RESULTS AND DISCUSSION

4. RESULTS AND DISSCUSSION

The presented data are graphically represented as mean values (ng/ml serum) along with table values. Moreover individual curves for each rabbit within a specific cell of all experiment was transformed to area under curve in order to be more indicative to cumulative effect of injected drug materials over the 16 hrs of each experiment. These areas under curve data were graphically exhibited and used for analysis of variance under each photoperiod (short darkness, equal dark / light and long darkness).

4.1 First experiment:

First experiment was planned and conducted as a trial to detect the possibility for improving the reproductive performance of does by magnifying the hormonal coordination related to productive activity with relation to different light regimes tested, to fulfill this aim applying human chorionic gonadotropin as well as other chemical compounds that thought to have considerable effect on hormonal regulation of reproduction. Melatonin as an important regulator of the circadian rhythms of several biological functions including reproduction , serotonin a neurotransmitter that effects growth and reproduction , bromocriptine which is considered as a dopamine agonist that is used in the treatment of hyperprolactinaemia, and indomethacin as antiprostaglandin drug. The effects of these

hormones and drugs on the level of serum progesterone are discussed as follows:

4.1.1. Serum progesterone level in untreated does subjected to different lighting regimes applied:

Table 3; Figures 1, 2 and 3 and Histograms 1, 2 and 3 shows that the variation in serum progesterone level due to either lighting regimes or experimental intervals. Averages of progesterone level were almost higher in does exposed to short lighting regime (16 hours (hrs) darkness / 8 hours (hrs) light) when compared to other lighting regimes, on the other hands, the higher level of serum progesterone were detected after two hours .This was quite true in all lighting regimes applied. Variation in serum progesterone level for does treated much saline solution due to estimation period may be attributed to the individual variation existed the reproductive activity rather than due to injected saline solution since the high serum progesterone levels were detected almost at the second hour after injection . The higher levels of serum progesterone found along the experimental period for does exposed to short lighting regime (16 hrs darkness and 8 hrs lighting) may lead to conclude that surge of LH hormone needs at least 8 hrs lighting to show its biological effect of ovulation in the animals of induced ovulation pattern like rabbits. Thus, the photoperiod regime shows its significant effect on ovulation mechanism in accordance

with the time of ovulation which occurred at approximately at the same time in all experimental animals.

4.1.2. Effect of HCG:

Data listed in **Table (3)** represented in **Figures (1, 2 and 3) and Histograms (1 , 2 and 3)** show averages of serum progesterone level in (ng/ml) in does exposed to different lighting regimes and injected with HCG compared with those of control group (injected with saline solution along 16 hrs after injection) .

Data obtained revealed an increase in serum progesterone levels as a result of injecting does with HCG, The rate of increment differed obviously according to the lighting regimes applied. Applying long lighting regime (8 hrs darkness and 16 hrs lighting) resulted in gradual increase in serum progesterone level reaching its lightest level (6.9526 ng /ml) at the 4th hr after injection then decreased up to the end of experimental period. Applying short lighting regime (16 hrs darkness (D) / 8 hrs light (L)) increased serum progesterone level with greater rate up to 4th hr after injection reaching highest level (13.0508 ng/ml) at the 2nd hr and (14.0844 ng /ml) at 4th hr then decreased steadily to reach lowest level at the 16th hr after injection . The same trend was found in does subjected to equal lighting regime (12 hrs D / 12 hrs L) but with lowest magnitude serum progesterone level increased after injection to reach its highest level (3.2797 ng/ml) after 2 hrs of

injection then decreased sharply and steadily towards the 16th hr after injection to reach the lowest level at this time (0.1384 ng/ml). Results obtained go in agreement with those of **Hilliard and Eaton (1971) and Lau *et al.*, (1978),** who reported that, peaks of progesterone and 20α-dihydroprogesterone in rabbit have been observed at 2 and 6 hrs after HCG injection respectively.

The obtained results concerning that, treating rabbit with HCG increased the level of serum progesterone during short photoperiod (16 hrs D/8 hrs L) compared to long photoperiod (8 hrs D/16 hrs L). Previously mentioned result may lead to conclude that, heat stress during long photoperiod (summer season) might result in higher glucocorticoid **(Roman – Ponce *et al.*, 1977)** and prolactin levels **(Peters and Tucker, 1977)** resulting in negative feedback effect on luteutropic hormone and consequently reduced progesterone secretion rates.

Analysis of variance for obtained data **(Table 4)** revealed significant variation in serum progesterone level due to lighting regime , injection and the interaction between them ($P < 0.01$) .

Obtained data agreed with results obtained by **kayisly *et al.*, (2003)** who stated that human chorionic gonadotropin interacts with the LHCG receptors and promotes the maintenance of corpus luteum during the beginning of pregnancy, causing it to secret the hormone progesterone. Progesterone enriches the uterus with thick

lining of blood vessels and capillaries so that it can sustain the growing fetus. He added that due to highly negative charge, HCG might repel the immune cells of the mother, protecting the fetus during the early pregnancy period. It has also been hypothesized that HCG may be placental link for the development of local maternal immunotolerance. The authors suggested that HCG may be a link in the development of peritrophoblastic immune tolerance and may facilitate the trophoblast invasions, which is known to expedite fetal development in the endometrium. Results obtained agree with those reported by **Caillol *et al.*, (1986)** who found that, progesterone concentrations are undetectable before mating or HCG treatment. However, transient rise in progesterone secretion was observed in the 24 hrs following mating or HCG injection which, occurs after the LH surge induced. **Batra *et al.*, (1979)** also reported that elevation in the level of progesterone in non-pregnant rabbits resulted from increased ovarian output through HCG injection has a pronounced and immediate depressing effect on the character of myometrial activity which is indicative of a shift from non-pregnant to pregnant type. These results are in accordance with **Browning *et al.*, (1980) and Dharmarajan *et al.*, (1989)**, who reported that the magnitude of estradiol (E_2) and progesterone (P_4), rise occurred in response to HCG injection.

Table (3): Progesterone serum levels (ng/ml) $\bar{x}$±SE in response to injection of HCG vs saline at short darkness photoperiod (8D/16L), equal dark and light photoperiod (12D/12L) and long darkness photoperiod (16D/8L).

Time after injection	Saline			HCG		
	8D/16L	12D/12L	16D/8L	8D/16L	12D/12L	16D/8L
0hr	0.3518±0.0448	0.5169±0.1248	0.8723±0.1633	0.0973±0.0516	0.2005±0.0821	0.1867±0.0371
1hr	0.6303±0.1729	0.3871±0.0445	0.7767±0.2260	0.1925±0.1273	1.2733±0.2450	0.2626±0.0610
2hr	0.6034±0.0897	1.4128±0.9812	1.2750±0.4254	5.3860±5.0900	3.2797±0.9767	13.0508±3.3939
4hr	0.5303±0.0887	0.5035±0.2030	0.6457±0.0937	6.9526±5.3762	0.8559±0.4248	14.0844±3.4214
8hr	0.4546±0.1422	0.2775±0.0173	0.5748±0.1137	1.3982±0.9838	0.2573±0.0884	6.0789±1.1813
16hr	0.2927±0.0323	0.4057±0.1030	0.6742±0.2112	1.1544±0.62064	0.1384±0.1768	1.4430±0.3942

Table (4): Analysis of variance of data (cumulative area under curve in units) collected after injection of HCG vs saline under different photoperiods.

S.O.V	d.f	S.S	M.S	F
Photoperiod	2	9079.57501	4539.7875	12.6317**
Injection	1	14258.2824	14258.28	39.673**
Interaction	2	10125.6967	5062.85	14.087**
Residual	25	8984.92118	359.39684	
Total	30	42448.47529	1414.9493	

** Significant at level of 0.01

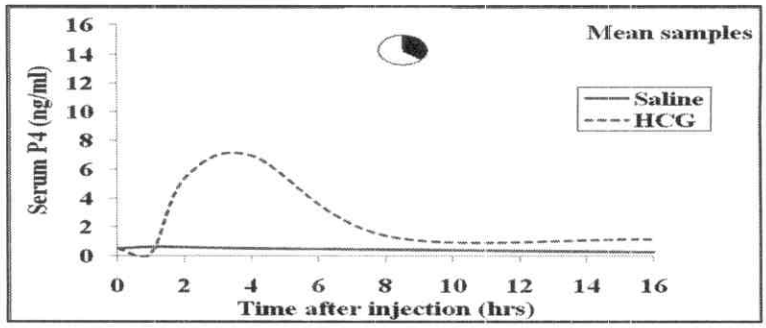


Figure (1) Serum progesterone level (ng/ml) in response to administration of HCG vs saline at short darkness photoperiod (8D/16L).

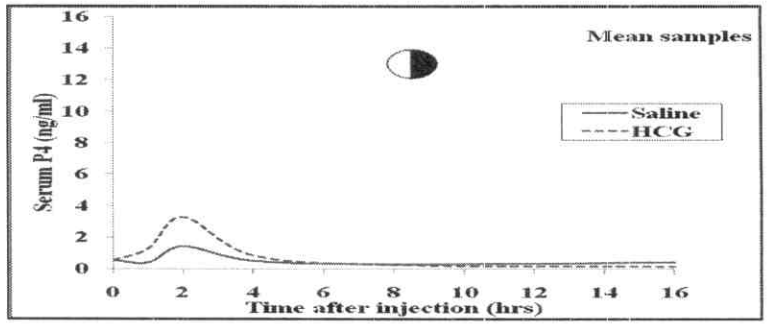


Figure (2) Serum progesterone level (ng/ml) in response to administration of HCG vs saline at equal dark / light photoperiod (12D/12L).

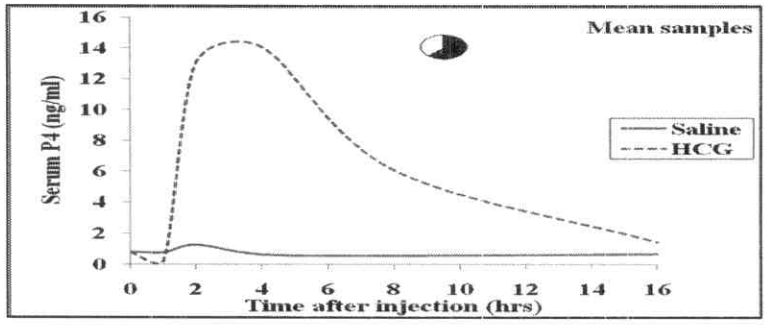
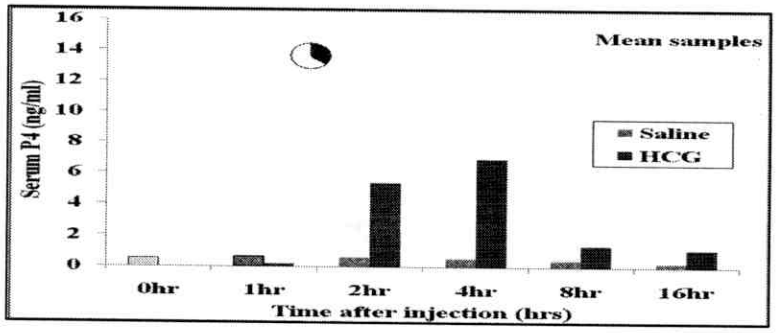
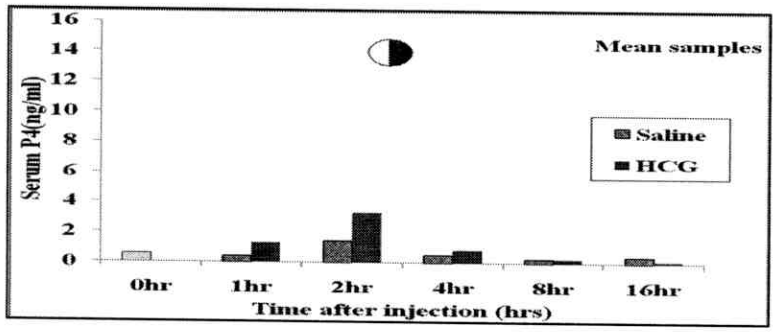


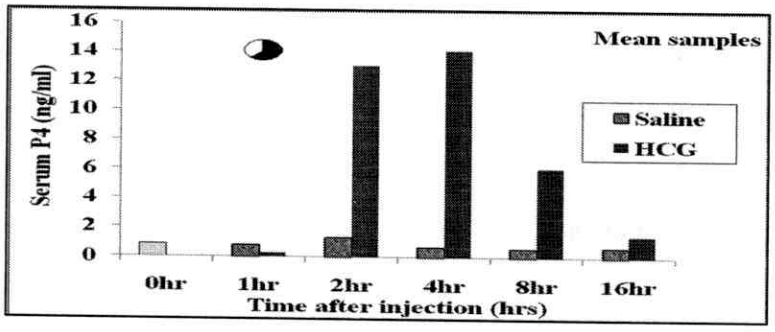
Figure (3) Serum progesterone level (ng/ml) in response to administration of HCG vs saline at long darkness photoperiod (16D/8L).



