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EFFECT OF DIFFERENT HORMONAL TREATMENTS AS THERAPEUTIC PROCEDURE ON REPEAT BREEDER BUFFALO HEIFERS

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SUMMARY

Total of 30 Egyptian buffalo heifers (20-25 months and 340-360 kg) failed to conceive after 3 services (repeat breeder heifers) were exposed to three hormonal protocols after exhibiting the 4th estrous cycle. In the 1st protocol, heifers (n=10) were injected with GnRH on day of estrus and service. In the 2nd protocol, heifers (n=10) were injected with GnRH on day of estrus (0 day), followed by $PGF_2\alpha$ on day 7. Heifers displaying estrus within 48 hr of $PGF_2\alpha$ injection were served, while those did not show estrus within 48 h were injected with a second dose of GnRH on day 9 and served on day 10. In the 3rd protocol, heifers (n=10) were injected with 1st $PGF_2\alpha$ injection starting on day 6 after exhibiting the 4th estrous cycle. Animals exhibiting estrus within 72 hr after 1st $PGF_2\alpha$ injection were served, while those did not exhibit estrus were re-injected after 11 days of the 1st injection with 2nd $PGF_2\alpha$ and served 24-48 hr later. Pregnancy was diagnosed by ultrascan after 35.d of service. At service, diameter of the largest follicles was recorded for the 1st and 2nd protocols, while diameter of corpus luteum (CL) was recorded for the 3rd protocol using ultrascan. Blood samples were collected pre- and post- hormonal injections of each protocol for determination of progesterone (P4) concentrations in blood serum of all animals. Results showed that conception rate (CR) in the 1st protocol was 80% and mean diameter of the largest follicle was 13.25 and 9.10 mm in conceived and non-conceived heifers, respectively. Concentration of P4 was lower pre-treatment and at service in conceived heifers (3.063 and 0.182 ng/ml) than in non-conceived heifers (5.828 and 0.296 ng/ml), while, an opposite trend was observed post-treatment (5.802 and 3.541 ng/ml), respectively. In the 2nd protocol, after $PGF_2\alpha$ injection, estrus rate was 50% (within 48 h) and CR was 80% based on inseminated animals. Mean diameter of the largest follicle was 8.3 and 7.0 mm in conceived and non-conceived heifers, respectively. After the 2nd GnRH and blind service, CR was 60% and mean diameter of largest follicle was 8.9 and 4.7 mm in conceived and non-conceived heifers, respectively. The final CR after GnRH-$PGF_2\alpha$-GnRH was 70%. The 1st GnRH injection increased P4 concentration in all treated heifers, while $PGF_2\alpha$ injection reduced P4 level to 0.433 and 1.420 ng/ml in conceived and non-conceived heifers, respectively. In the 3rd protocol, estrus rate was 50% within 72 h after the 1st $PGF_2\alpha$ injection and CR was 80%. Mean diameter of CL at service was 11.3 mm. All heifers (100%) injected with 2nd $PGF_2\alpha$ exhibited estrus (within 48-72 h), served and conceived. Mean CL diameter was 10.6 mm. For the whole 3rd protocol (after both $PGF_2\alpha$ injections), CR was 90% and mean diameter of CL was 10.9 mm. The 3rd protocol had the cheapest cost and the highest conception, followed by the 1st protocol, while the 2nd protocol showed the highest cost.

Keywords: Buffalo heifers, repeat breeder, hormonal treatment.

INTRODUCTION

Reproductive efficiency is the core of dairy farms profitability (Nebel and Jobst, 1998). Maximum economic return could be achieved when females conceive early with fewer services (less than three inseminations). Females those don't get pregnant by the third service are referred as 'repeat-breeders'. Repeat breeding, which occurs in 10–25% for cows, is one of the major gynecological problems affecting reproductive efficiency and profitability of dairy farms (Bartlett *et al.*, 1986).

Follicular dynamics and pituitary-ovarian axis are the main reasons for repeat-breeding phenomenon (Båge *et al.*, 1997). Within the six days post estrus, repeat breeders have lower progesterone concentrations relative to normal ones (Båge, 2002). To overcome this inconvenient phenomenon in dairy farms GnRH and prostaglandin F2α (PGF2α) have been used in different combinations to improve the reproductive efficiency in lactating dairy cows (Yaniz *et al.*, 2004) and Egyptian buffaloes (Hegazy, 2001).

Therefore, the aim of the current study is to test the more efficient hormonal protocols to improve reproductive performance of repeated breeder buffalo heifers.

MATERIALS AND METHODS

This study was carried out at Animal Production Experimental Station, Mehallet Moussa, Kafer El-sheikh Govemorate (located in the north of the Nile Delta), during the period from May to September 2008.

