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COMPARATIVE TREATMENTS OF ANESTROUS BALADY COWS USING $PGF_2\alpha$ AND/OR PMSG WITH SPECIAL REFERENCE TO SOME BIOCHEMICAL CHANGES AND CONSEQUENT FERTILITY

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ABSTRACT

A total number of 60 anestrous balady cows, divided into two main groups according to the ovarian findings (30 with persistent CL and 30 with smooth inactive ovaries)., Each main group was divided into 3 subgroups according to the hormonal treatment used. The first one, injected with 25 mg $PGF_2\alpha$ (Lutalyse)® two doses 11 days a part. The second subgroup injected with 0.25 mg PMSG (Folligon)® and the third injected with 25 mg $PGF_2\alpha$ (Lutalyse)® plus 0.25 mg PMSG (Folligon)®. Estimation of serum progesterone, concentration, estradiol 17-B, thyroid hormones (T_3 and T_4) and some serum biochemical constituents as inorganic phosphorus, total cholesterol, glucose and total protein concentrations were performed for all animals before and at the appearance of heat signs. The obtained results revealed that, balady cows with persistent CL showed a highly significant decrease in serum progesterone and glucose level after all hormonal treatment. However, a highly significant increase in of estradiol -17β, T_3, T_4, inorganic phosphorus and total cholesterol concentrations were observed after treatments with $PGF_2\alpha$ and/or PMSG. On the other hand, there was a significant increase in serum total protein level after treatment with $PGF_2\alpha$, PMSG alone and $PGF_2\alpha$ plus PMSG. Concerning the cow with smooth inactive ovaries, there were highly significant increase in level of serum progesterone and Estradiol -17β after all regimes used. There were highly significant increase in levels of serum T_3, T_4, were noticed. Whereas, serum total cholesterol and glucose were highly significantly decreased after all regimes. The values of serum inorganic phosphorus showed a highly significant increase after treatment with $PGF_2\alpha$ alone and $PGF_2\alpha$ plus PMSG. Morever non signficant increase in values of serum inorganic phospharus observed after treatment with PMSG alone. The mean value of serum total protein level showed a significant increase after treatment with $PGF_2\alpha$ alone and PMSG alone. Besides, there was a highly significant increase in the level of total protein after treatment with $PGF_2\alpha$ plus PMSG.

The present study revealed also that, higher percent of conception rate (70% and 60%) after 1.6 ± 0.22 and 1.9 ± 0.23 number of services recorded in animals treated with $PGF_2\alpha$ plus PMSG for both main groups having persistent CL and smooth inactive ovaries respectively. It could be concluded that injection of $PGF_2\alpha$ followed by PMSG 5 days later might give higher conception rate and lower number of services per conception in balday cows with persistent CL and smooth inactive ovaries.

Key words: $PGF_2\alpha$, PMSG, Anestrous, Balady cows, CL Hormonal and Biochemical changes; Conception rate.

INTRODUCTION

The problem of infertility in apparently healthy balady cows did not receive the desired attention. Various hormonal treatments were used to improve fertility such as GnRH **(Qayum et al., 1991)**; $PGF_2\alpha$ **(Abdel-Latief, 1993 and Sosa, 1994)**; and exogenous estrogen **(Zaabel et al., 1995a)**. The efficiency of reproductive process usually controlled by heriditary and environmental factors **(Hafez, 1993)** and dynamic activity of thyroid gland **(Afiefy et al., 1970)**.

Administration of $PGF_2\alpha$ results in the increase of adrenal, steroid plasma level and antagonizes some insuline effects **(Deaver et al., 1986)**. Thyroid hormones increase the basal metabolic rate by increasing oxygen consumption **(Knchans and Romson, 1989)**.

So, the present work was designed to study the effect of some medicaments on the level of ovarian and thyroid hormones in addition to some serum biochemical constituents of anestrous balady cow heifers before and after treatments with different hormones which may helpful in improving the reproductive efficiency of animals.