Histogram (1) Serum progesterone level (ng/ml) in response to administration of HCG vs saline at short darkness photoperiod (8D/16L).



Histogram (2) Serum progesterone level (ng/ml) in response to administration of HCG vs saline at equal dark / light photoperiod (12D/12L).



Histogram (3) Serum progesterone level (ng/ml) in response to administration of HCG vs saline at long darkness photoperiod (16D/8L).

4.1.3. Effect of Melatonin:

Data listed in **Table (5)** represented in **Figures (4, 5 and 6) and Histograms (4, 5 and 6)** show serum progesterone level in does subjected to different lighting regime applied and treated with melatonin.

Results obtained showed that treating experimental does with melatonin. Increase serum progesterone level at all intervals of estimation when compared with the corresponding values of does treated with saline solution. However, the rate of increase varied according to either lighting regime applied or interval of estimation after injection. The highest progesterone level was estimated after 1 hr of injection does subjected to long lighting regime (8 hrs D/16 hrs L) (14.5463 ng /ml) which sharply decrease to (7.1431 ng /ml) at 2 hrs after injection and still decrease up to 16 hrs after injection to reach the value of (1.7013 ng /ml).

Different pattern of change along the whole intervals of estimation was found in does subjected to other two lighting regimes. Serum progesterone level increased in does subjected to equal lighting regime (12 hrs D /12 hrs L) to reach its highest value at 2 hrs after injection (1.7679 ng /ml) then steadily decrease to reach a value of (0.8685 ng /ml) at 16 hrs after injection. Same trend of variation along all times of estimation was found in does subjected to short lighting regime (16 hrs D /8 hrs L) but with lower magnitude when compared to the other photo regimes applied.

The obtained results may lead to conclude that melatonin has been implicated as a key factor in the photoperiod reproductive cycles **(Thorpe and Herbert, 1976). Dodge and Badura (2002a)** reported that short day (SD) (16 hrs D/8 hrs L) exposure in female Syrian hamster reduces serum concentrations of LH and progesterone. Maximum differences in basal and peak of progesterone levels may have been observed between short day (16 hrs D/8 hrs L) and long day (8 hrs D/16 hrs L) **(Beasley *et al.*, 1981). Turek *et al.*, (1975)** indicated that the reproductive system of photoperiodic mammals is more likely to be responsive to exogenous melatonin.

However , analysis of variance for obtained data **(Table 6)** revealed in significant variation in serum progesterone level due to either lighting regime , injection or the interaction between them .Obtained results were scientifically logic since melatonin vary in daily cycle , there by regulating the circadian rhythms of several biological functions **(Altum and Agur-Altum , 2007)**. Many biological effects of melatonin are produced through activation of melatonin receptors **(Boutin *et al.*, 2005)** while other are due to its role as a pervasive and powerful anti-oxidant **(Hardeland, 2005)** with a particular role in protection of nuclear and mitochondrial DNA **(Reiter *et al.*, 2001)**. Finally, **Terzola (1993)** stated that melatonin also Lowes FSH levels, these hormonal changes could impair fertility.

Table (5): Progesterone serum levels (ng/ml) $\bar{x}$±SE in response to injection of melatonin vs saline at short darkness photoperiod (8D/16L), equal dark and light photoperiod (12D/12L) and long darkness photoperiod (16D/8L).

Time after injection	Saline			Melatonin		
	8D/16L	12D/12L	16D/8L	8D/16L	12D/12L	16D/8L
0hr	0.3518±0.0448	0.5169±0.1248	0.8723±0.1633	0.6828±0.0357	0.6810±0.1737	0.4703±0.0616
1hr	0.6303±0.1729	0.3871±0.0445	0.7767±0.2260	14.5463±6.2482	0.8314±0.1629	0.7528±0.3248
2hr	0.6034±0.0897	1.4128±0.9812	1.2750±0.4254	7.1431±1.9315	1.7679±1.2901	0.8729±0.1802
4hr	0.5303±0.0887	0.5035±0.2030	0.6457±0.0937	7.8530±1.3296	0.8750±0.3565	0.8863±0.2503
8hr	0.4546±0.1422	0.2775±0.0173	0.5748±0.1137	5.6212±2.5341	0.8742±0.2927	0.5104±0.0589
16hr	0.2927±0.0323	0.4057±0.103	0.6742±0.2112	1.7013±0.8089	0.8685±0.2342	0.5959±0.1875

Table (6): Analysis of variance of data (cumulative area under curve in units) collected after injection of melatonin vs saline under different photoperiods.

S.O.V	d.f	S.S	M.S	F
Photoperiod	2	3498.59945	1749.2995	3.3389
Injection	1	5817.320466	5817.32	11.03677
Interaction	2	647.87987	323.93995	0.6183
Residual	25	13097.73335	523.9092	
Total	30	23061.53314	768.7177	

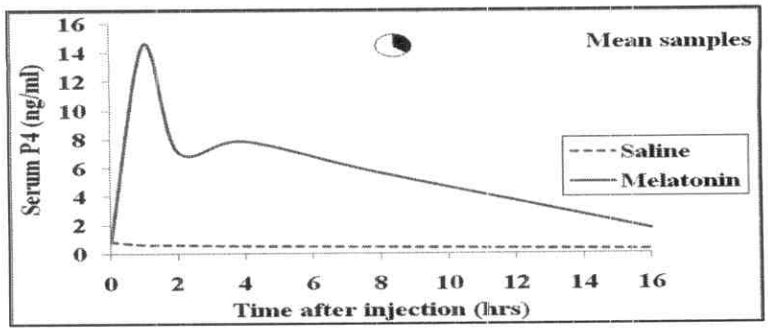


Figure (4) Serum progesterone level (ng/ml) in response to administration of melatonin vs saline at short darkness photoperiod (8D/16L).

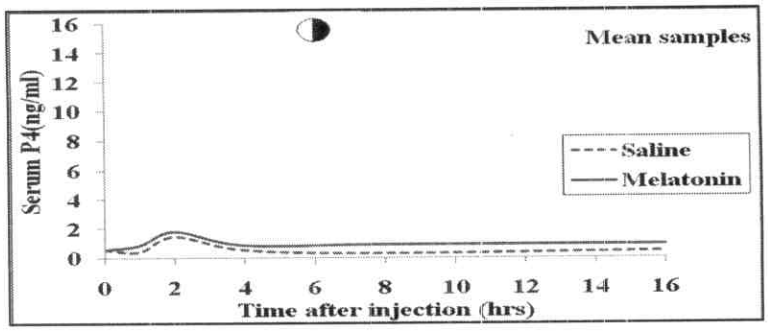


Figure (5) Serum progesterone level (ng/ml) in response to administration of melatonin vs saline at equal dark / light photoperiod (12D/12L).

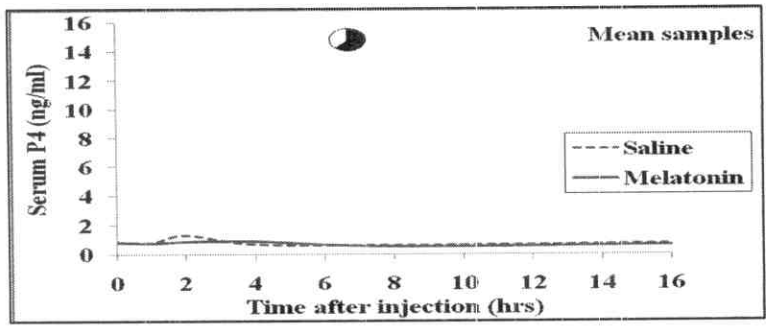
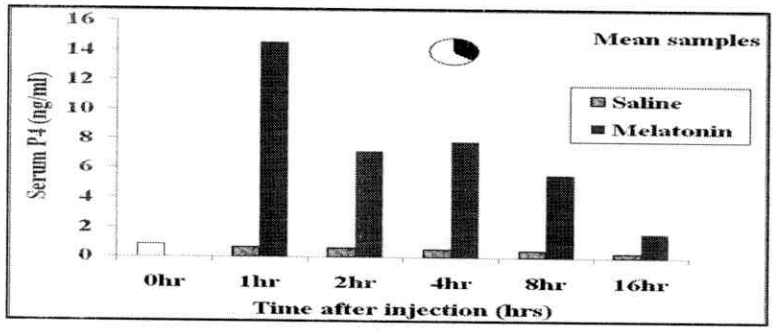
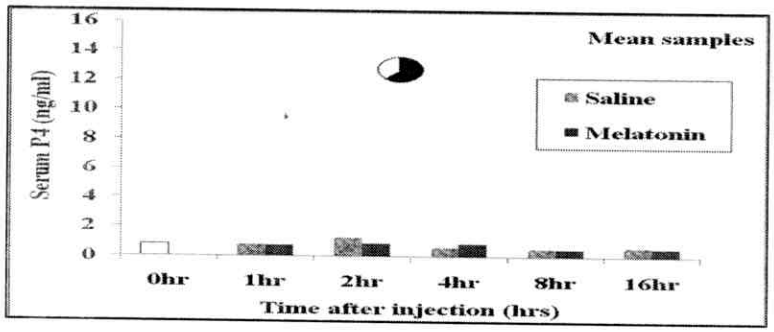


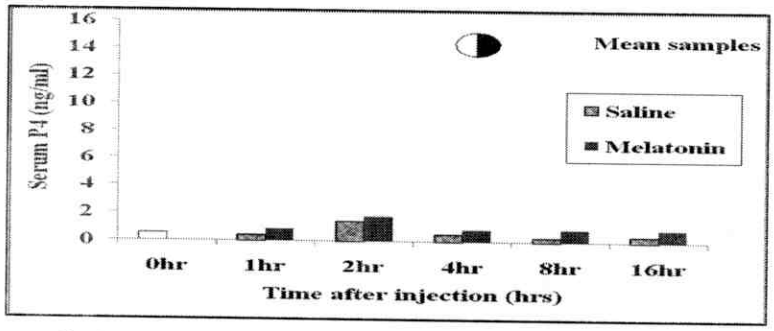
Figure (6) Serum progesterone level (ng/ml) in response to administration of melatonin vs saline at long darkness photoperiod (16D/8L).



