EFFECT OF ULTRASONOGRAPHY ON THE EFFICACY OF FSH IN TREATMENT OF ANOESTRUS BUFFALOES

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ABSTRACT

Buffaloes are apparently of low ovarian response, repeat breeders and silent heat due to the weak estrous symptoms. Different hormonal treatments failed to give a high percentage of success due to the diagnosis accuracy for the ovarian response.

The present study was conducted on 50 anoestrus buffalo-cows, 5-7 years old, divided into 2 groups (A and B). Group A included 25 buffalo-cows, their ovaries showed small-sized antral follicles based on ultrasonographic examination. Group B included 25 buffalo —cows randomly selected based on rectal examination that revealed structureless ovaries. Animals in both groups were injected with an activating dose of FSH-P (4 IU/animal) and if the animal does not respond, a second dose (4 IU) was injected after 2 weeks from the first one. All animals were examined rectally and sonographically twice weekly after treatment. They were closely observed for estrus detection and naturally bred by a fertile bull. Pregnancy was confirmed using sonography and rectal examination at days 25 and 60 respectively. Progesterone level was assayed before and after FSH-P treatment (one and two weeks post-treatment) to confirm the animal response and its cyclicity. The results of this investigation showed that, the responded cases were more in anestrous cases detected by ultrasonographic examination (18/25, 72%) if compared to those detected by rectal palpation of their ovaries (11/25, 44%). This was confirmed after mating by ultrasonographic examination for detection of embryo passing through detection of fetal parts and placentomes. Progesterone level in anoestrus cases selected ultrasonographically was higher than those selected randomly by rectal palpation indicating greater response for FSH in group A than B. It could be concluded that, ultrasonographic examination improved the efficacy of FSH-P treatment of anoestrus buffalo-cows.

INTRODUCTION

Swamp buffaloes are apparently of low ovarian response, repeat breeders and silent heat due to the weak estrous symptoms **(Nasir et al., 1986)**. Different hormonal treatments failed to give a high percentage of success due to the diagnosis accuracy for the ovarian response **(Jablonka-Shariff et al., 1996)**. In sheep, FSH-P gave good results in increasing medium and large size antral follicles beside it decreased the incidence of atresia **(Jablonka-Shariff et al., 1994)**. The radioimmunoassay technique to diagnose the blood plasma progesterone levels was useful in studying ovarian function and in evaluating the efficacy of hormonal therapy for ovarian dysfunction in swamp buffalo **(Jainudeen et al., 1981 and Kanai, 1987)**. It has been suggested that it was possible to improve the program of multiple ovarian stimulation by using real time B mode ultrasound for examination of ovarian response **(Echakumphu et al., 2000)**. The available quantity of FSH determines the selection of oocytes from the crop of growing follicles **(Baker, 1972)**.

The present study was designed to investigate the efficacy of FSH in treatment of anoestrus buffalo-cows diagnosed either by rectal palpation or ultrasonographic examination.

MATERIAL AND METHODS

Animals :

50 buffalo-cows, aged 5-7 years old and had been calved since nine months ago, were selected according to a regime of assortment in the period from August 2002 to January 2003 in a private farm in Dakahlia Province. The animals were fed ad libitium of straw and darawa in case of dry season and barseem in case of green season with the addition of 1 Kg of balanced concentrated ration daily in both seasons. Water was provided ad libitium.

Experiment :

Group A : this group included 25 buffalo-cows suffering from anaestrum and their ovaries showed small-sized antral follicles based on ultrasonographic examination using ultrascan 50, (Mitusbishi, Japan) with 7.5/5 MHz transrectal probe according to **Pierson and Ginther (1982)**.

Group B : this group included 25 buffalo-cows suffering from anaestrum. They were randomly selected based on rectal examination and the clinical examination revealed structurless ovaries.

Animals in both groups were injected with an activating dose of FSH-P (LH/FSH:40%, Laboratory of the Department of Reproductive Physiology, Faculty of Veterinary Medicine, University of Liege, Sar tilman B 4000, B 41, Belgium) (4 IU/animal), if the animal does not respond, a second dose (4 IU/animal) was injected after two weeks from the first one. All animals were examined

rectally and sonographically twice weekly after treatment. All buffaloes were closely observed for estrus detection. They were naturally bred using fertile bull. Pregnancy was confirmed using sonography and rectal examination at days 25 and 60 respectively.