MATERIALS AND METHODS

This study was carried out on 60 anestrous balady cows (3-6 years) on the village system in Kalubia province. All animals showed anestrous from the onset of puberty. Complete gynaecological examination has been carried out two times 10 days in between befor the start of experiments. Animals were classified into two main groups according to the rectal finding on the ovaries (30 with persistent CL and 30 with smooth inactive ovaries). Repeated rectal examination were performed to detect the persistence of CL. High values of progesterone were confirmed the rectal examination. Animals in each group were classified into 3 subgroups according to the drug used as follows :

Subgroup I: 10 cows injected I/m with 25 mg $PGF_2\alpha$ (Lutalyse)®* and repeated injection after 11 days in animals which not came in estrus.

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Subgroup II: 10 cows injected I/m with 0.25 mg PMSG (Folligon)®***
Subgroup III: 10 cows injected I/m with 25 mg $PGF_2\alpha$ (Lutalyse)® and after 5 days, the non responded heifers injected with 0.25 mg PMSG (Folligon)®.

Gynaecological examination and close observation have been carried daily after treatment to follow-up the effect of the drugs on heat response and conception rate. Pregnancy was diagnosed by rectal palpation 60 days after service. Blood samples were collected from all animals from the jugular vein immediatly before treatment and at the onset of heat signs. Serum was separated by centrifugation at 3000 r.p.m for 10 minutes and stored at -20°C untill analyzed for Progesterone, Estrodiol -17β, T_3 and T_4. In addition some blood biochemical constituents as serum inorganic phosphorus, total cholesterol, glucose and total proteins were determined. Serum progesterone and Estradiol-17β concentration were estimated by radio immuno assay (RIA) using commercial kits, provided by diagnostic products corporation, Los-angelos, USA, according to **Kubosik (1984) and Xing et al., (1983)** respectively. Serum T_3 (Tri-iodothyronine) and T_4 (Thyroxine) concentrations were determined also by RIA with a commercially available kits (Diagnostic products corporation, Los-Angelos, Cat. No., TKT31 and TKT41) for T_3 and T_4, respectively. Serum inorganic phosphorus, total cholesterol, glucose and total proteins were determined colorimetrically according to the methods of **Wootton, (1982), Zak et al., (1954), Trinder, (1969) and Weichselbaun (1946),** respectively. Data were statistically analysed according to **Snedecor and Cochran (1967).**

RESULTS AND DISCUSSION

The effect of hormonal treatments on ovarian hormones, thyroid hormones and some serum biochemical constituents in relation to reproductive efficiency in anestrous balady cows (with persistent CL or smooth inactive ovaires) were shown in table (1). The present results revealed that, there was a sharp decrease in serum progesterone level in cows with persistent CL in all treated groups (Table 1 and Fig. 1). These findings were in agrrement with those reported by **Singh et al., (1979)** who found a sharp fall in perogesterone level after treatment of subestrus buffaloes with $PGF_2\alpha$. The decreased perogesterone level after treatment with $PGF_2\alpha$ might be attributed to the luteolytic effect of $PGF_2\alpha$ on CL. These results confirmed by **Fogwell et al., (1978); and Sosa, (1994).** Moreover, decreased progesterone level after PMSG treatment might be attributed to the effect of FSH on estrus induction **(Qayum et al., 1991).** On the other hand the mean values of serum progesterone level were significantly increased after different treatments in animals, with static (smooth inactive) ovaries. These findings supported by **Essawy (1987),** who found that, small

elevation in progesterone concentration was noticed in buffaloes before ovulation, this elevations were originated from adrenal gland and /or lutenized follicles.

Ragarding serum estradiol -17β level, the abtained data (table 1 and Fig 2) revealed a highly significant increase of serum estradiol-17β after treatment with $PGF_2\alpha$ and /or PMSG in cows with persistent CL and smooth inactive ovaries. These results agreed well with that of **Zaabel et al., (1995a)** who reported that, estradiol-17β level showed a highly significant increase after treatment with $PGF_2\alpha$ plus Gn-RH and Gn-RH alone in heifers with and without CL, respectively. Moreover, **Pandey et al., (1982)** stated that, treatment of buffaloes with $PGF_2\alpha$ brings about abrupt release of estradiol within one day of injection. The elevation of serum estradiol -17β after treatment with $PGF_2\alpha$ and/or PMSG might be due to a direct effect of these drugs on the ovarian estrogen biosynthesis **(Qayum et al., 1991).**