Histogram (4) Serum progesterone level (ng/ml) in response to administration of melatonin vs saline at short darkness photoperiod (8D/16L).



Histogram (5) Serum progesterone level (ng/ml) in response to administration of melatonin vs saline at equal dark / light photoperiod (12D/12L).



Histogram (6) Serum progesterone level (ng/ml) in response to administration of melatonin vs saline at long darkness photoperiod (16D/8L).

4.1.4. Effect of serotonin:

Data related to the effect of injection serotonin on serum progesterone level in does subjected to the different lighting regime applied are listed in **Table (7)** represented in **Figures (7, 8 and 9) and Histograms (7, 8 and 9)**. Analysis of variance for data obtained is presented in **Table (8)**.

It was obviously clear that injected serotonin lowered the level of progesterone level in does subjected to all lighting regime applied. However, the rate of this decrement differed according the ratio between the periods of darkness to that of lighting. It was found that the decreasing rate of serum progesterone level increased as period of darkness increased. Does subjected to long lighting regime of (8 hrs D /16 hrs L) had higher progesterone level in this serum followed by those subjected to equal lighting regime (12 hrs D /12 hrs L). However, the lowest averages of serum progesterone levels were observed in does of short lighting regime (16 hrs D /8 hrs L). This trend of variation in serum progesterone level was true at all estimation intervals.

The previously mentioned results may lead to conclude that the secretion of GnRH is controlled by neuroendocrine signals that include: serotonin, norepinephrine, epinephrine, dopamine, acetylcholine, gamma amino butyric acid, opioid peptides, and glutamate **(Arias *et al.*, 1990 and Fernández-Fernandez *et al.*, 2005).** Serotonin may affect

GnRH secretion by either direct effect on GnRH neurons or indirectly by acting through other neurotransmitters, such as noradrenaline. An interrelationship between noradrenaline and GnRH neurons exists in the Preoptic area and median eminence **(Smith and Jennes, 2001).**

Serotonin had an adverse affect on ovulation which in closely affected by the level of LH surge which is effected by the length of daily lighting period from one side and the mating behavior of animals (specially of males) which correspondingly affect the incidence of ovulation in animals of inducing ovulation. Serotonin was found to be necessary for normal male mating behavior **(Loer and Kenyon, 1993)** and the inclination to leave food to reach for a mate **(Lipton *et al.*, 2004)**.

In mammals though insulin regulates blood sugar and insulin live growth factor IGF regulates growth, serotonin controls the release of both hormones so that serotonin suppresses insulin release from the beta cells in pancreas **(Paulmann *et al.*, 2009)** and exposure to selective serotonin reuptake inhibitor increase the extracellular level of serotonin and increase the level of serotonin in the synaptic cleft that becomes available to bind to post synaptic receptor. In addition, serotonin in mammals can also act as growth factor directly **(Wikipedia encyclopedia).**

Table (7): Progesterone serum levels (ng/ml) $\bar{x}$±SE in response to injection of serotonin vs saline at short darkness photoperiod (8D/16L), equal dark and light photoperiod (12D/12L) and long darkness photoperiod (16D/8L).

Time after injection	Saline			Serotonin		
	8D/16L	12D/12L	16D/8L	8D/16L	12D/12L	16D/8L
0hr	0.3518±0.0448	0.5169±0.1248	0.8723±0.1633	0.1056±0.0391	0.0188±0.0001	0.0188±O.0001
1hr	0.6303±0.1729	0.3871±0.0445	0.7767±0.2260	0.0227±0.0030	0.0371±0.0183	0.0283±O.0095
2hr	0.6034±0.0897	1.4128±0.9812	1.2750±0.4254	0.0574±0.0251	0.0188±0.0001	0.0380±O.0164
4hr	0.5303±0.0887	0.5035±0.2030	0.6457±0.0937	0.0496±0.0120	0.0188±0.0001	0.0341±O.0113
8hr	0.4546±0.1422	0.2775±0.0173	0.5748±0.1137	0.0345±0.0108	0.0270±0.0082	0.0203±O.0009
16hr	0.2927±0.0323	0.4057±0.1030	0.6742±0.2112	0.0383±0.0171	0.0191±0.0003	0.0193±O.0005

Table (8): Analysis of variance of data (cumulative area under curve in units) collected after injection of serotonin vs saline under different photoperiods.

S.O.V	d.f	S.S	M.S	F
Photoperiod	2	51.3809392	25.6905	47.0169**
Injection	1	541.8625145	541.8625	991.6775**
Interaction	2	244.7032583	122.352	223.9198**
Residual	28	15.2995939	0.54641	
Total	33	853.2463059	25.8559	

** Significant at level of 0.01

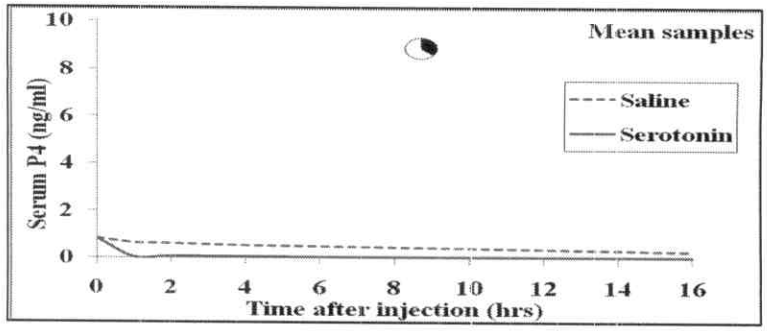


Figure (7) Serum progesterone level (ng/ml) in response to administration of serotonin vs saline at short darkness photoperiod (8D/16L).

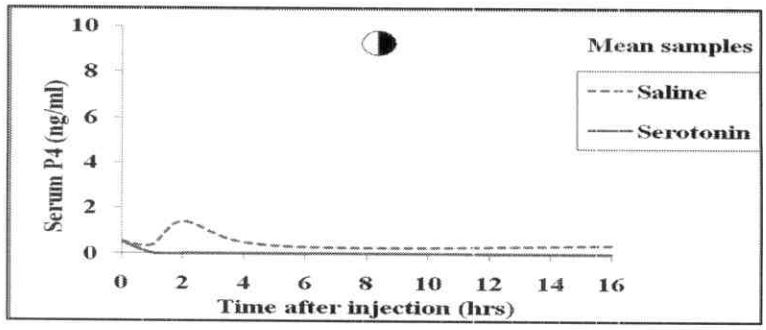


Figure (8) Serum progesterone level (ng/ml) in response to administration of serotonin vs saline at equal dark / light photoperiod (12D/12L).

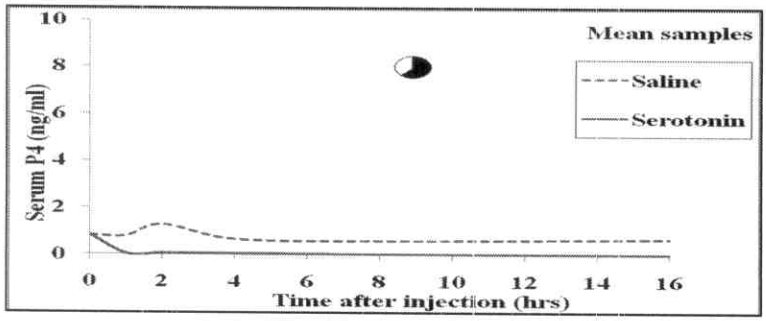
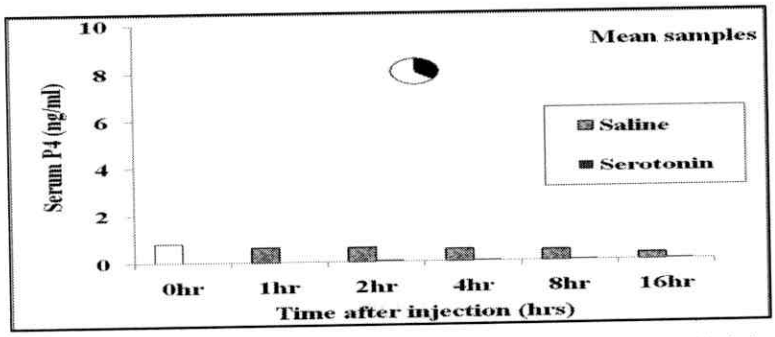
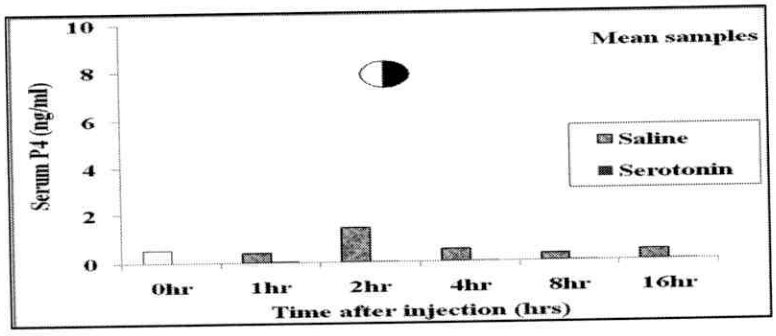


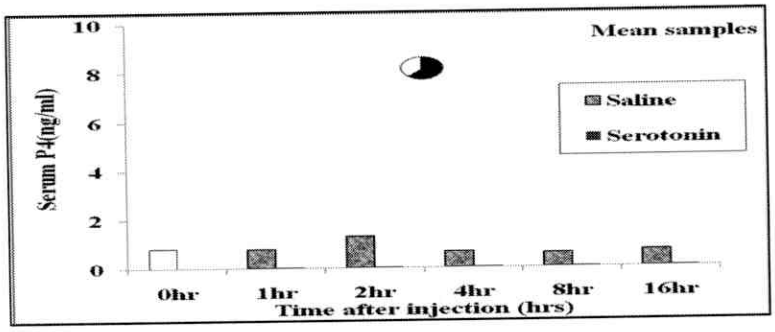
Figure (9) Serum progesterone level (ng/ml) in response to administration of serotonin vs saline at long darkness photoperiod (16D/8L).



Histogram (7) Serum progesterone level (ng/ml) in response to administration of serotonin vs saline at short darkness photoperiod (8D/16L).



Histogram (8) Serum progesterone level (ng/ml) in response to administration of serotonin vs saline at equal dark / light Photoperiod (12D/12L).



Histogram (9) Serum progesterone level (ng/ml) in response to administration of serotonin vs saline at long darkness photoperiod (16D/8L).

4.1.5. Effect of bromocriptine:

Data concerning the effect of injection experimental rabbits, subjected to three different lighting regimes, with bromocriptine are tabulated in **Table (9)** represented in **Figures (10, 11 and 12) and Histograms (10, 11 and 12)**, analysis of variance for obtained data is shown in **(Table 10)**.

Highly significant effect on serum progesterone level was found due to injection applied and the interaction between lighting regime and injection ($p<0.01$). While lighting regime showed significant effect ($P<0.05$).

