant follicle measured 1.0 cm in diameter and the second, when the uterus was turgid with high ed which agreed with the results obtained by Anouassi et al. (1994). The ovulating follicle continued to grow and reached a maximum diameter (3 - 6 and felt as goose egg with some fluctuation which agree with findings obta by Tibary and Anouassi (2000). Ultrasonographic examination of the preg uterus revealed that all parts of the embryo and his envelopes were e identified by ultrasonography in she-camels. While Barakat (2006) reported the application of the Cuboni test was a useful indicator for the early pregna detection in the she-camels. The corpus luteum of pregnancy was observe have two forms; the first form was compact CL with central hypoechogenic and the second form was large, dense and homogenous. Similar findings v reported by Skidmore and Adams (2000).While Abu-Nawwara (2006) found mature corpus luteum has a dense, hyperechoic central area in the shape star. Uterine involution was considered to be rapid in camelidae (20-25 d post partum) as the microcotyledonary and diffuse nature of the placentatic this species does not cause a great loss of uterine tissue and similar find were reported by Musa and Makawi (1989) and Tibary and Anouassi (19 While Ahmed (1990) noticed that the involution of the uterus completed by 3 42 days post partum. Vyas and Sahani (2000) reported that involution of ut completed in a period lower than 40±2.1 day. One animal developed l uterine cyst ≥4.55 cm diameter after 80 days postpartum. Similar finding observed by Tibary and Anouassi (2001) who examined 13 female camels detected uterine cysts in 3 females. the use of 1000 IU eCG (Folligon) in the breeding season resulted in activation of the follicular wave and two anir developed dominant follicles (≥ 1.5 cm) in diameter. These findings cam accordance with that recorded by Rai et al. (1990); Mckinnon and Tinson (19 Cooper et al. (1992); Skidmore et al. (1992); Vyas (1998) and Ismail and Eknah (2006).While Skidmore (2000) stated that, one of the most impor problems in superstimulation of the camel was the high incidence of responsive females (20-30%) which failed to produce follicles. No pregna occurred after treatment with eCG. Similar results were obtained by Dafalla e (1987); Rai et al., (1990) and Helimy (1991).While Minoia et al. (1992) reve that there was a higher pregnancy rate in the camels treated prior to the sta the breeding season (September to November) than those treated simi during the breeding season. Such difference in the pregnancy rate might be to environmental factors, temperature or sexual rest for male.

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ص العربى

ن الكفاءة التناسلية فى النوق بمساعدة الموجات الفوق صوتية

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هذه الدراسة على ١٤ ناقة وحيدة السنام (سلالة مغربى) وقسمت هذه الحيوانات إلى أربع
ات وتم فحصها أسبوعيا بواسطة جهاز الموجات الفوق صوتية عن طريق فتحة المستقيم.
عة الأولى ضمت أربع نوق تم حقنها بواسطة الهرمون المنشط للمبايض (Fertagyl) لحث
تنشيط المبايض ووجد نمو جريبات أكثر من ٨,. سم فى الحجم. المجموعة الثانية ضمت ثلاثة
فحصها بغرض التعرف على التغيرات التى تحدث للمبيض والرحم أثناء دورة الشبق وكذلك
س المبكر للحمل وللجسم الأصفر الخاص بالحمل بواسطة السونار. المجموعة الثالثة ضمت
ق بعد الولادة مباشرة ووجد أن إنغماد الرحم وعنق الرحم فى النوق سريع ويتراوح من ٢٠ –
بعد الولادة والجريبات لم تزد عن ١ سم حتى ٧٠ يوم بعد الولادة. المجموعة الرابعة ضمت
وق ووجد أن إستخدام ١٠٠٠ وحدة دولية من هرمون الفرس المشيمائى المحفز للمناسل
Fol) فى موسم عدم التزاوج (مايو- يونيو ٢٠٠٨) أدى إلى نمو جريبات تتراوح من (١-
م فى الحجم.