A significant increase in serum T_3 level after treatment with $PGF_2\alpha$ and/or PMSG in animals with persistent CL and with smooth inactive ovaries (Table 1 and Fig. 3). These results were nearly similar to those reported by **Zaabel et al., (1995b)** who observed a significant increase in serum T_3 level after $PGF_2\alpha$ and $PGF_2\alpha$ plus oestradiol benzoate therapy in buffalo-cows with and without palpable CL. Moreover **Ibrahim et al., (1990)** reported that, the serum T_3 concentration was increased significantly after injection of anestrous buffalo-cows with 25mg $PGF_2\alpha$. The higher levels of serum T_3 might be attributed to the stimulatory effect of $PGF_2\alpha$ on gonadotrophins secretion, steroid production by the adrenals and insulin release **(Johnson et al., 1973).**

Regarding serum T_4 level (Table 1 and Fig. 4), there was a highly significnat increase in their level after treatment with $PGF_2\alpha$ and/or PMSG in both groups. These results agreed well with that recorded by **Zaabel et al (1995b)**. The increased level of serum T_4 at the onset of estrus might be due to increased estrogen level at this period, as estrogen stimulated thyroid gland activity by direct action without the intervention of pituitary gland **(Kumar et al., 1991) and Megahed et al., (1995)**. On the other hand, **Kesler et al., (1981)** reported no changes in thyroid hormones concentrations during estrous cycle.

The serum inorganic phosphorus levels showed a highly significant increase after treatment with $PGF_2\alpha$ alone and $PGF_2\alpha$ plus PMSG in cows with persistent CL and smooth inactive ovaries (Table 1 and Fig. 5). Whereas, a non significant increase in the level of serum inorganic phosphorus was recorded after treatment with PMSG in cows with static ovaries. The increase in serum inorganic phosphorus level after PMSG treatment was closely similar to that reported by **Samy (1991)** who stated that, the serum inorganic phosphorus showed a non significant increase at 5th and 15th days, followed by a significnat increase at 20th and 30th after treatment with Gn-RH. The author attributed the

higher values of serum inorganic phosphorous levels during that time to the metabolic effect of Gn-RH on the anterior pituitary and stimulation of FSH and LH secretion which may also have an indirect role in the activation of some enzymes necessary for phosphorus absorption from the intestine. In contrary, **Hussein, et al., (1994)** recorded that, injection of buffaloes with Gn-RH significantly decreased serum inorganic phosphorus. Which might be attributed to the time of sampling and/or nutritional status of the animals and potency of drugs. On the other hand, **Dhoble and Gupta (1987) and El-Nour (1996)** found that, the level of inorganic phosphorus did not have a relationship to the clinical response following administration of Gn-RH and $PGF_2\alpha$.

The present results revealed that there was a highly significant increase in total cholesterol level after treatment with $PGF_2\alpha$ and/or PMSG in cows with persistent CL and significant decrease in these parameters in animals with smooth ovaries (Table 1 and Fig. 6). A non significant increase in serum total cholesterol level was noticed after treatment with Gn-RH and $PGF_2\alpha$ in buffaloes suffering from ovarian inactivity **(Samy, 1991 and Abdel-Latif, 1993)**. The increase in serum total cholesterol level after treatment with $PGF_2\alpha$ might be attributed to increased endogenous biosynthesis of cholesterol in endocrine gland tissue from acetate **(Purohit and Kohli, 1977)**. Whereas, decrease in serum total cholestorl level in heifers without CL after treatment with $PFG_2\alpha$ alone may be due to increase metabolic activity of animals under the effect of oestrogen hormone and hyperthyroidism **(Glomset and Williams, 1968)**.

The obtained data (Table 1 and Fig.7) indicated that there was a highly significnat decrease in serum glucose concentration of infertile animals with persistent CL and smooth inactive ovaries after different hormonal treatments. There results agreed well with those obtained by **Abdel-Latif (1993)** who recorded that, the serum glucose levels was significantly decreased in $PGF_2\alpha$ treated heifers with and without palpable CL. Similarly, **El-Nour (1996)** reported that, serum glucose level was decreased during both follicular and luteal phases of ewes synchronized with $PGF_2\alpha$. Although the decrease in serum glucose level still within the normal range, this decrease might be due to the effect of $PGF_2\alpha$ which cause stimulation of insulin release and have some insulin like effects on carbohydrate metabolism **(Goodman and Gilman, 1980)**, and the uterine cells became more permeable for glucose during estrus **(El-Baghdady, 1984)**. In contrary, **El-Shawaf (1984)** found that, serum glucose level was significanlty higher during follicular phase than in the luteal one in buffaloes.