From results obtained, it was obviously found that injection bromocriptine lowered the level of serum progesterone. However, the rate of decrease differed according to lighting regime and the time intervals after injection. The greatest decrease level was observed when applying animals to short lighting regime (16 hrs D /8 hrs L), while the lowest rate of decrease was found in the group of animals subjected to long lighting regime (8 hrs D /16 hrs L).Serum progesterone level in rabbits subjected to short lighting regime (16 hrs D /8 hrs L) decreased after injection bromocriptine and remained approximately constant along the whole experimental period with value mentioned (0.1665 ng/ml) **(Table 9)**. Different pattern of variation was found in rabbits subjected to either long lighting regime (8 hrs D/16 hrs L) or regime of equal dark and light

period (12 hrs D /12 hrs L).Serum progesterone level in rabbits subjected to long lighting regime increased at 1 hr after injection then decreased thereafter up to the 4th hr after injection, after that serum progesterone level increased at the 8th hr, then decreased sharply at the 16 hrs. However, serum progesterone level slightly and almost increased up to the end of the experimental period **(Table 9)**. Obtained results agreed with the scientific facts concerning the relationship between bromocriptine biological action as dopamine agonist from one side and the biological action of dopamine as a primary neuroendocrine inhibitor of secretion of prolactin from the pituitary gland **(Browman *et al.*, 2005)**. It is well known that bromocriptine is an ergoline derivative, which act as dopamine agonist used in the treatment of hyperprolactinaemia **(Boyed, 1995)**. Hyperprolactinaemia may stimulate hypothalamus negative feedback effects on gonadal steroids (**McNeilly, 1980)**. It is suggested that bromocriptine exerts its effect through the hypothalamic- hypophyseal-ovarian axis, which influence the processes of ovarian follicle formation and ovulation. This is tern affect the number of corpora lutea and correspondingly affect the serum progesterone level. Rabbit, which is induced ovulating animal, has a complex noradrenergic coital stimulus which is interrelated to gonadotropic releasing hormones **(Kaynard *et al.*, 1990)**. Obtained results go in agreement with those of **Yoshimura *et al.*, (1990)** who stated that prolactin in rabbits was found to

interfere directly with mechanical events within the ovary, thus preventing rupture of mature follicles via inactivation of plasminogen activator system. On the other hand inhibition of prolactin via bromocriptine injection in rabbits leads to stimulate ovulatory process ending with corpora lutea formation and progesterone secretion. Therefore, noradrenergic and dopaminergic neurons interrelated in hypothalamus is expected as a dopamine agonist (bromocriptine), which might result in enhancing the HCG – ovulation – induced rise in progesterone. This effect might have resulted from lifting the prolactin inhibitory effect on ovarian function **(McNeilly *et al.*, 1982).**

Inspection of data revealed that, effect of bromocriptine injection on progesterone levels increased during long photoperiod (8D/16L) compared to short photoperiod (16D/8L), where neuropeptide Y was found to stimulate GnRH release, which in turn is mediated via α1- adrenergic receptors. These receptors could be stimulated by increased levels of dopamine resulting from bromocriptine injection **(Berria *et al.*, 1991)**. Therefore, priming rabbit with dopamine further potentiates the effect of LH releasing hormone as manifested with higher progesterone levels **(Yoshimura *et al.*, 1990).**

Table (9): Progesterone serum levels (ng/ml) $\bar{x}$±SE in response to injection of bromocriptine vs saline at short darkness photoperiod (8D/16L), equal dark and light photoperiod (12D/12L) and long darkness photoperiod (16D/8L).

Time after injection	Saline			Bromocriptine		
	8D/16L	12D/12L	16D/8L	8D/16L	12D/12L	16D/8L
0hr	0.3518±0.0448	0.5169±0.1248	0.8723±0.1633	0.1665±0.0001	0.1665±0.0001	0.1672±0.0007
1hr	0.6303±0.1729	0.3871±0.0445	0.7767±0.2260	0.3444±0.1775	0.1704±0.0039	0.1665±0.0001
2hr	0.6034±0.0897	1.4128±0.9812	1.2750±0.4254	0.2058±0.0655	0.1712±0.0048	0.1665±0.0001
4hr	0.5303±0.0887	0.5035±0.2030	0.6457±0.0937	0.1950±0.0689	0.1928±0.0263	0.1665±0.0001
8hr	0.4546±0.1422	0.2775±0.0173	0.5748±0.1137	0.3493±0.2268	0.1665±0.0001	0.1665±0.0001
16hr	0.2927±0.0323	0.4057±0.103	0.6742±0.2112	0.1332±0.0333	0.2183±0.0518	0.1665±0.0001

Table (10): Analysis of variance of data (cumulative area under curve in units) collected after injection of bromocriptine vs saline under different photoperiods.

S.O.V	d.f	S.S	M.S	F
Photoperiod	2	17.07983412	8.53992	4.3383*
Injection	1	223.429331	233.4293	118.5829**
Interaction	2	279.115395	139.5577	70.89578**
Residual	28	55.1177544	1.968491071	
Total	33	574.0948941	17.39681515	

*Significant at level of 0.05

**Significant at level of 0.01

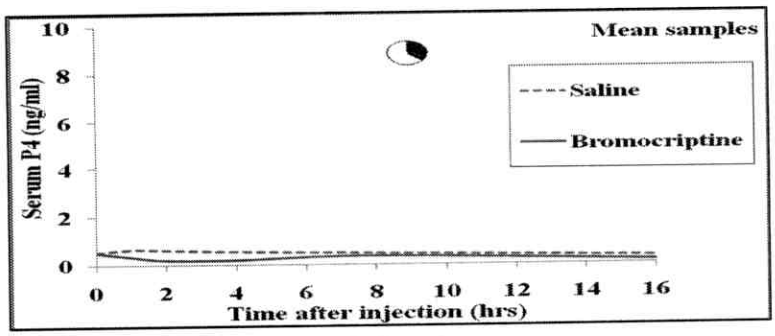


Figure (10) Serum progesterone level (ng/ml) in response to administration of bromocriptine vs saline at short darkness photoperiod (8D/16L).

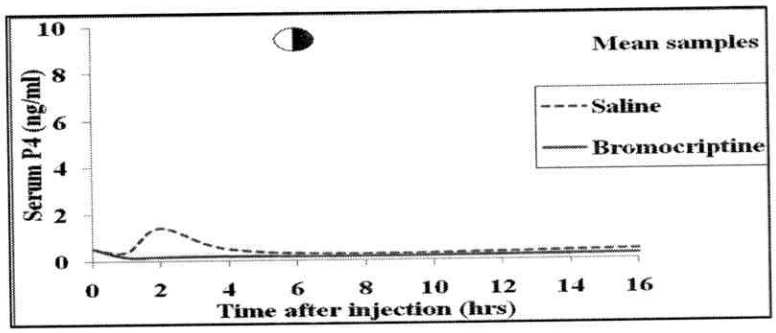


Figure (11) Serum progesterone level (ng/ml) in response to administration of bromocriptine vs saline at equal dark / light photoperiod (12D/12L).

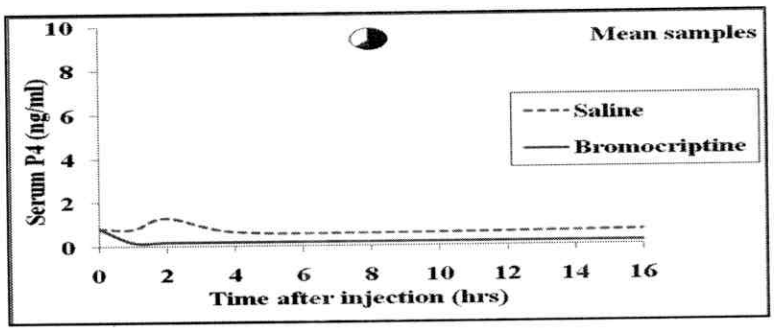
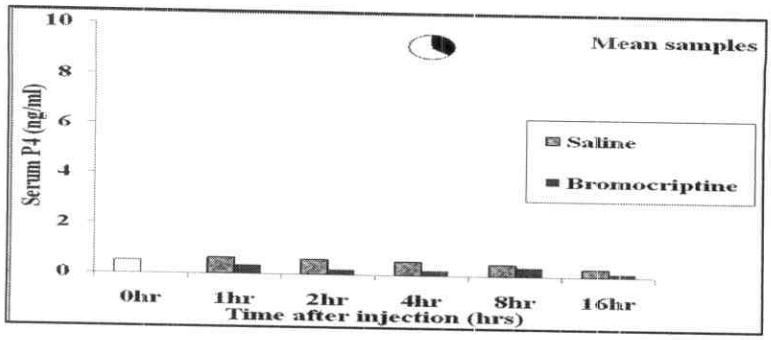
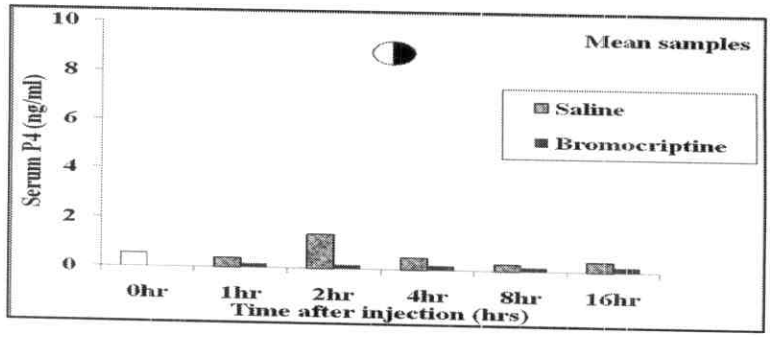


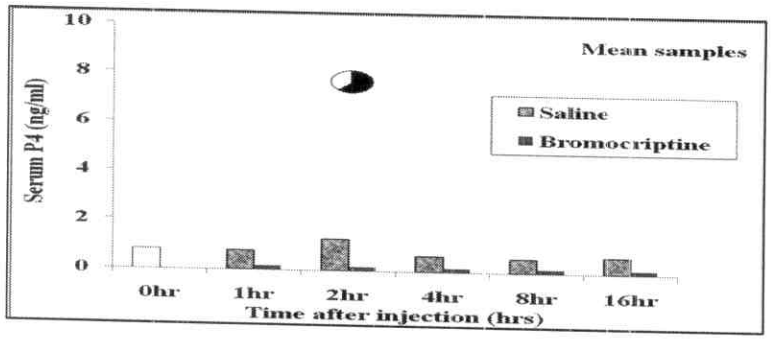
Figure (12) Serum progesterone level (ng/ml) in response to administration of bromocriptine vs saline at long darkness photoperiod (16D/8L).



Histogram (10) Serum progesterone level (ng/ml) in response to administration of bromocriptine vs saline at short darkness photoperiod (8D/16L).



Histogram (11) Serum progesterone level (ng/ml) in response to administration of bromocriptine vs saline at equal dark / light photoperiod (12D/12L).



Histogram (12) Serum progesterone level (ng/ml) in response to administration of bromocriptine vs saline at long darkness photoperiod (16D/8L).

4.1.6. Effect of indomethacin:

Data tabulated in **Table (11)** represented in **Figures (13, 14 and 15) and Histograms (13, 14 and 15)** show that means of serum progesterone levels in experimental rabbits subjected to different lighting regimes applied and treated with indomethacin (indometacin) drug that antagonist prostaglandin through its effect to inhibit its production. Analysis of variance for data obtained is listed in **ANOVA Table (12)**. Detecting obtained data related to the previously mentioned concern revealed insignificant variation in serum progesterone level in animals injected with indomethacin due to photoperiod applied. This could be considered as a logic result since photoperiod may affect surge of gonadotrophic hormone (especially LH hormone) rather than the level of progesterone in blood serum which is considered as a function of number of ovulated follicles and correspondingly number of corpora lutea succeeded to be developed of the ovulation and persistency for biosynthesizing and secreting progesterone hormone along the pregnancy period.