Progesterone assay: the assay was carried out using progesterone solid phase radioimmunoassay kits (DPC, USA). Progesterone was assessed according to **Abraham (1981)** before and after treatment with FSH (one and two weeks post-treatment) to confirm the animal response and its cyclicity. The sensitivity of the assay was 0.05 ng/ml.

RESULTS

The ultrasonographic examination of anestrous buffalo-cows revealed absence of any ovarian structure prior to FSH-P treatment (Fig. 1) except the presence of only small-sized antral follicles that cannot be detected by rectal palpation of the ovaries. After FSH-P treatment the follicular growth was obvious after about three weeks from FSH-P injection (Fig. 2). After mating of buffalo-cows showing estrus of both groups, ultrasonographic examination revealed non-responding cases for FSH-P treatment (Fig. 3) which were more recorded in anaestrus rectally palpated than ultrasonographically detected cases. At the same time, the responded cases were more in anestrous cases detected by ultrasonographic examination (18/25, 72%) than those detected by rectal palpation of their ovaries (11/25, 44%). The responded and mated cases were ultrasonographically examined for detection of embryo (Fig. 4), passing through detection of fetal parts and placentoms (Fig. 5) till detecting of even the details of the fetus as the scrotum (Fig. 6).

Fig. (7 a &b) showed that progesterone level in anoestrus cases selected ultrasonographically (Group A) was higher than those selected by rectal palpation (Group B) indicating the great response for FSH-P treatment in group A than group B.

DISCUSSION

Several hormonal treatments were tried for treatment of anaoestrus cases in most farm animals with special reference to buffalo-cows. However, the success for such treatments was limited for several known and unknown reasons. There are conflicting results concerning the use of FSH-P in treatment of anoestrus cases **(Jablonka-Shariff et al., 1996)**. In sheep, as in other mammals, preovulatory follicular development begins with recruitment and growth of a cohort of primary follicles within the ovary **(Fortune, 1994)**. Successful development to the preovulatory stage requires both proliferation and differentiation of follicular granulosa and thecal cells **(Mariana et al., 1991& Monniaux and Pisselet, 1992)**. The vast majority of ovarian follicles never

ovulate or form corpora lutea but rather become atretic at different stages of follicular development **(Hubbard and Oxberry, 1991)**.

Studies in several species have demonstrated that follicular growth and development is mediated by gonadotropins **(McNatty et al., 1993)**. Administration of exogenous gonadotrophic preparations containing FSH-like activity has been shown to change the balance between healthy and atretic follicles by preventing or delaying atresia **(NcNatty et al., 1985 & Hubbard and Oxberry, 1991)**. Also, administration of FSH is associated with advancing the maturation of gonadotrophin-dependent follicles without affecting the number of committed follicles **(Soboleva et al., 2000)**.

The obtained results are consisted with those obtained by previous studies **(Jablonka-Shariff et al., 1994 & Jablonka-Shariff et al., 1996)** who demonstrated in their first investigation that treatment of ewes with FSH-P increased the number of medium and large antral follicles and decreased the incidence of atresia compared with saline treatment and in their second study they found that FSH-P withdrawal was accompanied by a decrease in the number of follicles in all size classes compared with continuous FSH-P treatment. This decrease in the number of medium and large follicles after FSH-P withdrawal may have been due to the loss of gonadotropic support necessary to stimulate or maintain the growth of small and medium follicles. In support of this, FSH-P withdrawal was associated not only with decreased number of medium and large follicles, but also with an increased number of small follicles. Alternatively, part of the response may have been due to loss of medium and large follicles after withdrawal of FSH-P **(Jablonka-Shariff et al., 1996)**. At the same time, it was shown that ultrasonic screening showed that not only eCG (equine chorionic gonadotrophin), but also hMG (human menopausal gonadotrophin) given as a single application seem to provide a sufficient stimulus to achieve a satisfactory superovulatory response **(Riesenberg et al., 2001)**.