Concerning serum total protein (Table 1 and Fig. 8) revealed that, there was a highly significant increase in its level after treatment with $PGF_2\alpha$ plus PMSG in cows with persistent CL and smooth inactive ovaries and after treatmen with PMSG alone in animals with persistent CL. Whereas, a significant

increase in serum total protein level was noticed after treatment with $PGF_2\alpha$ alone in heifers with persistent CL and static ovaries. These results agreed with that reported by **Abdel-Latif (1993)** who observed that, injection of $PGF_2\alpha$ caused significant increase in serum total protein level in infertile buffalo-cows. The increase of serum total protein might be due to the anabolic action of estrogen during estrus that favoter for protein synthesis and retention of nitrogenous substances in the tissues.

Concerning the clinical reproductive efficiency the highest conception rate (70% and 60%) with lowest number of services per conception (1.6 ± 0.22 and 1.9 ± 0.23) was exhibited after treatment with $PGF_2\alpha$ plus PMSG in both cows with persistent CL and smooth inactive (static) ovaries (Table 2 and Fig. 9). Our results were higher than that obtained by **Graves et al. (1974)** who found the pregnancy rate of anestrous cows was 28.2% after $PGF_2\alpha$ plus Gn-RH treatment. The conception rat was 60% in animals with persistent CL which treated with $PGF_2\alpha$ alone. These findings nearly similar to those obtained by **Eissa et al. (1990)**. The treatment with PMSG exhibited 50% and 40% conception rate after 2.3 ± 0.26 and 3.0 ± 0.26 number of services per conception in cows with static ovaries and persistent CL resprectivity. These results disagreed with **Lee et al. (1983)** who cited that the conception rate of heifers treated with GnRH at first breeding did not differ greatly than control one.

From the present study it could be concluded that the best trial for treatment of anestrous balady cow-heifers with persistent CL and smooth inactive ovaries is exhibited by the administration of $PGF_2\alpha$ followed by PMSG injection 5 days latter. So we recommened the physician and farmers in Egypt to use these hormones for estrous induction and ovulation in subestrous and anestrous balady cows and improving their reproductive efficiency.

Table (1) : Effect of $PGF_2\alpha$ and/or PMSG on ovarian, thyroid hormones and some biochemical constituents in balady cows with persistent CL (PCL) and smooth inactive ovaries (S.I.O.).