On the other hand, significant variation ($P<0.05$) was observed in serum progesterone level due to injecting indomethacin. Rabbits treated with indomethacin had almost higher level of progesterone along the time passed after

injection. However, the rate of increment differed according to lighting regimes applied and estimation period. This was insured by the highly significant interaction effect between photoperiod and injection ($P<0.01$).

The previously mentioned results go in a harmony with many of scientific statement .It is well known now that indomethacin (or indometacin) is non-steroid drug which is non selective inhibitor of cyclooxygenase (COX) 1 and 2, enzymes, that participate in prostaglandin synthesis from arachidonic acid. So indomethacin is an effective tocolytic agent, able to delay premature labor by reducing uterine contractions through inhibition of PG synthesis in the uterus and possibly through calcium channel blockade. **(Giles and Bisits, 2007)**. In addition, treating pregnant does with indomethacin and or any of tocolytic medication may result in increasing the litter surge. On the other hand injection of indomethacin prior to LH inhibited the ovulatory process in the rabbit **(Grinwich *et al.*, 1972; O'Grady *et al.*, 1972a)** and indomethacin inhibited coitus induced ovulation in the rabbit **(O'Grady *et al.*, 1972a).**

Table (12): Analysis of variance of data (cumulative area under curve in units) collected after injection of indomethacin versus saline under different photoperiods.

S.O.V	d.f	S.S	M.S	F
Photoperiod	2	14.087074	7.043535	1.9693
Injection	1	16.823256	16.82326	4.7036*
Interaction	2	330.8706334	165.4353	46.254**
Residual	23	82.263366	3.576668	
Total	28	444.0433	14.0687	

*Significant at level of 0.05

**Significant at level of 0.01

Table (11): Progesterone serum levels (ng/ml) $\bar{x}$±SE in response to injection of indomethacin vs saline at short darkness photoperiod (8D/16L), equal dark and light photoperiod (12D/12L) and long darkness photoperiod (16D/8L).

Time after injection	Saline			Indomethacin		
	8D/16L	12D/12L	16D/8L	8D/16L	12D/12L	16D/8L
0hr	0.3518±0.0448	0.5169±0.1248	0.8723±0.1633	0.3869±0.2407	0.5996±0.2647	0.0860±0.032
1hr	0.6303±0.1729	0.3871±0.0445	0.7767±0.2260	0.7817±0.2021	0.5606±0.4232	0.2430±0.1337
2hr	0.6034±0.0897	1.4128±0.9812	1.2750±0.4254	0.4369±0.0945	0.2506±0.1109	0.1477±0.0531
4hr	0.5303±0.0887	0.5035±0.2030	0.6457±0.0937	1.0858±0.6363	0.2898±0.1499	0.5623±0.2919
8hr	0.4546±0.1422	0.2775±0.0173	0.5748±0.1137	0.4270±0.1301	0.5804±0.1614	0.2974±0.2030
16hr	0.2927±0.0323	0.4057±0.1030	0.6742±0.2112	0.2390±0.0409	0.1367±0.0136	0.1441±0.0790

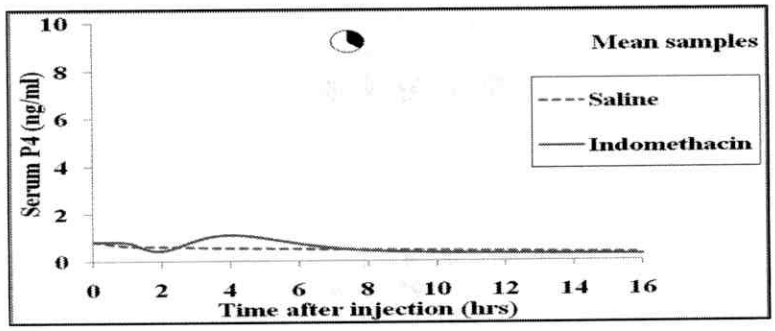


Figure (13) Serum progesterone level (ng/ml) in response to administration of indomethacin vs saline at short darkness photoperiod (8D/16L).

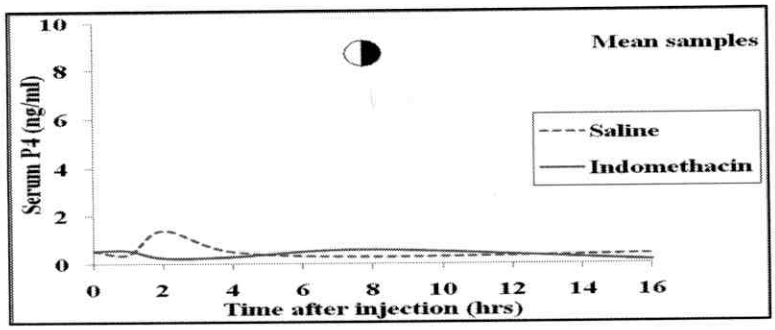


Figure (14) Serum progesterone level (ng/ml) in response to administration of indomethacin vs saline at equal dark / light photoperiod (12D/12L).

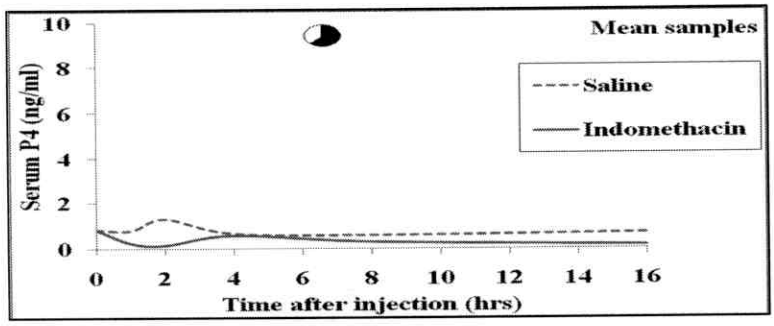
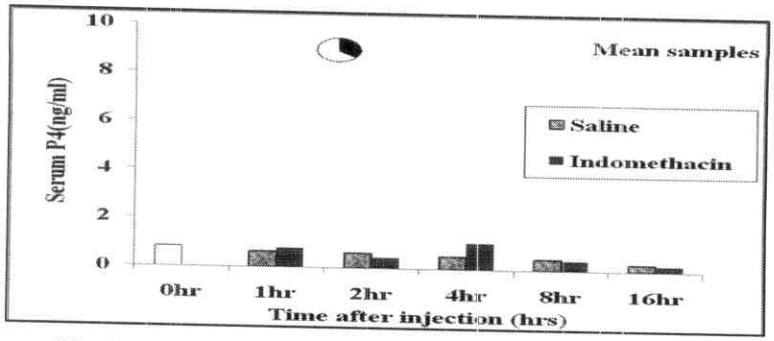
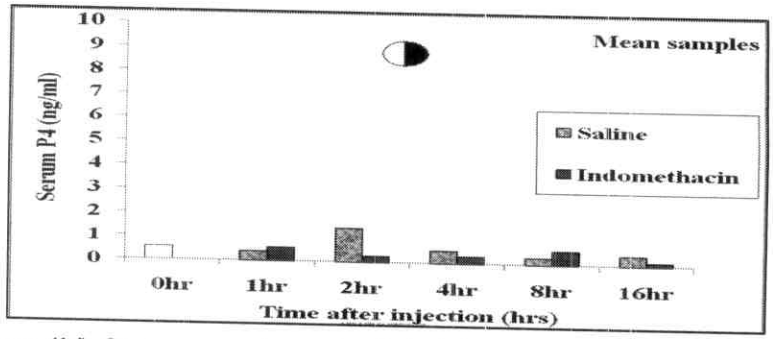


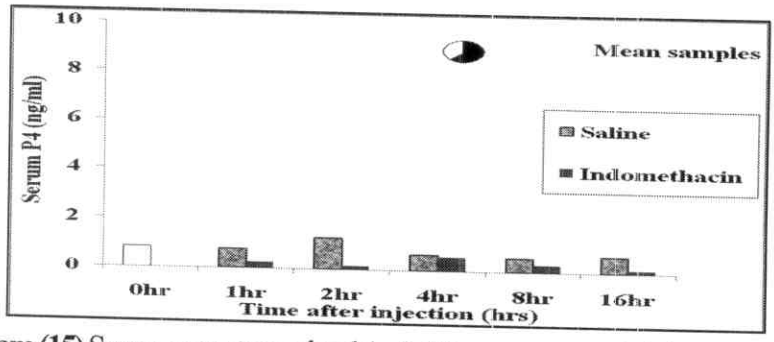
Figure (15) Serum progesterone level (ng/ml) in response to administration of indomethacin vs saline at long darkness photoperiod (16D/8L).



Histogram (13) Serum progesterone level (ng/ml) in response to administration of indomethacin vs saline at short darkness photoperiod (8D/16L).



Histogram (14) Serum progesterone level (ng/ml) in response to administration of indomethacin vs saline at equal dark / light photoperiod (12D/12L).



Histogram (15) Serum progesterone level (ng/ml) in response to administration of indomethacin vs saline at long darkness photoperiod (16D/8L).

4.2. Second experiment:

The second experiment was planned and conducted to point out the magnitude of improving the hormonal pattern in does subjected to different lighting regimes by treating animals with HCG alone or in combination with pharmaceutical preparations (melatonin, serotonin, bromocriptine, indomethacin) that are thought to have considerable effect on efficiency of biosynthesize and secretion of progesterone along a period of 16 hrs after injection.

4.2.1. Serum progesterone level in does treated with HCG:

Data presented in **Table (13)** represented in **Figures (16, 17 and 18) and Histograms (16, 17 and 18)** show the level of serum progesterone level in does subjected to different lighting regimes and treated with HCG (subcutaneous Injection). From data obtained it is obviously clear that serum progesterone level increased as time after injection passed up to 2 or 4 hrs after injection then decreased sharply thereafter to reach its minimum level after 16 hrs. However, the rate of increase or decrease differed according to the lighting regimes applied. The higher rate of increase occurred during the first 4 hrs after injection was found in does subjected to short lighting regime (16 hrs D/8 hrs L). It increased from (0.18665 ng/ml) at pre injection (0 hr) to (13.0508 ng/ml) at the 2nd hr and to (14.0844 ng/ml) at the 4th hr after injection .Similar trend of

increase, but with lower rate was found in does subjected to long lighting regime (8 hrs D/16 hrs L). Serum progesterone level in does of this group increased from (0.0973 ng/ml) at pre injection time (0hr) to (6.9526 ng/ml) at the 4th hr after injection quite different pattern of change in serum progesterone level was observed in does subjected to a lighting regime characterized by an equal light and dark periods (12 hrs D/12 hrs L). In this case, lower rate of increase was observed after 2hrs from injection time serum progesterone level decreased from (0.2005 ng/ml) before injection time (0hr) to (3.2797 ng/ml) after 2hrs from injection then decreased sharply up to the 16th hr.

From previously mentioned result, it could be concluded that progesterone biosynthesis and/or surge is directly affected by lighting regime. It seems that short lighting regime (16hrs darkness and 8hrs lighting) is the most convenient lighting regime for progesterone biosynthesis and/or surge by corpus luteum. In addition, results obtained go in harmony with various statements concerning the biological function of HCG. It worth to mention that HCG interacts with LHCG receptor and promotes the maintenance of corpus luteum during the beginning of pregnancy, causing it to secrete the hormone progesterone **(Cole, 2009)**. In addition it was suggested that HCG may be a link in the development of peritrophoblastic immune tolerance, and may facilitate the

trophoblast invasion, which is known to expedite embryonic development in the indomethacin **(Kayisly *et al.*, 2003)**.Because of its similarity LH, HCG can also be used clinically to induce ovulation in the ovaries **(www.IVF.com)**.