However, unless antral follicles, containing granulosa cells that possess FSH receptors, were present on the ovaries, the cases will not respond to FSH-P treatment **(Greenwald and Terranova, 1988)**. An increased proliferation of not only granulosa cells, but also thecal cells **(Jablonka-Shariff et al., 1996)** . Since thecal cells probably do not possess FSH receptors, however, a direct effect of FSH on thecal cells proliferation seems unlikely and a paracrine interaction between the granulosa and thecal cells may be involved **(Mariana et al., 1991& Monniaux and Pisselet, 1992)**.

Thus, in the present investigation, the used ultrasonographic examination to select anoestrus buffalo-cows to be treated with FSH-P, resulted in a higher response (18/25, 72%) than those anoestrus cases selected by rectal palpation of their ovaries (11/25, 44%). Ultrasonograph-

ic examination enabled us to select anoestrus cases with antral follicles on their ovaries, whereas in rectal palpation the accuracy of detecting such cases was questionable and difficult. It was possible to improve the program of multiple ovarian stimulation by using real-time B mode ultrasound for examination of ovarian responses **(Echakumphu et al., 2000)**. In our investigation, we planed to confirm the responded cases after mating by a fertile bull until detecting embryo proper, fetal parts, placentoms and even the sex of the fetus.

The present study was based also on the radioimmunoassay determination of the serum progesterone level before and after FSH-P treatment as a useful guide for the ovarian function and a valuable evaluating technique for the efficacy of hormonal therapy for ovarian dysfunction in buffalo-cows **(Kamonpatana et al., 1979, Jainudeen et al., 1981 & Kanai, 1987)**. The present results (Fig. 7 a&b) indicated that there was an obvious increase in progesterone serum level in anoestrus buffalo-cows selected by ultrasonographic examination (group A) than anoestrus cases selected by rectal palpation (group B). This was a prove for the greater response, obtained in group A (72%) than in group B (44%). On the other hand, the progesterone assay before FSH-P treatment proved that there were a number of cyclic cows more in group B than in group A indicating the accuracy of ultrasonographic examination in detecting smooth inactive ovaries containing antral follicles than rectal palpation. In rectal palpation, some of the smooth inactive ovaries may contain either embedded or regressed corpus luteum, which may be miss-diagnosed.

In conclusion, ultrasonographic examination improved the efficacy of FSH-P treatment of anoestrus buffalo-cows.