Groups		Persistent Corpus luteum (P.C.L.)			Smooth inactive ovaries (S.I.O.)		
Subgroups / Parameters		$PGF_2\alpha$ (Lutalyse)	PMSG (Folligon)	$PGF_2\alpha$+ PMSG (Lutalyse + Folligon)	$PGF_2\alpha$ (Lutalyse)	PMSG (Folligon)	$PGF_2\alpha$+ PMSG (Lutalyse+ Folligon)
Progesterone ng/ml	B	2.166 ± 0.013	1.267 ± 0.028	2.054 ± 0.028	0.470 ± 0.024	0.419 ± 0.017	0.416 ± 0.316
	A	0.827± 0.026**	0.529± 0.032**	0.363± 0.228**	1.127± 0.035**	1.154± 0.040**	1.428± 0.148**
Estradiol 17-β pg/ml	B	11.46 ± 0.32	15.53 ± 0.20	14.65 ± 0.24	14.00 ± 0.14	16.13 ± 0.10	18.02 ± 0.15
	A	24.44 ± 0.26 **	28.44 ± 0.14**	26.61 ± 0.22**	26.94 ± 0.20**	35.72 ± 0.27**	27.89 ± 0.23**
T3 (Triiodothyronine) ng / dl	B	45.96 ± 0.09	37.41 ± 0.44	54.09 ± 0.29	34.80 ± 0.26	38.23 ± 0.14	44.89 ± 0.30
	A	66.25 ± 0.30**	61.19 ± 0.16**	81.08 ± 0.19**	60.52 ± 0.27**	66.81 ± 0.33 **	71.52 ± 0.27**
T4 (Thyroxine) ug/dl	B	2.98 ± 0.22	2.09 ± 0.32	1.58 ± 0.11	2.18 ± 0.08	2.71 ± 0.11	1.55 ± 0.28
	A	6.18 ± 0.19**	5.47 ± 0.32**	7.26 ± 0.23**	6.55 ± 0.21**	6.28 ± 0.09**	5.11 ± 0.13**
Inorganic Phosphorus mg/dl	B	3.68 ± 0.17	4.37 ± 0.11	3.97 ± 0.15	3.32 ± 0.15	4.93 ± 0.16	3.54 ± 0.15
	A	4.80 ± 0.16 **	4.96 ± 0.12**	6.16 ± 0.16**	4.19 ± 0.17**	5.02 ± 0.11	5.19 ± 0.21 **
Total cholesterol mg/dl	B	105.96 ± 0.39	144.60 ± 0.44	205.93 ± 0.68	150.99 ± 0.37	172.94 ± 0.51	213.40 ± 0.77
	A	55.44 ±0.54**	160.77 ± 0.38**	244.41 ± 0.32**	117.90 ± 0.67**	159.18 ± 0.60**	194.98 ± 0.51**
Glucose mg/dl	B	55.72 ± 0.96	54.42 ± 0.32	47.71 ± 0.27	72.72 ±0.65	53.82 ± 0.84	76.54 ± 0.62
	A	47.00 ± 0.70**	47.15 ± 0.22**	41.47 ± 0.25**	59.67 ± 0.62**	45.73 ± 0.60**	50.58 ± 0.54**
Total protein gm/dl	B	5.04 ± 0.32	6.47 ± 0.15	7.43 ± 0.21	5.78 ± 0.42	7.01 ± 0.50	7.87 ± 0.52
	A	6.04 ± 0.26*	7.99 ± 0.30**	11.55 ± 0.25**	7.50 ± 0.48*	8.75 ± 0.30*	9.75 ± 0.36**

* Significant differences ($P < 0.05$).
B : Before treatment.
Data are presented as mean ± S.E.

** Highly significant differences ($P < 0.01$)
A : After treatmnet
S.E: Standard error.

Table (2): Estrus number of services / conception and conception rate in balady cows after treatment with $PGF_2\alpha$ and/or PMSG.

Hormones used	Time elapsed till 1st heat (days)			No. Of services/ conception		Conception rate	
		PCL	S.I.O.	PCL	S.I.O.	PCL	S.I.O.
PGF2α (Lutalyse)	1st inj.	4.00 ± 1.00	5.60 ± 0.56	2.0±0.21 (a)	4.0± 0.26 (a)	60%	40%
	2nd inj.	14.00 ± 0.53	16.20 ± 0.53				
PMSG (Folligon)	singl inj.	15.60 ± 0.52	4.80 ± 0.39	3.0±0.26 (aab)	2.3± 0.26 (aab)	40%	50%
PGF2α + PMSG (lutalyse + Folligon)	1st inj.	8.00 ± 0.30	8.30 ± 0.26	1.6 ± 0.22 (bbc)	1.9± 0.23 (aabc)	70%	60%
	2nd inj.	18.20 ± 0.29	19.50 ± 0.27				

- PCL : Persistent corpus luteum
- S.I.O. : Smooth inactive ovaries
- Values with the same single alphabetic were significantly differs ($P<0.05$)
- Values with the same double alphabetics were highly significnat differs ($P<0.01$)

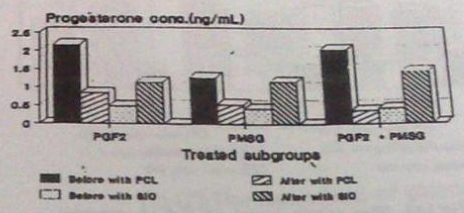

Fig.(1): The mean level of progesterone hormone in cows with persistent CL(PCL) and smooth inactive ovaries (SIO).