4.2.2. Serum progesterone level in does treated with HCG in combination with melatonin:

Data concerning the level of serum progesterone in does subjected to applied lighting regimes and injected with HCG in combination with melatonin are tabulated in **Table (13)** represented in **Figures (16, 17 and 18) and Histograms (16, 17 and 18)**. Analysis of variance for obtained data is shown in **Table (14)**.

From data obtained it could be reported that injecting does with HCG in combination with melatonin decreased the level of progesterone in blood serum when compared with injecting animals with HCG only. The rate of decrement differed according to the lighting regime applied. The higher decreasing rate occurred when applying short lighting regime (16 hrs D / 8 hrs L) followed by applying lighting regime of equal light-dark period (12 hrs L / 12 hrs D). However, less rate of decrease was observed when applying long lighting regime (8hrs D / 16 hrs L). On the other hand serum progesterone level increased as time of injecting HCG in combination with melatonin passed reaching its maximum level at the 8th hr in short lighting regime (16 hrs D / 8 hrs L) and long

lighting regime (8 hrs D / 16 hrs L) after injection .This was quite true in all lighting regimes applied but with great variation within them. The higher increasing rate was observed in does of long lighting period (8 hrs D/16 hrs L). In this case serum progesterone level increased from (1.2092 ng/ml) at 0hr to (7.4325 ng/ml) at the 8th hr after injection. The rate of increase was very low when applying short lighting regime. It increases from (0.1334 ng/ml) at 0hr to (0.4351 ng/ml) at the 8th hr after injection. However, the higher serum progesterone level was observed at the 4th hr after injecting does subjected to lighting regime of equal Light /Dark period (12 hrs D/12 hrs L). Analysis of variance for obtained data **(Table 14)** shows significant variation in serum progesterone level due to lighting regime applied only ($P < 0.05$). Results obtained were scientifically logic and agree with different statements related to the biological action of either HCG or melatonin. It is well known that melatonin lowers FSH levels but it improves the thyroid function **(Bellipanni *et al.*, 2005)**.

Table (13): Progesterone serum levels (ng/ml) $\bar{x}$±SE in response to injection of melatonin+HCG vs HCG at short darkness photoperiod (8D/16L), equal dark and light photoperiod (12D/12L) and long darkness photoperiod (16D/8L).

Time after injection	HCG			Melatonin+HCG		
	8D/16L	12D/12L	16D/8L	8D/16L	12D/12L	16D/8L
0hr	0.0973±0.0516	0.2005±0.0821	0.18665±0.0371	1.2092±0.2311	0.0680±0.0492	0.1334±0.0950
1hr	0.1925±0.1273	1.2733±0.2450	0.2626±0.0610	6.1484±1.9946	0.1515±0.1327	0.0354±0.0083
2hr	5.3860±5.0900	3.2797±0.9767	13.0508±3.3939	6.7445±3.2104	0.4652±0.3526	0.0356±0.0102
4hr	6.9526±5.3762	0.8559±0.4248	14.0844±3.4214	4.3127±1.8072	2.0006±1.5144	0.0662±0.0242
8hr	1.3982±0.9838	0.25727±0.0884	6.0789±1.1813	7.4325±2.8376	0.5933±0.3604	0.4351±0.2040
16hr	1.1544±0.62064	0.1384±0.1768	1.443±0.3942	3.4128±0.3831	0.0757±0.0458	0.0447±0.0447

Table (14): Analysis of variance of data (cumulative area under curve in units) collected after injection of melatonin+HCG versus HCG under different photoperiods.

S.O.V	d.f	S.S	M.S	F
Photoperiod	2	13879.29693	6939.65	4.5709*
Injection	1	1215.96854	1215.969	0.80092
Interaction	2	10133.342	5066.67	3.3373
Residual	14	21255.0175	1518.2157	
Total	19	46483.62497	2446.5063	

*Significant at level of 0.05

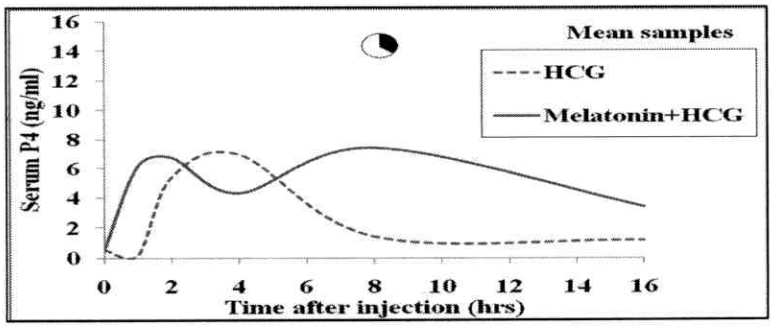


Figure (16) Serum progesterone level (ng/ml) in response to administration of melatonin+HCG vs HCG at short darkness photoperiod (8D/16L).

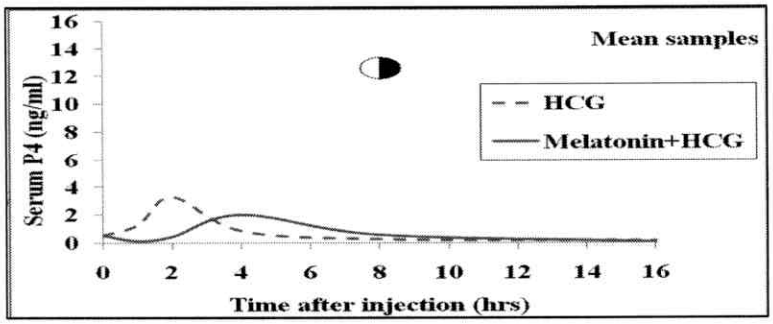


Figure (17) Serum progesterone level (ng/ml) in response to administration of melatonin+HCG vs HCG at equal dark / light photoperiod (12D/12L).

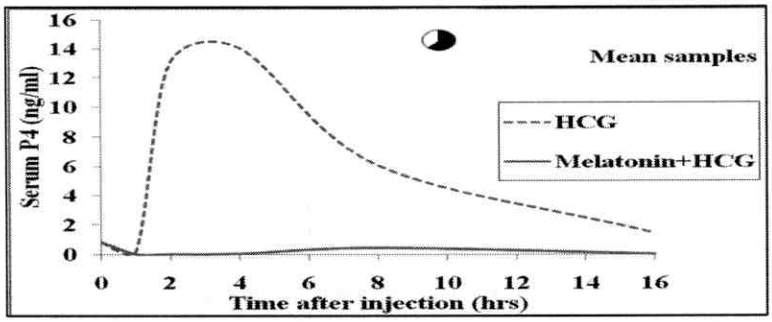
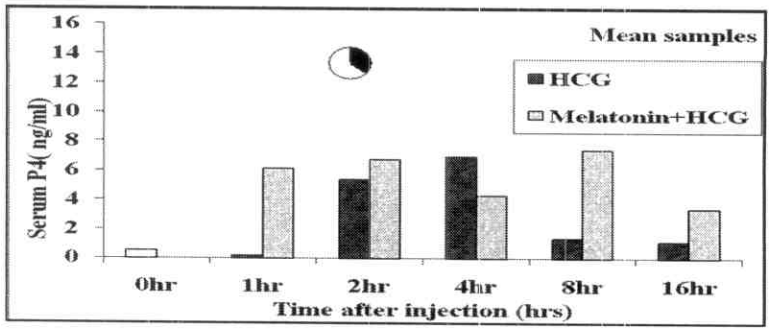
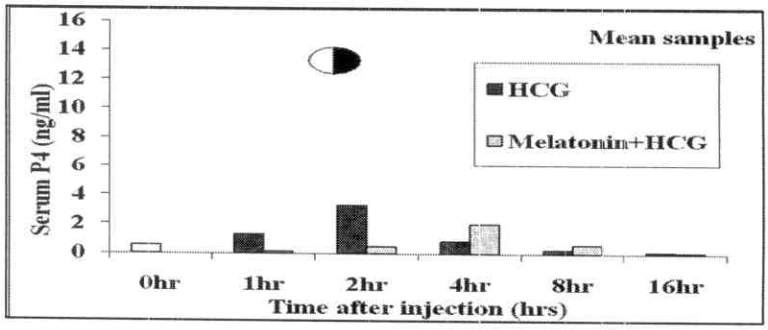


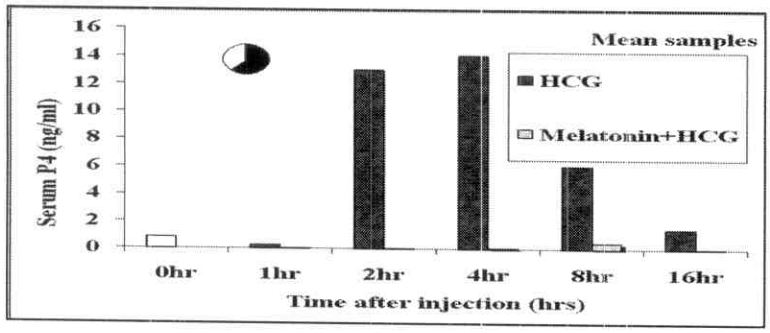
Figure (18) Serum progesterone level (ng/ml) in response to administration of melatonin+HCG vs HCG at long darkness photoperiod (16D/8L).



Histogram (16) Serum progesterone level (ng/ml) in response to administration of melatonin+HCG vs HCG at short darkness photoperiod (8D/16L).



Histogram (17) Serum progesterone level (ng/ml) in response to administration of melatonin+HCG vs HCG at equal dark / light photoperiod (12D/12L).



Histogram (18) Serum progesterone level (ng/ml) in response to administration of melatonin+HCG vs HCG at long photoperiod (16D/8L).

4.2.3. Serum progesterone level in does treated with HCG in combination with serotonin:

Data concerning the level of serum progesterone level in does subjected to different lighting regimes and treated with HCG in combination with serotonin are tabulated in **Table (15)** represented in **Figures (19, 20 and 21) and Histograms (19, 20 and 21)**. Analysis of variance for this data is presented in **Table (16)**.

Obtained data revealed that injecting experimental does with HCG in combination with serotonin resulted into decreasing serum progesterone level when compared by those injected with HCG alone. However, the rate of decrease differed within the various lightening regime applied. Higher serum progesterone levels (0.1983 ng/ml) were observed at the 4th hr after injection in does subjected to long lighting regime (8 hrs D/16 hrs L) and to (1.8238 ng/ml) in does subjected to equal lighting regime (12hrs D/12 hrs L). However, higher progesterone levels in blood serum was observed at the 8th hr after injection for does subjected to short lighting regime (16hrs D / 8hrs L) that mounted (2.5732 ng/ml). However, no characterized pattern was found in variation of serum progesterone level as time after injection passed.

The decrease in serum progesterone level due to treatment with HCG in combination with serotonin may attribute to the effect of serotonin, which acts as growth factor directly. In addition,

serotonin was found to suppress insulin release from beta cells in the pancreas (**Paulmann *et al.*, 2009**). Correspondingly affect the energy supply needed to reproductive activity in general and development of corpora lutea .This may be reflected as decrease in serum progesterone level.

Table (15): Progesterone serum levels (ng/ml) $\bar{x}$±SE in response to injection of serotonin+HCG vs HCG at short darkness photoperiod (8D/16L), equal dark and light photoperiod (12D/12L) and long darkness photoperiod (16D/8L).