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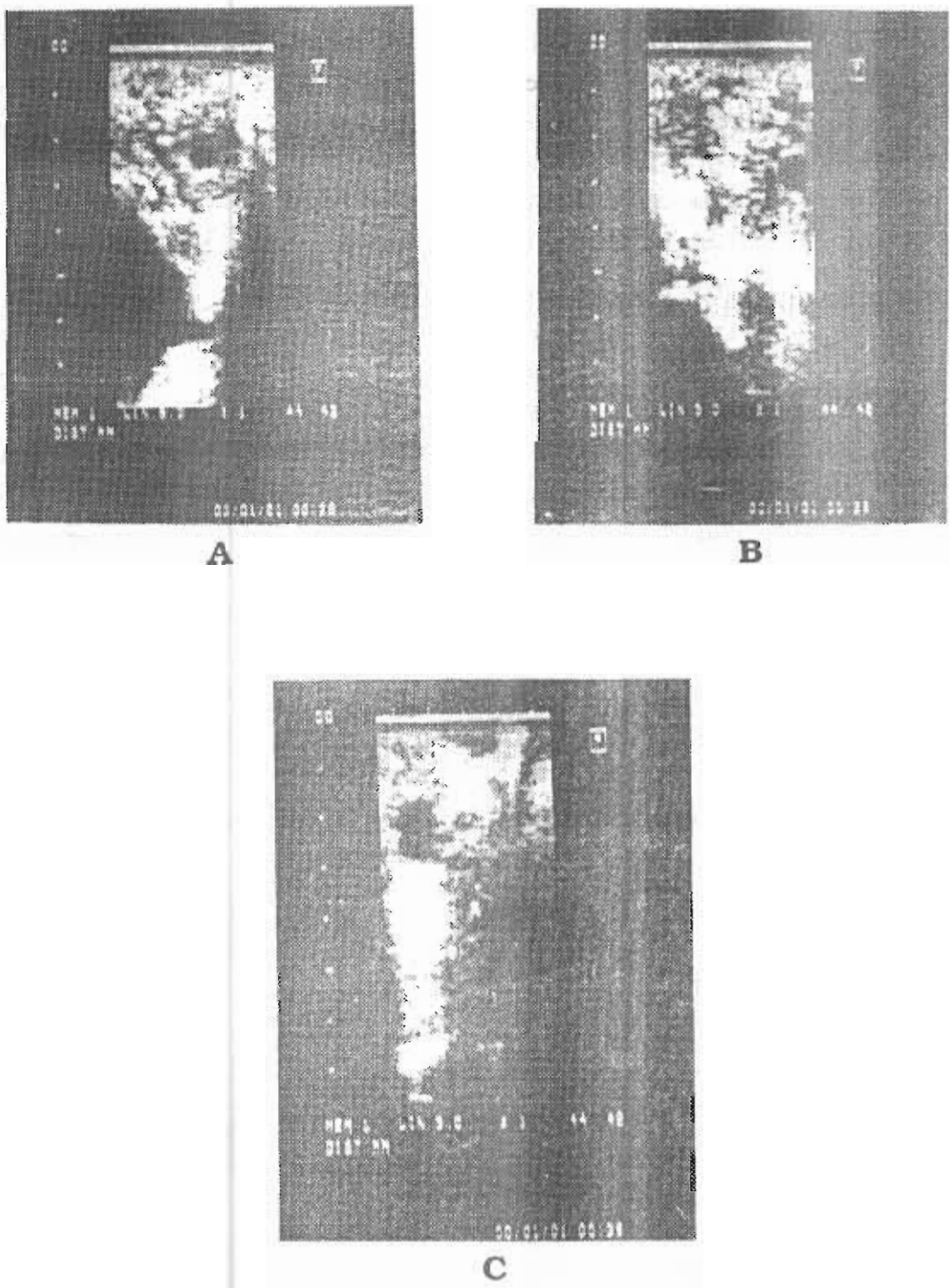

Figure 1 : Absence of Any Ovarian Structure Prior To FSH Treatment.

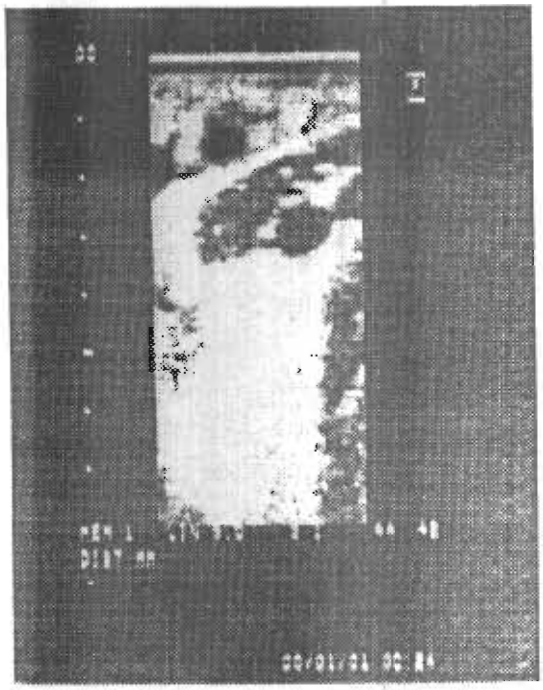

Figure 2 : 5 MM Follicle On Right Ovary 20 Days Post Treatment With FSH.

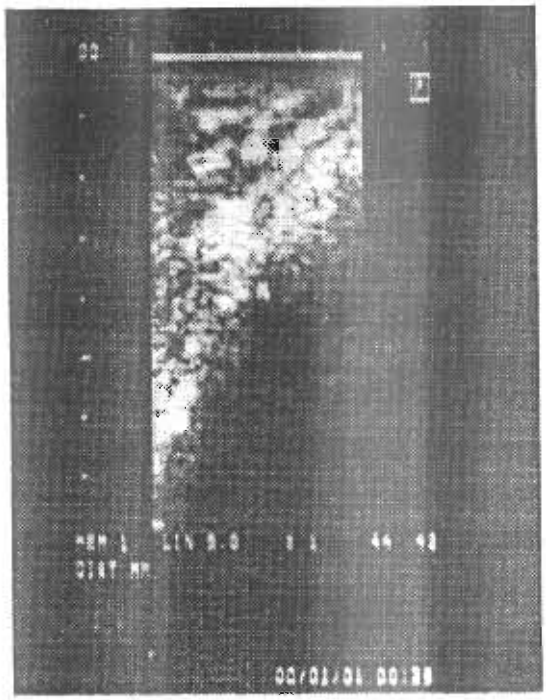

Figure 3 : Non-Graved Uterine Horn 30 Days Post Mating.

