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DIAGNOSTIC LABORATORY TESTS TO VERIFY OVULATION OCCURRENCE WITH EVALUATION OF ACCURACY OF RECTAL PALPATION IN BUFFALOES

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SUMMARY

The present investigation was carried out in cycling and noncycling buffaloes. Rectal palpation, plasma progesterone, testosterone concentration and plasma plasminogen activity were measured to predict and verify ovulation and to evaluate the accuracy of rectal palpation.

The results revealed that plasma testosterone concentration increased significantly during the luteal phase than during the follicular phase, its reliability to verify ovulation was 84.21% when compared whit measurement of progesterone, plasminogen activity did not differ during the follicular phase than during the luteal phase, its reliability to detect ovulation was 0%. the accuracy of rectal palpation in verification of ovulation occurrence was 76.02% while its overall accuracy in diagnosis of ovarian function was 77.38%. Misdiagnosis of follicles as corpora lutea was 23.98%, while, misdiagnosis of corpora lutea as follicles was 25%. The percentage of embeded corpora lutea was 19.35%.

Thus, we can conclude that measurement of plasma progesterone is still the most reliable method to verify ovulation and to diagnose accurately the ovarian function.

Keywords: Verify, ovulation, palpation, accuracy

INTRODUCTION

Repeat breeding is one of the important factors causing reproductive failure in buffaloes. Shrivastava and Kharche (1986) pointed out that the incidence of repeat breeding in buffaloes was 16.35%. This problem have many causes, of which are; anovulation, improper time of insemination, failure of conception, inadequate corpus luteum function and inaccurate diagnosis of ovarian function.

Until recently, and for economic reason, rectal palpation of the ovaries is used to check ovarian function and indicates whether or not buffaloes respond to treatment. El-Wishey et al.(1971) noticed that 40% of cyclic buffaloes have embeded corpora lutea in their ovaries, while, Sharifuddin and Jainudeen (1983) reported that 29% of the follicles were misdiagnosed as corpora lutea by rectal palpation. In addition, Kamonpatana et al. (1989) recorded that palpation is subjective and even experienced individuals are not always accurate where the accuracy reached to 33.77%, while Ghoniem et al. (1992) pointed out that the precision of rectal palpation for identifying suboestrus and anoestrus in buffaloes was 60% and 62.5%.

It was found that accurate assessing of ovarian status of animals is by determination of plasma progesterone which is more accurate than milk progestérone (Hussein et al.,1992). Kamonpatana et al. (1989) recorded that progesterone level blow 0.3 ng/ml indicated presence of follicle (F) and above 0.6 ng/ml indicated presence corpus luteum (C.L). On the other hand, Khattab et al. (1990) reported that in 35.3% of ovulations, the formation of C.L. was not followed by an increase in blood progesterone. Moreover, Singh and Madan (1987) reported that mean plasma testosterone concentration was lowest at oestrus, and remain low up to day 5 after oestrus but it was highly increased on day 8 and 9.

Enzymatic degradation of the follicular wall is probably the best hypothesis explaining follicular rupture. The plasminogen activator-plasminogen hypothesis is the most recent and most likely explanation of the mechanism initiating the cascade that leads to follicular rupture. Politis et al. (1990) reported that plasmin activity in follicular fluid was increased just before the expected time of ovulation in

sow. Smokovitis et al. (1988) pointed out that follicular fluid showed increasing plasminogen activator activity with the growth of the follicles in sow.

The objective of the present investigation was to develop and evaluate the different measures used to predict and verify ovulation occurrence which may be helpful in spreading artificial insemination and reducing the breeding interval in buffaloes.

MATERIALS AND METHODS

The present study was performed on buffaloes in governmental farms, private sector farms and with smallholder farmers. Before any interference, complete reproductive case history was taken. Animals were rectally palpated for examination of the ovaries and reproductive tract. Six individuals carried out rectal palpation throughout the experiment. Blood samples were collected from the jugular vein into plain vacutainers in the follicular phase and 7-10 days after oestrus part of the sample was taken where heparin (5 i.u/ml) was added to get plasma for determination of progesterone and testosterone conc., while sodium citrate (3.8%) was added to the other part at a rate of (1:9) for determination of plasminogen activity plasma was separated and stored at -20°C in a deep freeze.