Time after injection	HCG			Serotonin +HCG		
	8D/16L	12D/12L	16D/8L	8D/16L	12D/12L	16D/8L
0hr	0.0973±0.0516	0.2005±0.0821	0.18665±0.0371	0.1599±0.0686	0.2270±0.1357	0.3116±0.0812
1hr	0.1925±0.1273	1.2733±0.2450	0.2626±0.0610	0.1284±0.0263	0.1439±0.0526	0.3111±0.1970
2hr	5.3860±5.0900	3.2797±0.9767	13.0508±3.3939	0.1180±0.0120	1.1678±0.8608	0.3903±0.2827
4hr	6.9526±5.3762	0.8559±0.4248	14.0844±3.4214	0.1983±0.1070	1.8238±0.8977	0.7255±0.4929
8hr	1.3982±0.9838	0.25727±0.0884	6.0789±1.1813	0.1652±0.0547	0.4613±0.1910	2.5732±2.2926
16hr	1.1544±0.62064	0.1384±0.1768	1.443±0.3942	0.1606±0.0346	0.2016±0.1103	0.6513±0.4200

Table (16): Analysis of variance of data (cumulative area under curve in units) collected after injection of serotonin +HCG versus HCG under different photoperiods.

S.O.V	d.f	S.S	M.S	F
Photoperiod	2	12969.05293	6484.525	16.3403**
Injection	1	8457.804431	8457.804	21.3127**
Interaction	2	11713.83551	5856.92	14.7588**
Residual	14	5555.809439	396.8435	
Total	19	38696.50231	2036.6579	

**Significant at level of 0.01

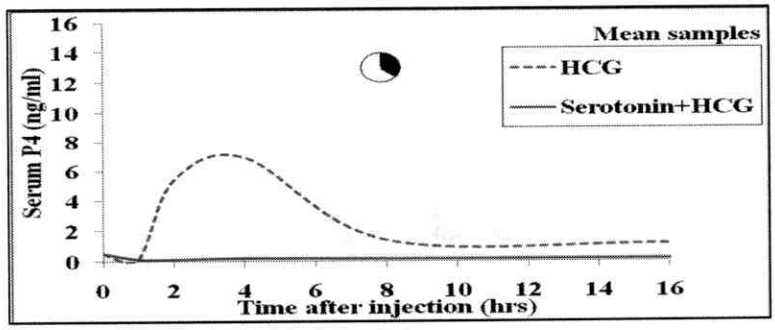


Figure (19) Serum progesterone level (ng/ml) in response to administration of serotonin+HCG vs HCG at short darkness photoperiod (8D/16L).

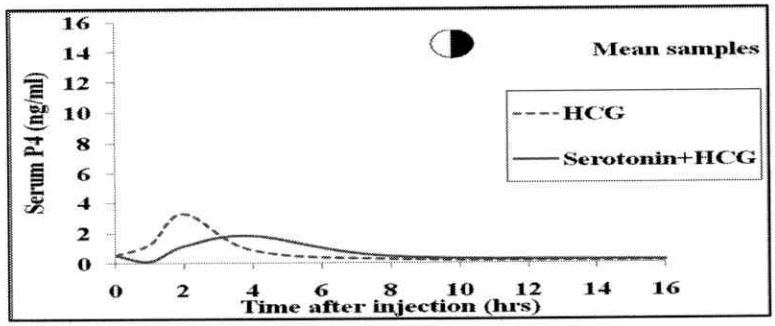


Figure (20) Serum progesterone level (ng/ml) in response to administration of serotonin+HCG vs HCG at equal dark / light photoperiod (12D/12L).

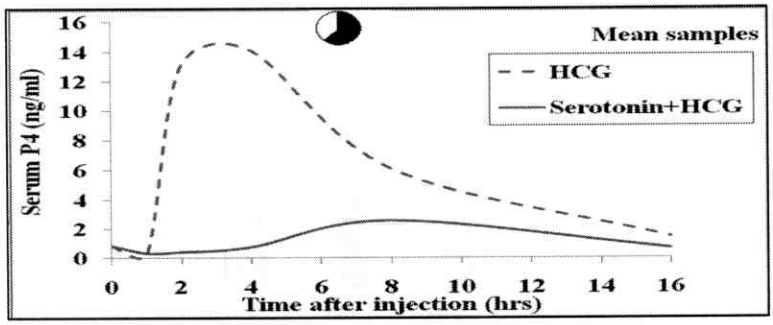
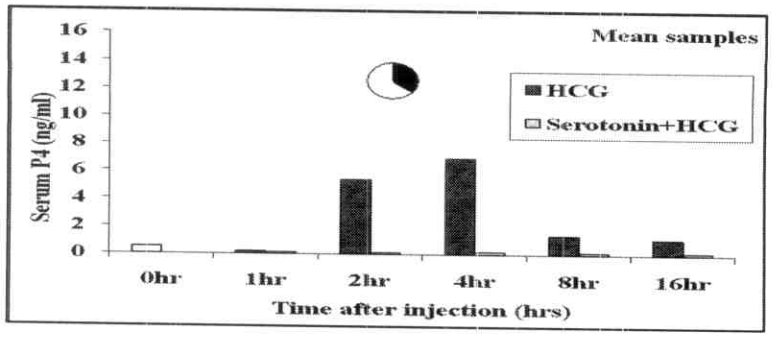
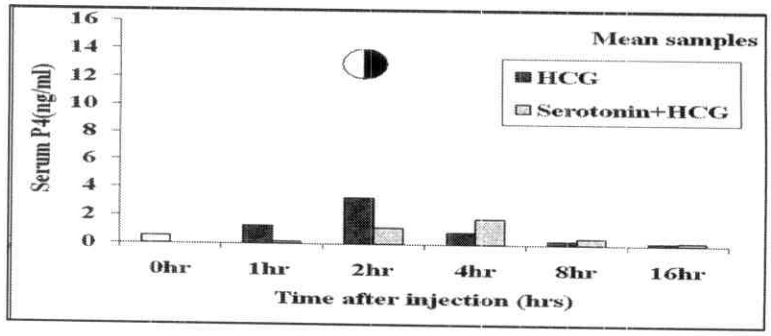


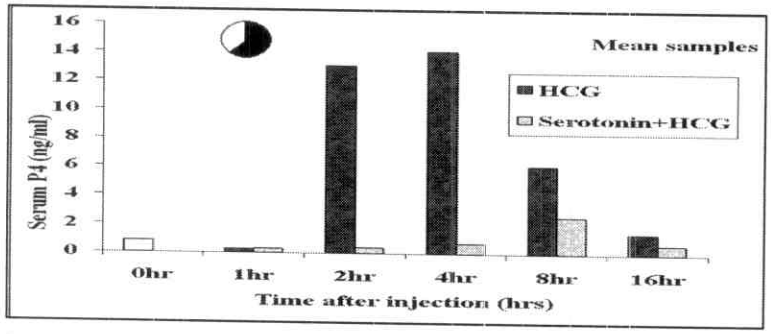
Figure (21) Serum progesterone level (ng/ml) in response to administration of serotonin+HCG vs HCG at long darkness photoperiod (16D/8L).



Histogram (19) Serum progesterone level (ng/ml) in response to administration of serotonin+HCG vs HCG at short darkness photoperiod (8D/16L).



Histogram (20) Serum progesterone level (ng/ml) in response to administration of serotonin+HCG vs HCG at equal dark / light photoperiod (12D/12L).



Histogram (21) Serum progesterone level (ng/ml) in response to administration of serotonin+HCG vs HCG at long darkness photoperiod (16D/8L).

4.2.4. Serum progesterone level in does treated with HCG in combination with bromocriptine:

Table (17) represented in **Figures (22, 23 and 24) and Histograms (22, 23 and 24)** shows averages of serum progesterone level in experimental does exposed to different lighting programs and treated with either HCG alone or in combination with bromocriptine (Dopamine agonist drug that blocks D_2 receptor in adenohypophysis to inhibit prolactin release).

Results obtained revealed that injecting HCG in combination with bromocriptine mostly increase the level of serum progesterone than did injecting HCG alone. This was quite true in does of all lighting regimes applied but with different increment magnitudes. The higher increment rate was obviously found in does subjected to short lighting program (16hrs D/ 8hrs L). When compared with those subjected to other two lighting regimes applied (8hrs D/16hrs L) or (12 hrs D /12 hrs L). Approximately similar trend of variation in serum level of progesterone was observed in does of these two lightening regimes mentioned.

Serum progesterone level in does injected with HCG in combination with bromocriptine increased as time after injection passed. It reached its highest level at the 3rd hr after injection. At this time average of serum progesterone level weighed (3.12616 ng/ml , 3.5317 ng/ml and 28.7236 ng/ml) in does subjected to

lighting program of (8 hrs D/16 hrs L , 12 hrs D/12 hrs L and 16 hrs D/8 hrs L), respectively **(Table 17)**. Analysis of variance for data obtained is listed in **ANOVA Table (18)**.

It could be concluded that injecting does with HCG in combination with bromocriptine becomes more efficient in increasing the level of serum progesterone. This may be due to the inhibiting action of bromocriptine on prolactin that may stimulate LH surge, which has positive effect on maintenance of corpora lutea, and correspondingly increase progesterone secretion. It may be also suggested that adding bromocriptine to HCG improve either the rate of follicular formation and development by diminishing the antagonist action of prolactin against gonadotrophic hormones in general and luteinizing hormone in particular. This LH surge triggers ovulation thereby not only release the egg, but also initiating the conversion of residual follicle into a corpus luteum that, in turn, produces progesterone to prepare indomethacin for a possible implantation. In addition, LH is necessary to maintain luteal function along the gestation period. Obtained results go in agreement with those of **Lafond *et al.*, (1986),** who reported that inhibition of prolactin via bromocriptine treatment potentiates the HCG stimulatory effect. Furthermore, bromocriptine (a dopamine agonist) administration elicits a dopamine rise in blood levels of the neurotransmitter. High dopamine levels inhibits prolactin secretion

(buys *et al.*, 1990), whereas elevated concentrations of blood prolactin were found to directly inhibit ovarian functions and decrease progesterone levels **(McNeilly *et al.*, 1982).** It could be concluded that inhibition of prolactin via bromocriptine treatment would further potentiates the effect of HCG in ovulation induction and subsequent corpus luteum function.

Table (17): Progesterone serum levels (ng/ml) $\bar{x}$±SE in response to injection of bromocriptine +HCG vs HCG at short darkness photoperiod (8D/16L), equal dark and light photoperiod (12D/12L) and long darkness photoperiod (16D/8L).

Time after injection	HCG			Bromocriptine +HCG		
	8D/16L	12D/12L	16D/8L	8D/16L	12D/12L	16D/8L
0hr	0.0973±0.0516	0.2005±0.0821	0.18665±0.0371	1.5770±0.3026	1.3418±0.4245	3.1927±0.5687
1hr	0.1925±0.1273	1.2733±0.2450	0.2626±0.0610	1.4760±0.2359	1.6364±0.2426	7.6859±2.4423
2hr	5.3860±5.0900	3.2797±0.9767	13.0508±3.3939	1.5848±0.5753	1.7510±0.4316	18.8177±6.1949
4hr	6.9526±5.3762	0.8559±0.4248	14.0844±3.4214	3.1262±0.5344	3.5317±0.6712	28.7236±9.2569
8hr	1.3982±0.9838	0.25727±0.0884	6.0789±1.1813	1.9163±0.6560	6.2311±1.3188	13.1143±1.7458
16hr	1.1544±0.62064	0.1384±0.1768	1.443±0.3942	1.4406±0.5183	4.7947±2.1990	6.8879±1.7410

Table (18): Analysis of variance of data (cumulative area under curve in units) collected after injection of bromocriptine +HCG versus HCG under different photoperiods.