Figure 4 : 40 Days Embryo, 18 Mm. The Echogenic Line Above Embryo Is Amnion, Echogenic Bulge Represent Embryo Proper.

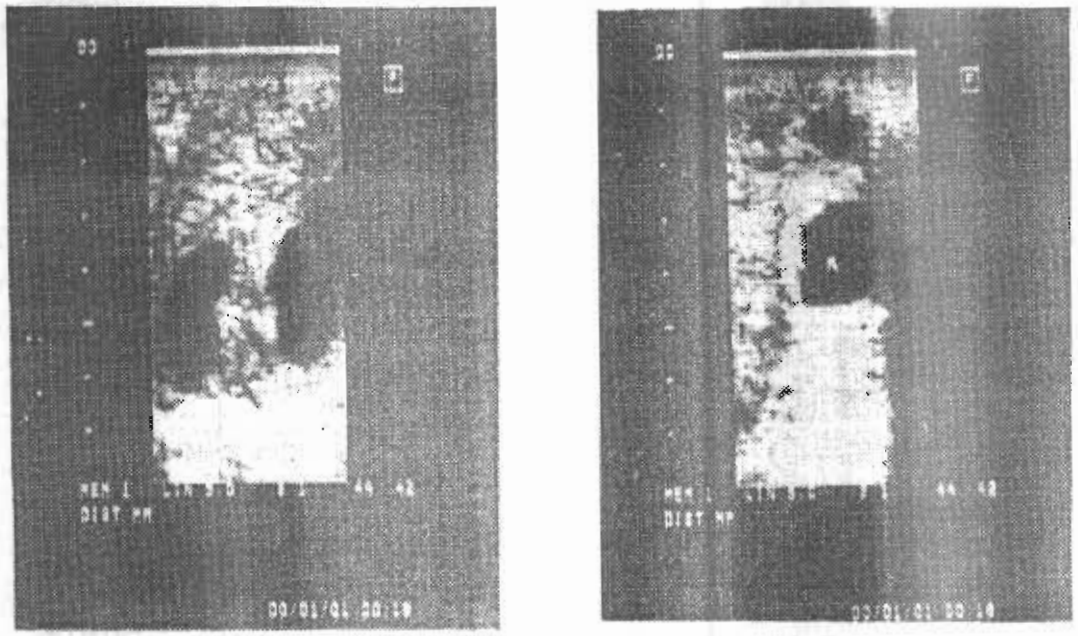

Figure 5 : Sagital Section Through Fetus Showing Buds Of Fore And Hind Limbs, Fetal Head And Circular Shaped Placentom Length Of Embryo 30mm .

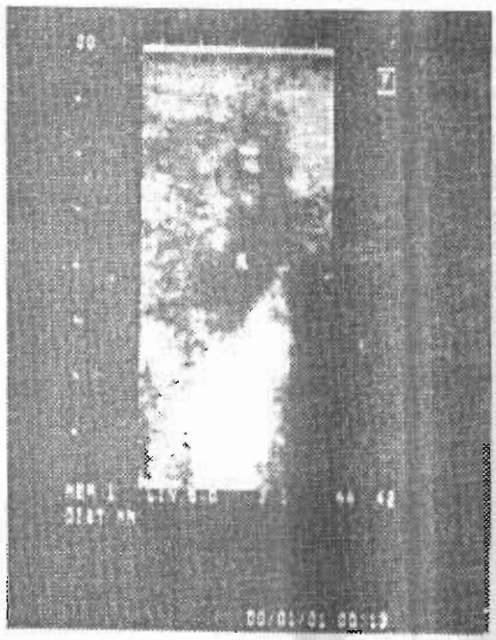

Figure 6 : Sagital Section Through 18mm Length Embryo (Day 60).