Animals:

A total of 30 Egyptian buffalo heifers those failed to conceive after 3 services (repeat breeders) was used in this study. Heifers had 20-25 month old and body weight of 340-360 kg. Animals were fed on concentrate feed mixture, maize silage, rice straw, and berseem (*Trifolium alexandrinum)* hay according to the systems adopted by Animal Production Research Institute (APRI). Fresh water was made available all times, since heifers were housed loose in semi-open sheds.

Treatment protocols:

In all treatments, heifers exhibiting the 4th estrous cycle (failed to conceive after 3 services) were exposed to therapeutic treatments.

Heifers in the 1st protocol (n=10) were treated with intramuscularly injected at the time of service with a single dose of 5 ml GnRH analogue (Receptal, Hoechst, Germany) containing 20 µg Buserelin GnRH.

In the 2nd protocol, heifers (n=10) were i.m. injected with 5 ml Receptal at day of estrus (0 day), followed by i.m. injection on day 7 with 3 ml $PGF_2\alpha$ analogue (Synchromate, Bremer Pharma 27540 Bremerhaven, Germany) containing 0.750 µg cloprostenol. Heifers those did not display estrus signs within 48 hr after $PGF_2\alpha$ were i.m. injected with a second dose of 5 ml Receptal (day 9), followed by a natural mating 24 hr later (day 10).

In the 3rd protocol, heifers were injected with i.m dose of 3 ml $PGF_2\alpha$ analogue. Animals those exhibit heat symptoms within 72 hours after treatment were served naturally. Animals which did not exhibit estrous activity were re-treated after 11 days with a second dose of 3 ml Synchromate and naturally mated 24-48 h after the 2nd dose.

Estrous detection, service and pregnancy diagnosis:

Estrus was detected within 3 days post GnRH injection (1st protocol), post-$PGF_2\alpha$ injection (2nd protocol) and post 1st and 2nd $PGF_2\alpha$ injections (3rd protocol) using a teaser buffalo bull for four times/day at 8 and 12 a.m. as well as 4 and 8 p.m. Heifers detected in heat were naturally inseminated with fertile buffalo bull.

Pregnancy diagnosis was carried out on day 35 post-insemination for animals non-returned to estrus after 24 days using ultrascan (Falco, Easote/Piemedical, Maastricht, 6-8 MH2 Linear array transducer, Alliance Medical Int.) and confirmed by rectal palpation on day 60 post-insemination. Also, ultrascan was used for estimation of the largest follicles following the insemination (1st and 2nd protocols) and for estimation of corpus luteum (CL) diameter on day of 1st and 2nd-$PGF_2\alpha$ in the 3rd protocol.

Blood sampling:

At 8 a.m. before feeding, blood samples were collected from the jugular vein for progesterone (P4) determination. Blood samples were centrifuged for 15 minutes at 3000 r.p.m. for serum separation, which stored at -20 °C till the P4 assay. Blood samples were collected from all animals in each protocol as follows: The 1st protocol: 48 h pre-GnRH, service and 48 h post-GnRH. The 2nd protocol: 24 hr pre- and post 1st GnRH, 48 hr post-$PGF_2\alpha$ and 24 h post-insemination. The 3rd protocol: 24 hr pre- and 48 hr post- 1st $PGF_2\alpha$, as well as 24 hr pre- and 48 hr post-2nd $PGF_2\alpha$. In all protocols, blood samples were taken on day 24 post-insemination.

Progesterone (P4) assay:

Direct radioimmunoassay technique (RIA) was performed for determination of P4 concentrations in blood serum samples using ready antibody coated tubes kit (Diagnosis Systems Laboratories, Texas, USA) according to the procedure outlined by the manufacturer.

According to the manufacture's information, the cross reaction of progesterone antibody (at 50% binding), was 100% with P4, while was 6.00, 2.50, 1.20, 0.80, 0.48, and 0.10% with 5α-pregnone-3, 20-dione 11-Deoxycorticosterone, 17α-Hydroxyprogesterone, 5β-pregnone-3, 20-dione 11-Deoxycortisol, and 20α-Dihydroxyprogesterone, respectively and less than 0.1% with any of the other steroids.

The standard curve of P4 ranged from 0.0 to 60.0 ng /ml. The sensitivity value was reported to be 0.12 ng /ml. The intra and inter-assay coefficients of variation were 8.0% and 13.1.

Economic evaluation:

Economic evaluation of each hormonal protocol was calculated as total cost based on price and number of injections for each treated animal, and then for 10 animals.

Statistical Analysis:

Results were statistically analyzed according to Snedecor and Cocharn (1982). Analysis of variance using one way design was used to compare P4 concentrations between conceived and non-conceived heifers. While, conception and estrus rate was tested using Chi-square analysis.

RESULTS AND DISCUSSION

1st Protocol: "GnRH at estrus"

Results (Tabll 1 and figure 1) of this treatment revealed that conception rate (CR) of treated heifers was 80% (8/10). Mean diameter

of the largest follicles was wider in conceived heifers than in non-conceived heifers (13.25 *vs.* 9.10 mm, Plate 1 A and B). Pregnancy in conceived heifers was proved by ultrasound examination (Plate 1 C) on day 35 post-insemination and confirmed on day 60 post-insemination by rectal palpation.