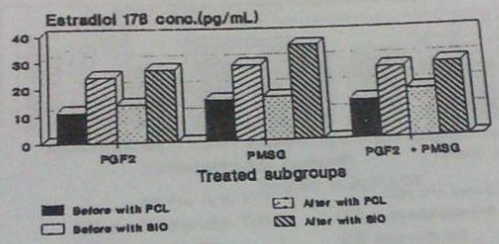

Fig.(2): The mean level of estradiol 17β hormone in cows with persistent CL (PCL) and smooth inactive ovaries (SIO).

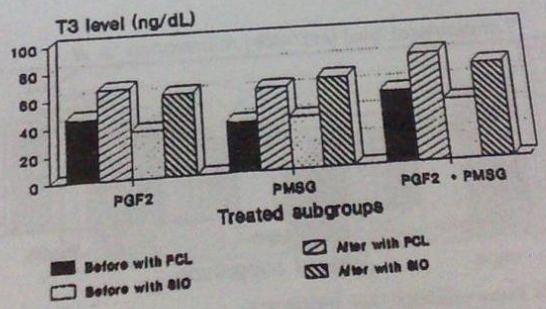

Fig.(3): The mean level of T3 hormone in cows with persistent CL (PCL) and smooth ovaries (SIO).

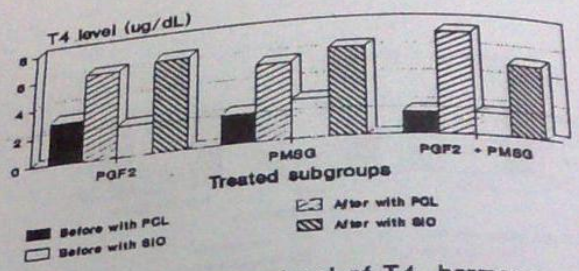

Fig.(4): The mean level of T4 hormone in cows with persistent CL (PCL) and smooth inactive ovaries (SIO).

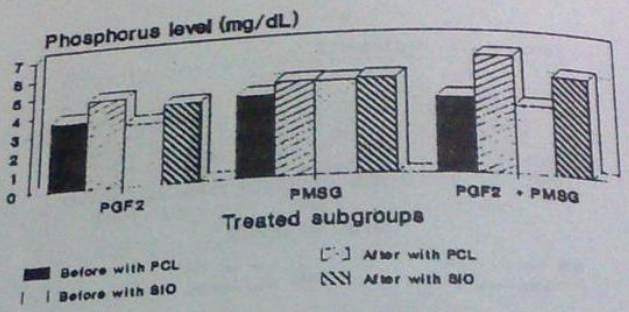

Fig.(5): The mean level of inorganic phosphorus in cows with persistent CL (PCL) and smooth inactive ovaries (SIO).

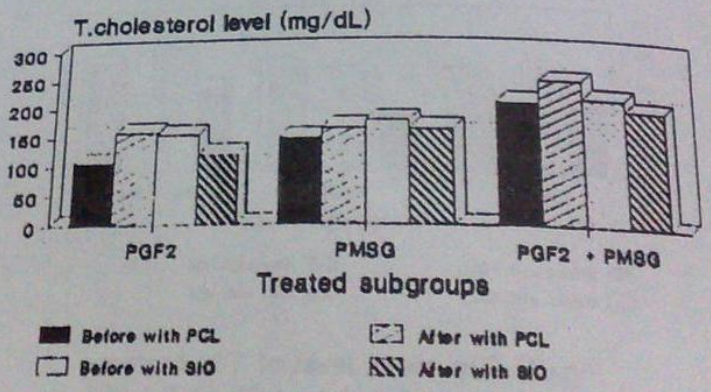

Fig.(6):The mean level of total cholesterol in cows with persistent CL (PCL) and smooth inactive ovaries (SIO).

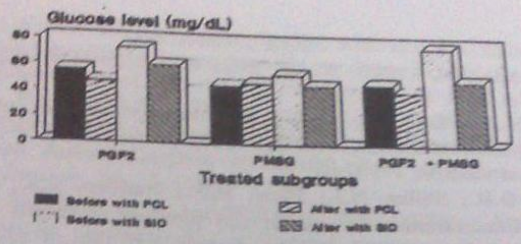

Fig.(7):The mean level of glucose in cows with persistent CL (PCL) and smooth inactive ovaries (SIO).