* Buds For Hind-forelimbs Appear And Scrotum Appears Between 2 Hind Limbs .

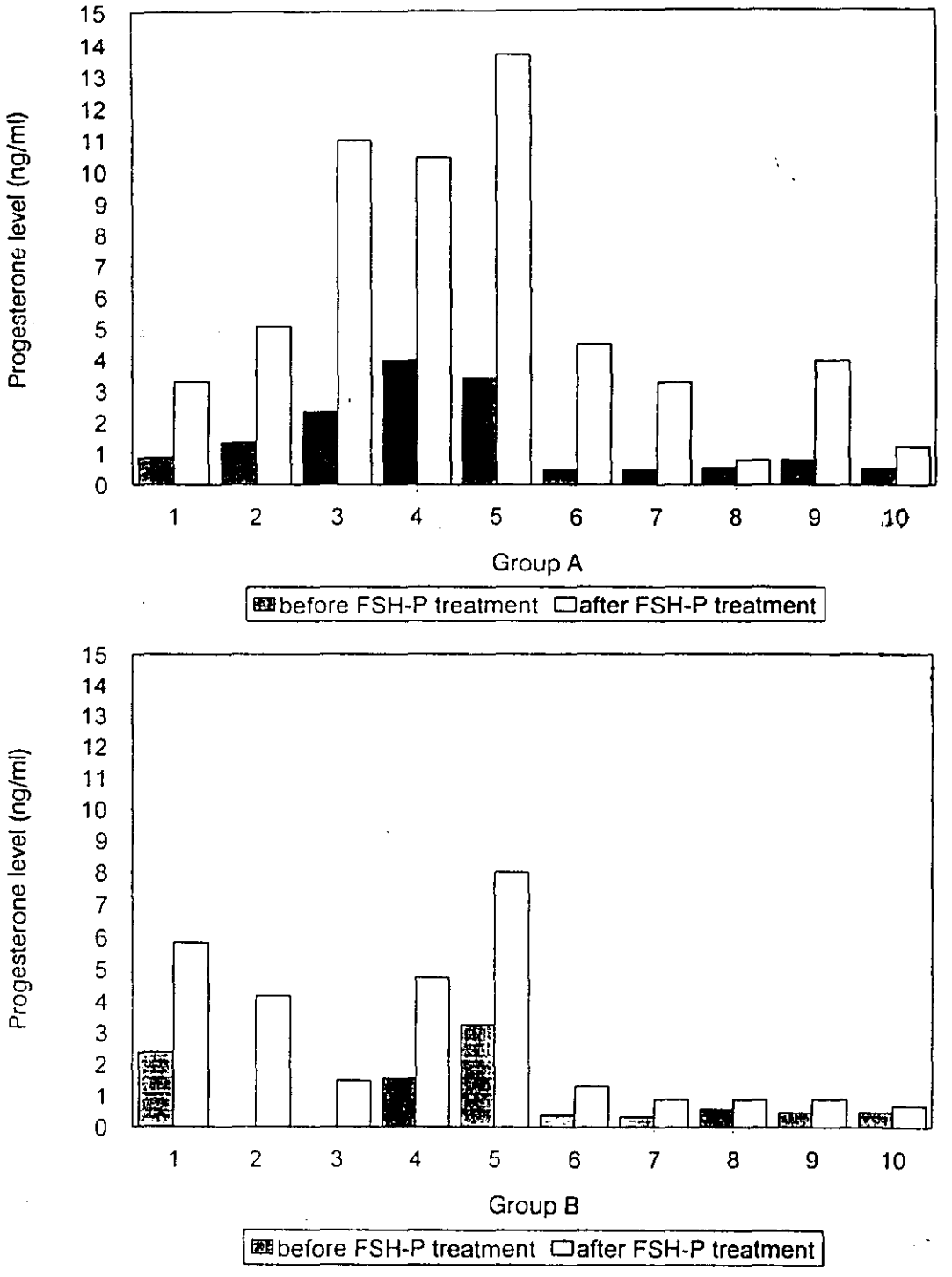

Figure 7 : Progesterone Levels (Ng/ml) Before And After Fsh-p Treatment In Ultrasonographically (Group A) And Rectally (Group B) Detected Cases Of Anoestrum.