Similarly, Kaim *et al.* (2003) found that GnRH injection at estrus increased CR of cows from 41.3 to 55.5% across seasons compared to control. They reported that if GnRH treatment was given at estrus, failure or delay of ovulation might be prevented and CR might increase. Also, Nakao *et al.* (1984) mentioned that administering GnRH at estrus might prevent ovulation failure or reduce the variation in the interval to ovulation. These findings could be related to the fact that repeat breeder heifers have a smaller preovulatory LH surge than virgin heifers, therefore, an increase in the spontaneous surge that results from the administrating GnRH at estrus affects the CR favorably (Kaim *et al.,* 2003). Also, in most other studies, GnRH administered at artificial insemination may induce a second LH peak and did not increase the spontaneous one (Ryan *et al.,* 1994).

On the other hand, Stevenson, *et al.* (1988) suggested that GnRH-induced ovulation may provide greater synchrony between the time of insemination and the time of ovulation. In this respect, Stevenson *et al.* (1984) found that conception rates increased when cows were treated in the first 5 to 8 hours of estrus. Furthermore, Peters (2005) indicated the use of GnRH as a 'holding' injection on the day of insemination led to improve the chances of successful pregnancy, particularly in repeat breeder cows.

The present study indicated that pregnancy of treated animals was associated with wider diameter of the largest follicle. Ovulation of large follicle yields higher CR (Mussard *et al.,* 2003). In this respect, Perry *et al.* (2005) reported induced ovulation of large follicles (11.3 mm in diameter). Also, Perry *et al.* (2007) found that heifers having ovulated follicles with diameter of 10.7-15.7 mm at time of artificial insemination were more likely to become pregnant than that ovulated a follicle with a more diameter.

In comparing P4 profile during the fourth estrous cycle pre-treatment and post-GnRH treatment in treated animals, it was found that P4 concentration was higher in non-conceived heifers than in conceived heifers at different sampling days of estrous cycle. However, the opposite situation was observed during post-treatment period (24 days post-service), which can indicate the incidence of pregnancy (Figure 1 and Table 1)..

As illustrated in Figure 1, conceived and non-conceived repeat breeder heifers exhibited progressive increases in P4 concentrations from 0 day to 8 days of the cycle, being lower in conceived than non-conceived heifers. The conceived animals had serum P4 concentrations of about 6 to 9.5 ng/ml during luteal phase, which were comparable to luteal concentrations reported by Foster *et al.* (1997) from 11-18 day of estrous cycle. The sharp reduction in P4 concentration in non-return/non-conceived heifers post-treatment may be related to failure in embryo implantation or early embryonic mortality. In this respect, Moore *et al.* (2005) observed lower concentrations of serum P4 coincident with embryo loss between day 24 and 28.

The results in Table (1) illustrated that P4 concentration pre-treatment increased in non-conceived compared with conceived heifers, being in an opposite trend at service. The observed increased in P4 level of repeat breeding animals was reported and the origin of the excessive P4 during estrus in repeat breeder heifers is still unknown, but it could be due to either an incomplete CL luteolysis or a release from sources other than the ovary (Waldmann, 2001).

In treated repeat breeder heifers (non-conceived), marginally elevated plasma P4 level at estrus, so-called supra-basal P4 levels, has earlier been measured and is believed to impair fertility.

This P4 could be of extra-gonadal origin (Båge *et al.,* 2000). It is possible that the supra-basal P4 concentrations present in non-conceived repeat breeder heifers during estrus reduced tubal contractility, resulting in an impaired or delayed sperm transport from the sperm reservoir to the site of fertilization (Singh *et al.,* 2005).

Concentration of P4 post-treatment was higher in conceived than in non-conceived animals, being more than 1 ng/ml. Such finding may be attributed to presence of CL, regardless conception of animals. Mee *et al.* (1993) mentioned that GnRH induced increase in blood P4 levels in repeat breeder cows, which might be one of the causes of the increased CR in these cows.

Concentration of P4 on day 24 post-insemination was 5.855 ng/ml in conceived versus less than 0.5 ng/ml in non-conceived heifers (0.131 ng/ml). Similarity, El-Moghazy (2003) mentioned that P4 concentration was almost higher than 1 ng/ml in pregnant and less than 1 ng/ml in those failed to conceive.

The present results indicated benefits of GnRH treatment at the time of insemination to synchronize the onset of estrus and ovulation

with insemination and consequently incidence of pregnancy of most repeat breeder heifers.

2nd Protocol: "GnRH-PGF2α-GnRH":

Results in Table (2) revealed that 50% of treated heifers (5/10) exhibited estrous activity within 48 hr post-$PGF_2\alpha$ injection (day 7). Conception rate (CR) was 80% (4/5) of inseminated animals. Mean diameter of the largest follicles after $PGF_2\alpha$ injection was 8.3 and 7 mm in conceived and non-conceived heifers, respectively.