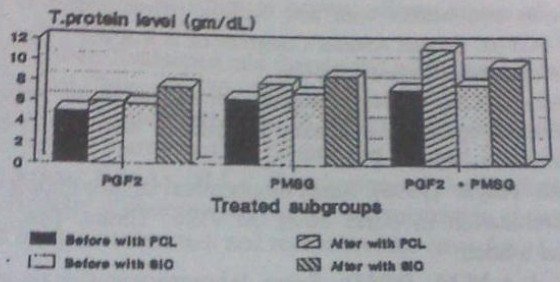

Fig.(8):The mean level of total protein in cows with persistent CL (PCL) and smooth inactive ovaries (SIO)

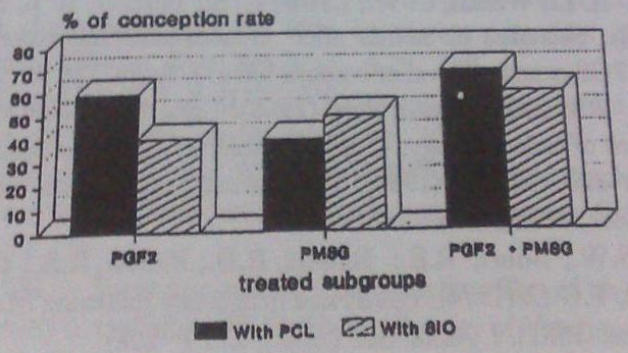

Fig.(9):The percentage of conception rate in cows with persistent CL (PCL) and smooth inactive ovaries (SIO).

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الملخص العربي

علاجات مقارنة لظاهرة عدم الشياع فى الأبقار البلدية باستخدام هرمونى البروستاجلاندين (F2α) والجونادو تروبين (PMSG) وعلى وجه الخصوص بعض التغيرات البيوكيميائية والخصوبة المستقبلية.

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أجرى هذا البحث على ٦٠ بقرة بلدية (٣٠ تعانى من خمول المبايض و ٣٠ تعانى من وجود الجسم الأصفر) وقد قسمت كل مجموعه أساسيه إلى ثلاثة مجاميع تبعاً لنوع الهرمونات المستخدمة فى العلاج بحيث اشتملت كل مجموعه على ١٠ بقرات من كلاً من حالتا الأضطرابات التناسلية.

المجموعة الأولى:- أعطى كل حيوان ٢٥ ملليجرام من البروستاجلاندين F2- ألفا فى العضل وقد تكررت الجرعه مرة بعد ١١ يوماً فى الحيونات التى لم تستجيب للشياع بعد الجرعه الأولى.

المجموعة الثانية: أعطى كل حيوان ٢٥, ملليجرام من الـ PMSG فى العضل.

المجموعة الثالثة: أعطى كل حيوان ٢٥ ملليجرام من البروستاجلاندين F2- ألفا وبعدها بخمسة أيام تم حقن ٢٥, ملليجرام من الـ PMSG فى العضل ولقد جمعت عينات الدم قبل الحقن وعند ظهور الشبق بعد إستجابة الحيوانات للعلاج لدراسة تأثير العلاج على مستوى هرمونات المبيض (الأستراديول ١٧ - بيتا و البروجستيرون) وكذلك هرمونات الغدة الدرقيه (T4،T3) بالأضافه إلى بعض المكونات البيوكيميائية فى المصل مثل الفسفور الغير عضوى، الكلستيرول الكلى، الجلوكوز، والبرتين الكلى. وقد أسفرت النتائج على الأتى:

بالنسبة للأبقار البلدية التى تعانى من وجود الجسم الأصفر كان هناك نقص عالى المعنوى فى مستوى هرمون البروجستيرون والجلوكوز وذلك بعد كل العلاجات المختلفه بينما كان هناك زيادة عالية المعنويه فى مستوى الأستراديول ١٧ - بيتا، T4،T3 ، الفسفور الغير عضوى وتركيز الكلستيرول الكلى بعد العلاج بالبروستاجلاندين F2- ألفا and / or الـ PMSG. من ناحية آخرى أظهرت النتائج وجود زياده معنوية فى مستوى البروتين الكلى بعد العلاج بهرمون البروستاجلاندين F2- ألفا، الـ PMSG كلاً على حده وأيضاً بعد العلاج بهرمون البروستاجلاندين F2- ألفا مع الـ PMSG.