Hormonal assays

Plasma progesterone and testosterone were assayed in duplicate by using radioimmunoassay kits purchased from DPC (Diagnostic Products corporation, los Angeles,USA) and based on methods adopted by (Kubasik et al., 1984) for progesterone. The antiserum used was highly specific for progesterone (100%), with a particularly low cross reactivity (ND-2.4%) to other naturally occurring steroids in plasma. The concentration of the slandard used ranged between (0-40 ng/ml). Testosterone was measured according to the method adopted by. Jaffe and Beherman (1974). The antiserum used was highly specific for testosterone, percentage of cross reactivity to other steroids in plasma ranged between 0-22%. The concentration of the standard used ranged between (0-1600 ng/dl).

The plasminogen activity was measured chromogenically by using Berichrom plasminogen reagents obtained from

(Behringwerke, AG, Germany) according to the method of Soria et al. (1978).

Statistical analysis

All data were subjected to statistical analysis according to procedures reported by Sendecor and Cochran (1967). T-test was performed to evaluate the difference between groups at the 5% level of probability.

RESULTS AND DISCUSSION

Diagnostic laboratory tests are important to make correct assessment of ovarian function. The present investigation was designed to develop and evaluate the validity and accuracy of diagnostic methods of ovarian function.

Results of the present study (Table 1) indicated that the mean plasma progesterone concentration during the follicular phase (before ovulation) was 0.25 ng/ml but it increased significantly up to 3.96 ng/ml in the presence of functional corpus luteum (7-10 days after oestrus occurrence which is in agreement with (Kanai and Shimizu, 1984, Barkawi et al., 1986 and Vale et al., 1990) but not with khattab et al. (1990) who reported that in 35.3% of ovulation in the Egyptian buffaloes, the formation of corpus luteum was not followed by an increase in blood progesterone concentration.

Table 1. Reliability of some trials to predict and verify ovulation occurrence in buffaloes

Assessments	Follicular Phase	Luteal phase	Reliability % Compared to progesterone
Plasma Progesterone (ng/ml)	0.25 ± 0.04^{a} (n=40)	3.96 ± 0.36^{a} (n=40)	100%
Plasma Testosterone (ng/dl)	6.07 ± 0.41^{b} (n= 40)	9.41 ± 0.71^{b} (n=40)	84.21%
Plsminogen activity %	16.22±0.90 (n=40)	16.21±0.74 (n=40)	0%
Rectal palpation			76.02%

Values having the same letter in the same raw are significantly different from each other at $P<0.05$.

Regarding plasma testosterone levels during follicular (before ovulation) and luteal (7-10) days after oestrus, it was found that plasma testosterone increased significantly during the luteal phase than that during the follicular phase which is in agreement with Sighm and Madan (1987). Testosterone assessment may be helpful to predict ovulation but its accuracy is only 84.21% when compared with progesterone.

According to the multifactor hypothesis of ovulation (Lipner, 1988), plasminogen activator secreted by the granulosa cells converts plaminogen present in the follicular fluid into plasmin which in turn, converts latent collagenase on the follicular wall collagen into collagenase. Collagenase convert collagen into telopeptide-free collagen which converted by protease into degraded collagen accompanied by follicular wall rupture and ovulation. An increase in the activity of plasminoyen activator (Smokovitis et al., 1988) and plasminogen (Politis et al., 1990) in the follicular fluid occurred with the growth of the follicles. These dynamic changes was not reflected on the plasminogen activity in plasma (Table 1) during the follicular and luteal phases which makes this test unreliable to verify ovulation.