In comparable with the obtained results, El-Moghazy (2003) found that the percentage of cyclic buffalo cows that responded to the GnRH-$PGF_2\alpha$ injection was 48.3%. However, Hegazy (2001) found that the percentage of animals responding to 1st GnRH injection was lower in cyclic animals (13%). The variation in incidence of estrous activity in cyclic buffaloes following the 1st GnRH-$PGF_2\alpha$ injection may be related to the luteal phase of treated animals (El-Moghazy, 2003) or incidence of silent ovulation in some animals (Hegazy, 2001).

After the 2nd GnRH (day 9) and blind insemination (day 10) as shown in Table (2), CR was 60% (3/5) treated/inseminated animals.

In this respect, Martel (2008) reported that the 2nd GnRH injection subsequently induced ovulation in 87% of the cows. The timing insemination applied in this study was according to the results of Sterry *et al.* (2007), who found that CR increased when the 2nd GnRH injection was 72h post-$PGF_2\alpha$ compared with 48 hr post- $PGF_2\alpha$ injection. Data in Table (2) revealed that mean diameter of the largest follicle after 2nd GnRH injection was larger in conceived than in non-conceived heifers (8.9 *vs.* 4.7 mm). Perry *et al.* (2005) found decreased conception rates in female beef cattle induced to ovulate follicles of a smaller diameter within a CO-Synch program.

The final CR was 70% after GnRH-$PGF_2\alpha$-GnRH protocol (7/10). Mean diameter of the largest follicles was significantly ($P<0.05$) lower in non-conceived than in conceived heifers (Table 2). Improving CR of repeat breeder heifers treated with this protocol was mainly related to that administration of GnRH leads to an LH surge during any stage of the estrous cycle, which will promote the ovulation of a dominant follicle or induce luteinization and/or atresia of pre-dominant follicles (Guilbault *et al.,* 1990). Also, increasing CR of this protocol requires the presence of a dominant follicle at the time of the 1st GnRH injection (De Rensis *et al.,* 2005). In buffaloes, the Ovsynch protocol has been observed to synchronize ovulation, ranging between 78% (Baruselli, 2001) and 90% (Paul and Prakash 2005) of animals, with conception rates, averaging 60% (Baruselli, 2001) or ranging between 33 and 50% (Paul and Prakash, 2005). The main synchronizing effect seems to reside in the 2nd GnRH injection, whereas the importance of the 1st GnRH is in prolonging the luteal phase in animals treated late in the cycle (Peters, 2005). Finally, a 2nd GnRH injection is administered 48 hours after $PGF_2\alpha$ to induce a preovulatory LH surge that triggers ovulation within an 8 hr period, beginning approximately 24 hr after the injection (Pursley *et al.,* 1995).

Induced ovulation of heifers following the GnRH-$PGF_2\alpha$-GnRH protocol would be anticipated approximately 28 hr after the 2nd GnRH injection, whereas spontaneous ovulation of heifers exhibiting estrus would be anticipated approximately 31 hr after the onset of estrus or approximately 19 hr after insemination (Pursley *et al.,* 1995). In this respect, Doležel *et al.* (2002) hypothesized that GnRH administration would be performed in all experimental cows by the end of the growing phase or at the beginning of the static phase of the dominant follicle when GnRH would be administered 72, 48 and 24 hr after $PGF_2\alpha$ in cows bearing small, medium and large follicles at the time of initial treatment.

Concentration of P4 pre-treatment tended to be slightly lower in conceived than non-conceived heifers. However, post 1st GnRH injection, P4 concentration increased in conceived and non-conceived heifers indicating presence of functional CL after the ovulation induced by 1st GnRH. Post- $PGF_2\alpha$ injection, P4 concentration sharply reduced to 0.433 ng/ml in conceived animals, being significantly ($P<0.05$) lower than 1.420 ng/ml in non-conceived heifers (Table 3 and Figure 2).

Such reduction may indicate a complete regression of the functional CL in all conceived heifers and some of non-conceived heifers. Based on serum progesterone, complete luteal regression after $PGF_2\alpha$ and higher ovulatory responses to GnRH contributed to higher conception to timed artificial insemination in Ovsynch protocol (Cordoba and Fricke, 2002). Post-2nd GnRH injection, P4 concentration increased in all treated heifers, being significantly ($P<0.05$) higher in conceived than in non-conceived heifers (5.214 *vs.* 3.645 ng/ml).

The 2nd GnRH injection induced growing of new dominant follicle, which ovulated within 24 hr and subsequent increased P4 concentrations by an average of 24 hr relative to animals just receiving a GnRH-$PGF_2\alpha$ regimen (Peters *et al.,* 1999).