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الملخص العربى

إحـــداث الولادة إصطناعيـــاً فى النعـــــاج

المشتركون فى البحث

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مع قدوم التقنيات الحديثة للتحكم فى تناسل الأغنام بات من الممكن تلقيح النعاج فى معظم أوقات العام، وقد أجريت دراستنا على ١٠٠ نعجة بصحة جيدة وتظهر التغيرت دورة الشبق الطبيعية، وقد تم حقنها بحقنتين فى العضل تفصلها أربع ساعات، من البروستاجلاندين إف ٢ ألفا فى اليوم العاشر من دورة الشبق حتى يتم تزامن الشبق فى وقت واحد، وقد تم ملاحظتها لظهور أعراض الشبق عليها وتم تلقيحها بواسطة كبش خصب، وقد أعتبر آخر يوم فى التلقيح هو اليوم صفر من الحمل، وقد تم إستبعاد النعاج التى لم تظهر عليها علامات الشبق من التجربة، وبعد ١٧-٢٣ يوماً من التلقيح تم إدخال كبش خصب مره أخرى (مرتين يومياً) فى كل مرة لمدة ساعة على الأقل للكشف عن النعاج التى من الممكن أن تأتى فى الشبق مرة أخرى حتى يتم تلقيحها.

وقد تم تشخيص الحمل باستخدام جهاز فحص الموجات فوق الضوئية حيث تم فحص النعاج بالموجات فوق الصوتية إسبوعياً إبتداءاً من الإسبوع الرابع وحتى الإسبوع الثامن من التلقيح، وقد تم تشخيص الحمل فى ٨٠ نعجة فقط وهى بالتالى التى أستخدمت فعلياً فى هذه الدراسة وتم تتبعها.

وفى اليوم ١٤٠ من الحمل، تم تقسيم النعاج العشار لأربع مجموعات كل منها يتكون من ٢٠ نعجة وتم حقن المجموعة الأولى عضلياً بـ ١٥ مجم من البروستاجلاندين إف ٢ ألفا، والمجموعة الثانية بـ١٦ مجم من الديكساميثازون، والمجموعة الثالثة بـ ١٦ مجم ديكسا ميثازون بالإضافة إلى ١٥مجم من البروستاجلاندين إف، والمجموعة الرابعة بـ ٤ مللى من ٩ر.٪ محلول ملح معقم، وقد كانت هناك فروق معنوية فى متوسط الزمن من العلاج حتى حدوث الولادة.

ولم تلد نعاج خلال ٧٢ ساعة من الحقن سواءاً فى المجموعة الحاكمة أو المجموعة المحقونة بالبروستاجلاندين إف ٢ ألفا، بينما ولدت ١٣ نعجة (٦٥٪) خلال ٤٨ ساعة من حقن الديكساميثازون مقابل ٥ نعاج فقط (٢٥٪) ولدت فى المجموعة المحقونة ديكساميثازون وبروستاجلاندين إف ٢ ألفا معاً. كما أن كل النعاج قد ولدت خلال ٧٢ ساعة من حقن الديكساميثازون لوحده أو الديكساميثازون مع البروستاجلاندين إف٢ ألفا معاً.

ولم يكن هناك أى فروق معنوية بين الأربع مجموعات فى زمن نزول المشيمة بالساعات، وقد تم ملاحظة إحتباس مشيمى فى حالات قليلة موزعة على كل المجموعات فيما عدا مجموعة الديكساميثازون، ولم تسجل حالات عسر ولادة فى كل المجموعات، كما أن كل الحملان قد ولدت حية فيما عدا أربع حملان، ولم يكن هناك فروق تذكر فى عدد الحملان المولودة فى الأربع مجموعات.

ومن الممكن إذن إستنتاج أن حقن الديكساميثازون لوحده فى النعاج بالقرب من زمن الولادة الطبيعى يؤدى إلى ولادة حملان حية وسليمة صحياً وذلك خلال فترة زمنية صغيرة ومعقولة من حقن العقار بدون حدوث حالات عسر ولادة أو إحتباس مشيمى.