The overall accuracy of rectal palpation as diagnostic method for assessment of ovarian function was 77.38% when compared with plasma progesterone (Table 2). kamonpatana (1989) reported that the percentage of correct rectal palpation of stages of C.L and follicle development was not higher than 33.77 % and that the palpator's ability to classify the actual C.L or follicle is uncertain. In the present investigation, 23.98% of the palpated corpra lutea were actually follicles but misdiagnosed as corpora lutea which seems to be lower than that previously reported by sharifuddin and Jainudeen (1983). In addition, 25% of corpora lutea were misdiagnosed as follicles (Table 2). The percent of embeded corpora lutea was 19.35% which seems to be about half the value previously pointed out by El-Wishey (1971). Thus, rectal palpation only is not absolutely reliable method, but due to economic causes it still widespread mean for diagnosis of ovarian activity. Thus, we can conclude that measurement of plasma progesterone is still the most reliable method to verifly ovulation occurrence and to diagnose ovarian activity.

Table 2. Accuracy of rectal palpation as a diagnostic method for ovarian function

Diagnostic method	Ovarian Structures						Overal accuracy
A) Rectal palpation	C.L 171		F 72		NSS 124		
	Correct diagno-sis	Misdiagnosis of F as C.L	Correct diagno-sis	Misdiagnosis of C.L as F	Correct diagno-sis	Embeded C.L	
B) After progesterone assay % of correct & misdiagno-sis	130 76.02%	41 23.98%	54 75%	18 25%	100 80.65%	24 19.35%	77.38%

NSS = Non specific structures (anoestrus)
F = Follicle
C.L = Corpus luteum
* Verified by smooth ovary (NSS) on palpation accompained with progesterone conc.< 1.0 ng/ml.

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إختبارات تشخصية معملية للتحقق من حدوث التبويض مع تقييم الدقة الجس فى الجاموس

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١- قسم الفسيولوجيا طب بيطرى القاهرة، ٢- معهد بحوث التناسليات بالهرم، ٣- الخدمات البيطرية بالقوات المسلحة

أجريت هذه الدراسة على الجاموس فى مختلف الحالات التناسلية وقد تم جس هذه الحيوانات لفحص المبايض والقناة التناسلية وبعد الجس تم سحب عينات من الدم لقياس تركيزات البروجستيرون والتستوستيرون ونشاط انزيم البلازمينوجين لنفس الحيوان فى مختلف المراحل وقد اظهرت النتائج ما يلى:

١- انه بمقارنة تركيزات هرمون البروجستيرون والتستوستيرون فى حالة وجود حويصلة جراف فى المبيض (المرحلة الحويصلية) بمستوياتها فى حالة وجود جسم أصفر عمره ٧ - ١٠ ايام (المرحلة اللوتينيئية) تبين ان مستوى البروجستيرون يزداد معنويا فى المرحلة اللوتينئية عن مستواه فى المرحلة الحويصلية فى جميع الحيوانات اما مستوى هرمون التستوستيرون فقد زاد فى المرحلة اللوتينيئة فى ٨٤,٢١٪ فقط من الحيوانات

٢- تبين ان النسبة المئوية لنشاط انزيم البلازمنيوجين لم يتغير معنويا فى المرحلة اللوتينئية عن المرحلة الحويصلية.

٣- وجد من دقة الجس فى تشخيص الجسم الأصفر فى المبيض كانت ٧٦,٠٢٪ وفى حالة وجود حويصلة جراف ٧٥٪ اما دقة تشخيص المبيض الخامل فقد كانت ٨٣,٠٦٪ والنسبة المئوية للجسم الاصفر المدفون كانت ١٩,٣٥ ٪ فى الجاموس اما النسبة العامة لدقة الجس فقد كانت ٧٨,٢٠٪.

مما سبق يتضح ان البروجستيرون يبقى كأهم مؤشر للتحقق من حدوث التبويض وتشخيص الحالة التناسلية للحيوان . اما نسبة الاعتماد على قياس هرمون التستوسيترون للتحقيق من الجس فقد كانت ٨٤,٢١٪ والبلازمينوجين صفر٪ والجس ٧٦,٠٢٪.