Results in Table (3) also indicated that P4 concentration was significantly ($P<0.05$) higher in conceived than in non-conceived heifers on day 24 post-insemination (7.694 *vs.* 0.146 ng/ml).

3rd protocol ($PGF_2\alpha$-$PGF_2\alpha$):

Results in Table (4) show that estrus/insemination rate of heifers within 48-72 hr Post-1st $PGF_2\alpha$ injection was 50% (5/10) and CR was 80% (4/5). Ultrasound examination on day of 1st $PGF_2\alpha$ injection showed that mean diameter of CL was 11.3 mm.

Post-2nd $PGF_2\alpha$ injection of other non-responded animals (n=5), all heifers (100%) exhibited estrous activity within 48-72 hr and conceived after insemination, being higher ($P<0.05$) than those post-1st $PGF_2\alpha$ injection. Mean diameter of CL on day of the 2nd $PGF_2\alpha$ injection was 10.6 mm, being insignificantly lower than that post- 1st $PGF_2\alpha$ injection (Table 4). The higher response to estrus post-2nd $PGF_2\alpha$ (100%) may indicate that at the 2nd $PGF_2\alpha$ injection 11 days later, the initially responsive group should be on days 7 to 9 (early cycle) of a new cycle, and the remainder would be at a broader range of a new estrous cycle (days 6 to 15), but still responsive to the luteolysin. Therefore, the majority of animals should be in early stages of the estrous cycle at the 2nd $PGF_2\alpha$ injection (Hardin *et al.,* 1980).

In this study, estrus incidence was within 48-72 hr post-1st or 2nd $PGF_2\alpha$ and follicular diameter ranged between 10.6 and 11.6 mm on day of both injections (Table 4). The time interval between $PGF_2\alpha$ treatment and the onset of estrus in buffalo varies according to the stage of estrous cycle at the time of $PGF_2\alpha$ administration. De Rensis and Pez-Gatius (2007) observed that animals treated when follicles are in the pre-dominance stage of development display estrus 4–6 days later, whereas animals treated in the presence of a dominant follicle display estrus 2-3 days after $PGF_2\alpha$ administration. Since the intervals between treatment, estrus, and ovulation vary after $PGF_2\alpha$ administration, a timed artificial insemination protocol cannot be applied. The present interval from $PGF_2\alpha$ treatment to estrus reported in this study is in line with other reports (Sahasrabudhe and Pandit 1997), while it was slightly shorter than 88 hr (Brito *et al.,* 2002). This interval was shorter when $PGF_2\alpha$ is given during the early luteal phase of the estrous cycle in the presence of a dominant follicle (Baruselli, 2001).

At the end of 3rd protocol, only one out of 10 heifers failed to conceive after 2nd $PGF_2\alpha$ injection and CR was 90%. In comparable the present results with other reports, Brito *et al.* (2002) found that a single or double-treatment regimens of $PGF_2\alpha$ in buffalo induced estrus and ovulation in 60–80% of animals. The obtained higher CR in this study post-1st or 2nd $PGF_2\alpha$ injections was attributed to an appropriate time of estrus incidence and consequently good time of insemination and fertilization. However, Répási *et al.* (2006) found that more animals became pregnant ($P>0.05$) if they were inseminated within 4 days after $PGF_2\alpha$ treatment in dairy cows.

Concerning P4 profile (Table 5) the results indicated that P4 concentration was above 1 ng/ml pre-treatment. This was associated with high diameter of CL of $PGF_2\alpha$-treated animals.

Meanwhile, post-1st $PGF_2\alpha$ injection, P4 level markedly decreased below 1 ng/ml in animals responded to estrus (conceived and non-conceived heifers) as a result of CL regression, initiation of new follicular wave and onset of estrus. In non-responded heifers (n=5), P4 concentration was 4.471 ng/ml, indicating no incidence of estrus or silent ovulation in these animals. On day 24 post-insemination, P4 concentration was 6.2 ng/ml in responded animals, indicating incidence of pregnancy. After the 2nd $PGF_2\alpha$ injection of non-responded animals to the 1st $PGF_2\alpha$ injection, P4 concentration pre- and post-injection as well as on day 24 post-service showed similar trend to those conceived after the 1st $PGF_2\alpha$ injection (Table 5).

In buffaloes, the effect of $PGF_2\alpha$ administration herein is very similar to that observed in cattle. The administration of $PGF_2\alpha$ from day 5 of the estrous cycle causes regression of CL. Thereafter, P4 declines rapidly to basal concentrations within 24 hr resulting in induction of estrus and ovulation (Chohan *et al.,* 1995).

Reproductive and economic evaluation of different protocols:

From the reproductive point of view, 24 out of 30 treated buffalo heifers (80%) were conceived using all hormonal protocols, being the highest in $PGF_2\alpha$-$PGF_2\alpha$ (90%), moderate (80%) in GnRH at insemination and the lowest (70%) in GnRH-$PGF_2\alpha$-GnRH protocol. Also, the economic evaluation, based on cost of hormal injections, indicated that $PGF_2\alpha$-$PGF_2\alpha$ had the cheapest cost, followed by GnRH at insemination, while GnRH- $PGF_2\alpha$ -GnRH protocol showed the highest cost. Generally, using $PGF_2\alpha$-$PGF_2\alpha$ protocol showed the lowest cost (1.8 L.E/treated animal) with the highest conception rate (90%, Table 6).

According to the foregoing results, using $PGF_2\alpha$-$PGF_2\alpha$ protocol as therapeutic treatment was mor efficient in improving reproductive erformance of repeat breeder

buffalo heifers with the lowest cost as compared to other hormonal protocols (GnRH at service or GnRH-$PGF_2\alpha$-GnRH) used in this study.

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Table 1. Profile of P4 (Mean±SE) in blood serum of conceived and non-conceived heifers treated with GnRH at insemination during treatment

P4 concentration (ng/ml):	Conceived (n=8)	Non-conceived (n=2)
Pre-treatment	3.063±0.97	5.828±1.12
At estrus	0.182±0.03	0.596±0.04
Post-treatment	5.802±0.54	3.541±0.81
24 days post-insemination	5.855±0.25	0.131±0.04

Table 2. Conception rate, estrus rate and diameter of largest follicle of conceived and non-conceived heifers in the 2nd protocol.

Post-GnRH + $PGF_2\alpha$ injections:	
Number of treated animals	10
Estrus/insemination rate, %	50 (5/10)
Conception rate, % (based on inseminated animals)	80 (4/5)
Diameter of largest follicle of conceived heifers, mm	8.3±1.52
Diameter of largest follicle of non-conceived heifers, mm	7.0±0.00
Post-2nd GnRH injection (timing insemination):	
Number of treated animals	5
Estrus/insemination rate	100 (5/5)
CR (%) (based on timing insemination)	60 (3/5)
Diameter of largest follicle of conceived heifers, mm	8.9±1.41
Diameter of largest follicle of non-conceived heifers, mm	4.7±0.80
The whole protocol:	
Number of treated animals	10
Total CR (%)	70 (7/10)
Diameter of largest follicle of conceived heifers, mm	8.60 ± 1.64^{a}
Diameter of largest follicle of non-conceived heifers, mm	5.85 ± 1.20^{b}

a and b: Means denoted within the same column with different superscripts are significantly different at P<0.05.

Table 3. Progesterone profile (ng/ml) pre-, during and post- GnRH- $PGF_2\alpha$-GnRH treatment.

Sampling time	Conceived		Non- conceived	
	n	P4 (ng/ml)	n	P4 (ng/ml)
Pre-treatment	7	3.014±0.25	3	3.588±0.41
Post 1st GnRH injection	7	4.682±0.74	3	5.209±0.54
Post- $PGF_2\alpha$ injection	7	0.433 ± 0.021^{b}	3	1.420 ± 0.032^{a}
Post 2nd GnRH injection	3	5.214 ± 0.82^{a}	2	3.645 ± 0.76^{b}
24 days post-service	7	7.694 ± 1.02^{a}	3	0.146 ± 0.042^{b}

Means denoted within the same row with different superscripts are significantly different at P<0.05. NS: Not significant. * Significant at P<0.05.

Table 4. Conception rate, estrus rate and follicular diameter of heifers treated with $PGF_2\alpha$-$PGF_2\alpha$ protocol

Pre-treatment:	
Number of treated animals	10
Diameter of corpus luteum, mm	11.3±1.04
Post- 1st $PGF_2\alpha$:	
Estrus/insemination rate, %	$50\ (5/10)^{b}$
Conception rate, % (inseminated based on heat)	$80\ (4/5)^{B}$
Pre-2nd $PGF_2\alpha$:	
Number of treated animals	5
Diameter of corpus luteum, mm	10.6±0.98
Post- 2nd $PGF_2\alpha$:	
Estrus/insemination rate, %	$100\ (5/5)^{a}$
Conception rate, % (inseminated based on heat)	$100\ (5/5)^{A}$
Total protocol:	
Number of treated animals	10
Diameter of corpus luteum, mm	10.9±0.99
Estrus/insemination rate, %	100 (10/10)
Conception rate, %	90 (9/10)

Means denoted within the same column with different superscripts are significantly different at P<0.05.

Table 5. Progesterone (Mean±SE) profile (ng/ml) pre-, during and post-$PGF_2\alpha$-$PGF_2\alpha$ treatment

Item	Responded animals				Non-responded*	
	Conceived		non-conceive			
	N	P4 (ng/ml)	N	P4 (ng/ml)	n	P4 (ng/ml)
Pre-treatment	4	4.469±0.257	1	3.928±0.0	5	3.143±0.584
1st $PGF_2\alpha$ injection:						
Post- injection**	4	0.081±0.011	1	0.798±0.0	5	4.471±0.425
Day 24 post-service	4	6.203±0.542	1	0.313±0.0		-
2nd $PGF_2\alpha$ injection:						
Pre injection	5	4.471±0.425	-	-	-	-
Post injection**	5	0.291±0.005	-	-	-	-
Day 24 post-service	5	7.619±1.02	-	-	-	-

* Non-responded to the 1st $PGF_2\alpha$ injection. ** On day of estrus incidence(at estrus).

Table 6. Reproductive evaluation and economic efficiency of different hormonal treatments

Item	Hormonal protocol			Total
	GnRH(1)	G-P-G(2)	P-P(3)	
Reproductive evaluation of treatment:				
Treated animals (n)	10	10	10	30
Conceived animals (n)	8	7	9	24
Non conceived (n)	2	3	1	6
Conception rate (%)	80	70	90	80
Economic efficiency of treatment:				
Treatment period (day)	0	10	11	-
Price of 1st injection (L.E.)	200	200	120	520
Price of 2nd injection (L.E.)	-	120	60	180
Price of 3rd injection (L.E.)	-	100	-	100
Total cost (L.E./group)	200	420	180	800

(1): GnRH at service (2): GnRH-$PGF_2\alpha$-GnRH (3): $PGF_2\alpha$-$PGF_2\alpha$

Price of each injection from GnRH and $PGF_2\alpha$ was 20 and 12 L.E, respectively.

Plate (1): Ultrasonography examination of heifer treated with GnRH at insemination showing: ovarian follicle with 10.04 mm in diameter in conceived animal (A) and ovarian follicle with 9.00 mm in diameter in non-conceived animal (B) at insemination as well as embry on day 35 post-insemination, indicating the incidence of pregnancy.

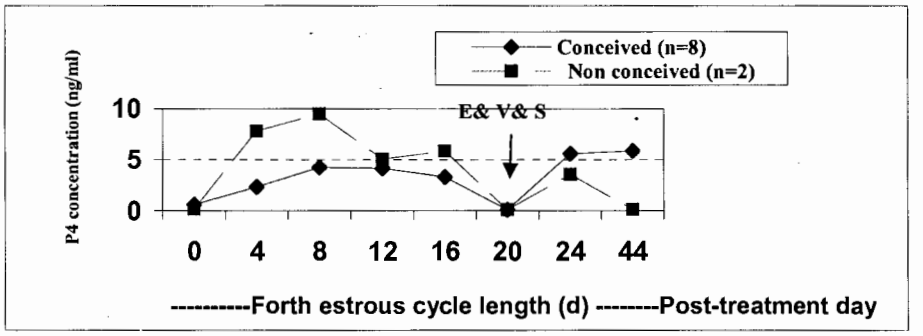

Fig. 1. Progesterone profile during the fourth estrous cycle (pre-treatment) and post- GnRH treatment in conceived and non-conceived heifers. (E: estrus, V: ovulation and S: service)

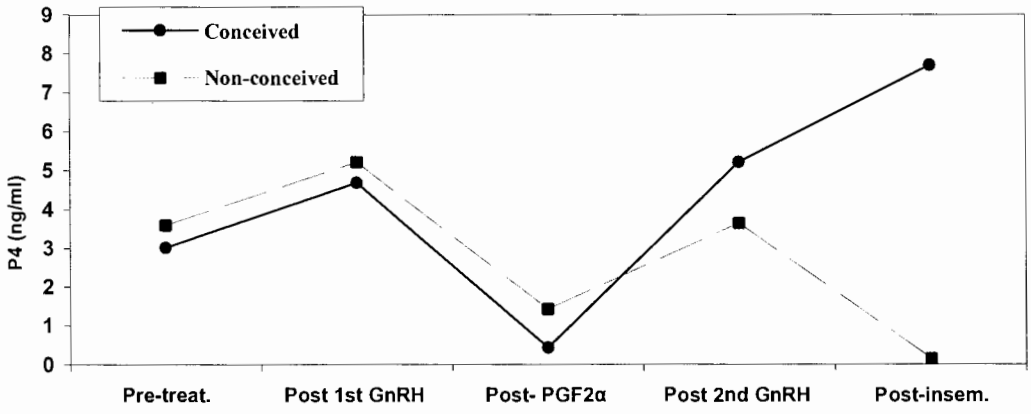

Fig. 2. Progesterone profile pre-, during and post- GnRH-$PGF_2\alpha$-GnRH treatment.

تأثير المعاملات الهرمونية كإجراء علاجي على عجلات الجاموس متكررة التلقيح

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استخدم في هذه الدراسة ٣٠ عجلة جاموس مصري (أعمارها من ٢٠-٢٥ شهر وأوزانها من ٣٤٠-٣٦٠كجم) فشلت فى الحمل بعد تلقيحها ٣ تلقيحات متتاليه (عجلات متكررة التلقيح) وذلك باستخدم ثلاثة بروتوكولات هرمونية بعد أظهارها دورة الشياع الرابعه. فى البروتوكول الأول تم حقن عجلات (١٠ عجلات) ب GnRH في يوم الشياع والتلقيح. فى البروتوكول الثاني تم حقن عجلات (١٠ عجلات) ب GnRH في اليوم الأول وب $PGF2\alpha$ بعد سبع أيام. تم تلقيح العجلات التى أظهرت شياع خلال ٤٨ ساعه من الحقن ب $PGF2\alpha$، بينما العجلات التى لم تظهر شياع كانت تحقن ب GnRH بعد تسعة أيام من بداية المعامله والتلقيح في اليوم العاشر بدون الأعتماد على حدوث شياع. بينما فى البروتوكول الثالث تم حقن عجلات (١٠ عجلات) بـ$PGF2\alpha$ وتم تلقيح العجلات التى أظهرت شياع خلال 48-24 ساعه من الحقن الأول، بينما العجلات التى لم تظهر شياع كانت تحقن الحقن الثانيه من بـ$PGF2\alpha$ فى اليوم الحادى عشر من المعامله وتلقح خلال ٢٤-٤٨ ساعه بدون الأعتماد على حدوث شياع. وكانت جميع التلقيحات طبيعيا باستخدام فحل مختبر وكان يتم تشخيص الحمل بواسطة جهاز السونار بعد ٣٥ يوم من التلقيح. تم الكشف عن السياع بالملاحظه الشخصيه. تم قياس قطر أكبر حويصلة أثناء التلقيح في البروتوكول الأول والثاني وكذلك قطر الجسم الأصفر عند التلقيح فى البروتوكول الثالث باستخدام السونار. تم جمع عينات الدم قبل وبعد الحقن بالهرمونات في كل البروتوكولات لتحديد تركيز هرمون البروجيستيرون في سيرم الدم لجميع الحيوانات.

أظهرت النتائج أن معدل الأخصاب في البروتوكول الأول كان ٨٠٪ ومتوسط قطر أكبر حويصلات كان ١٣,٢٥ و ٩,١٠ مم للعجلات المخصبه والتي لم تخصب، على التوالي. كان تركيز هرمون البروجيستيرون منخفض قبل المعاملة وعند التلقيح في العجلات المخصبه (3.063 و ٠,١٨٢ نانوجرام/مل، على الترتيب) عن تلك التى لم تخصب (5.828 و٠,٢٩٦ نانوجرام/مل، على الترتيب)، في حين لوحظ موقف معاكس لذلك في مرحلة ما بعد المعاملة (٥,٨٠٢ و ٣,٥٤١ نانوجرام/مل، على التوالي). في البروتوكول الثاني: بعد حقن $PGF2\alpha$، كان معدل الشياع ٥٠٪ (خلال ٤٨ ساعة) وكان معدل الأخصاب ٨٠٪ على أساس الحيوانات التي تم تلقيحها. وكان متوسط قطر أكبر الحويصلات ٨,٣ و ٧,٠ مم في العجلات المخصبه والتي لم تخصب، على التوالي. كان معدل الأخصاب النهائي بعد الحقن الثانى ب-GnRH والتلقيح ٦٠%. وكان متوسط قطر أكبر الحويصلات ٨,٩ و٤,٧ مم في العجلات المخصبه والتي لم تخصب، على الترتيب. كان معدل الأخصاب النهائى للبروتوكول الثانى هو ٧٠%. بعد أول حقن للـ GnRH زاد تركيز البروجيستيرون في جميع العجلات المعامله، في حين خفض حقن $PGF2\alpha$ من مستوى البروجيستيرون إلى ٠,٤٣٣ و ١,٤٢٠ نانوجرام/مل في العجلات المخصبه والتي لم تخصب ، على التوالي. في البروتوكول الثالث كان معدل ظهور الشياع ٥٠٪ خلال ٧٢ ساعة بعد الحقن الأول للـ $PGF2\alpha$ و كان معدل الأخصاب ٨٠٪ ومتوسط قطر الجسم الأصفر عند التلقيح ١١,٣ مم. أظهرت جميع العجلات المعمله شياع خلال ٤٨-٧٢ ساعة بعد الحقن الثاني للـ $PGF2\alpha$ وكان متوسط قطر الجسم الأصفر١٠,٦ مم. عموما، بعد الحقن الأول والثاني للـ $PGF2\alpha$ كان معدل الأخصاب ٩٠ % ومتوسط قطر الجسم الأصفر كان ١٠,٩ مم.

تشير هذه الدراسة إلى أن البروتوكول الثالث ($PGF2\alpha$-$PGF2\alpha$) أقل تكلفة ، تلاها البروتوكول الأول (GnRH عند التلقيح) ، في حين أن البروتوكول الثاني (GnRH-$PGF2\alpha$-GnRH) أظهر أعلى تكلفة.