S.O.V	d.f	S.S	M.S	F
Photoperiod	2	119607.4	59803.7	2455.5705**
Injection	1	48359.28	48359.28	1985.6567**
Interaction	2	44374.86	22187.43	911.0272**
Residual	17	414.0231	24.3543	
Total	22	212655.499	9670.7045	

**Significant at level of 0.01

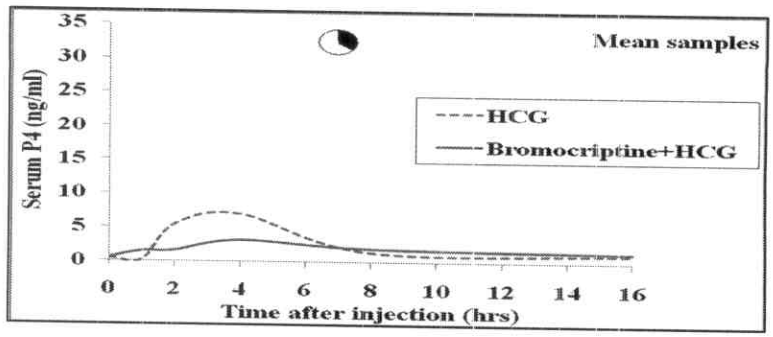


Figure (22) Serum progesterone level (ng/ml) in response to administration of bromocriptin+HCG vs HCG at short darkness photoperiod (8D/16L).

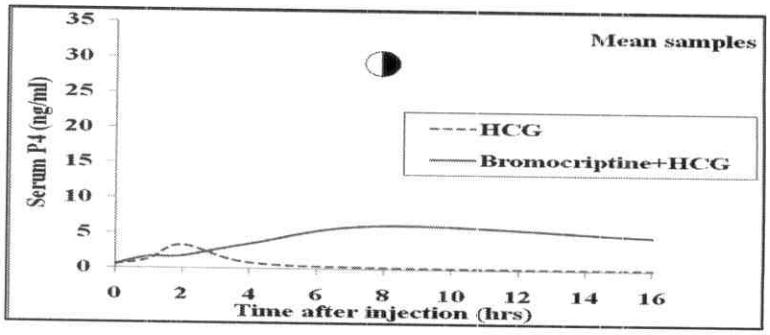


Figure (23) Serum progesterone level (ng/ml) in response to administration of bromocriptin+HCG vs HCG at equal dark / light photoperiod (12D/12L).

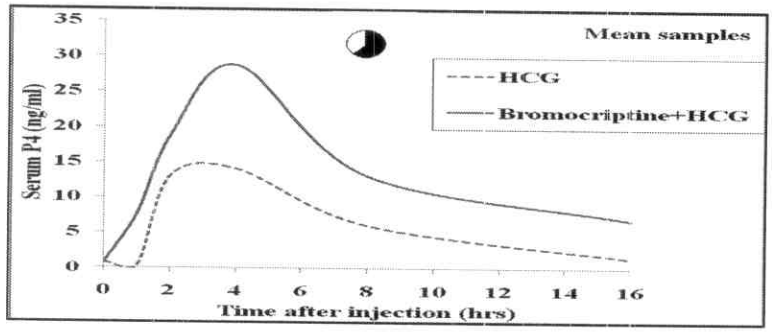
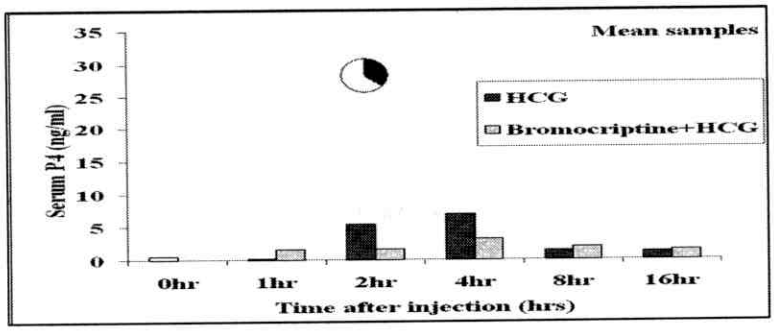
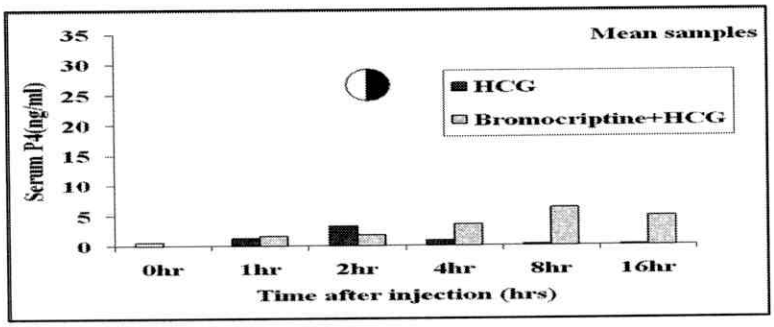


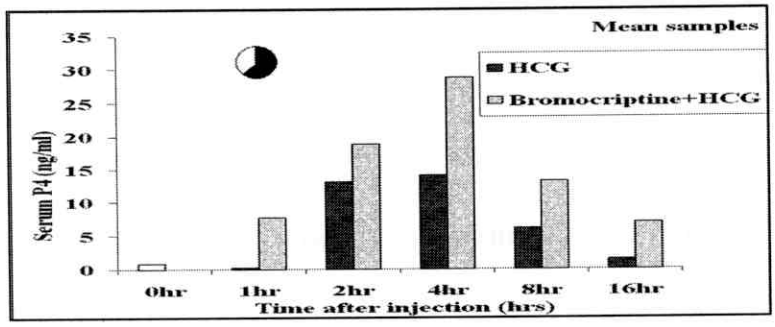
Figure (24) Serum progesterone level (ng/ml) in response to administration of bromocriptin+HCG vs HCG at long darkness photoperiod (16D/8L).



Histogram (22) Serum progesterone level (ng/ml) in response to administration of bromocriptine+HCG vs HCG at short darkness photoperiod (8D/16L).



Histogram (23) Serum progesterone level (ng/ml) in response to administration of bromocriptine+HCG vs HCG at equal dark / light photoperiod (12D/12L).



Histogram (24) Serum progesterone level (ng/ml) in response to administration of bromocriptine +HCG vs HCG at long darkness photoperiod (16D/8L).

4.2.5. Serum progesterone level in does treated with HCG in combination with indomethacin:

Averages of serum progesterone level in does exposed to three lighting programs and treated with either HCG only or in combination with indomethacin are tabulated in **Table (19)** represented in **Figures (25 , 26 and 27) and Histograms (25, 26 and 27)**. Indomethacin was applied as medication inhibit production of prostaglandins and therefore inhibit uterus contraction .Inspecting the obtained data, it was found that injection HCG in combination with indomethacin increased the level of serum progesterone level when compared with injection HCG alone. However, the rate of increase differed according lighting regime applied the greater increment rate was found in does subjected to long darkness photoperiod of (16 hrs D and 8 hrs L). Does of long lighting period regime (8 hrs D / 16 hrs L) shows lowest rate of serum progesterone level. While does of an equal dark and light regime (12 hrs D /12 hrs L) shows intermediate serum progesterone level values.

The level of serum progesterone increased during the early period of the injection then decreased there after reaching its lowest level at the 16th hr after injection. The highest serum progesterone level was found at the 4th hr after injecting does subjected to short lighting regime (16 hrs D/ 8 hrs L), it

mounted (201.6806 ng/ml) However, the highest values were found at the second hour after injecting does subjected to equal lighting regime (12 hrs D/ 12 hrs L) (35.1933 ng/ml) and at the 16th hr after injecting does subjected to long lighting regime (8 hrs D/ 16 hrs L) (30.5246 ng/ml). Analysis of variance of obtained data showed highly significant variation in average serum progesterone level due to lighting regime and injection ($P<0.01$) **(ANOVA Table 20)**. Increasing the average of serum progesterone level due to injecting does with HCG in combination with indomethacin may be attributed to cumulative biological function of HCG and indomethacin. HCG, which interacts with LHCG receptor and promotes the maintenance of corpus luteum during the beginning of pregnancy, causing it to secrete the hormone progesterone **(Kayisly *et al.*, 2003)**. In addition, indomethacin works by inhibiting the production of prostaglandin and inhibit the uterine contraction **(Ferreia *et al.*, 1971)**.

Table (19): Progesterone serum levels (ng/ml) $\bar{x}$±SE in response to injection of indomethacin +HCG vs HCG at short darkness photoperiod (8D/16L), equal dark and light photoperiod (12D/12L) and long darkness photoperiod (16D/8L).

Time after injection	HCG			Indomethacin +HCG		
	8D/16L	12D/12L	16D/8L	8D/16L	12D/12L	16D/8L
0hr	0.0973±0.0516	0.2005±0.0821	0.1867±0.0371	1.5653±0.1089	8.2714±1.1120	1.5488±0.2956
1hr	0.1925±0.1273	1.2733±0.2450	0.2626±0.0610	27.6202±14.6004	9.1089±2.5677	21.5399±5.4167
2hr	5.3860±5.0900	3.2797±0.9767	13.0508±3.3939	15.1631±0.8003	35.1933±13.3733	70.7313±17.0880
4hr	6.9526±5.3762	0.8559±0.4248	14.0844±3.4214	12.8135±2.7953	30.7422±7.0919	201.6806±46.5550
8hr	1.3982±0.9838	0.25727±0.0884	6.0789±1.1813	19.4920±2.4440	23.1776±3.9866	86.9393±20.5270
16hr	1.1544±0.62064	0.1384±0.1768	1.443±0.3942	30.5246±10.6894	16.9155±1.7647	46.1649±9.8367

Table (20): Analysis of variance of data (cumulative area under curve in units) collected after injection of indomethacin+HCG versus HCG under different photoperiods.

S.O.V	d.f	S.S	M.S	F
Photoperiod	2	1186364.181	593182	7.22**
Injection	1	2469703.965	2469704	30.05**
Interaction	2	113514.2532	56757.1266	0.6905347
Residual	13	1068509.133	82193.01023	
Total	18	4838091.532	268782.89	

**Significant at level of 0.01

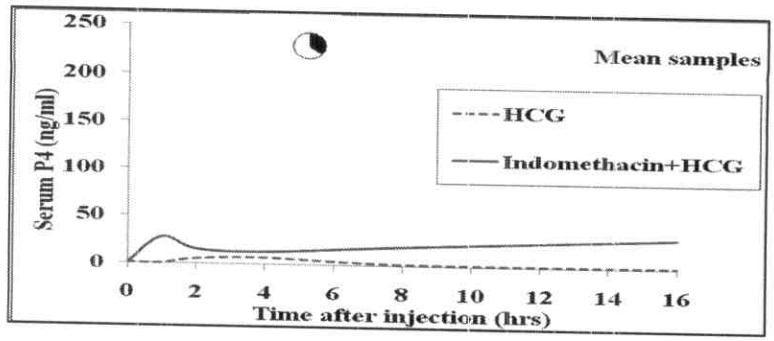


Figure (25) Serum progesterone level (ng/ml) in response to administration of indomethacin +HCG vs HCG at short darkness photoperiod (8D/16L).

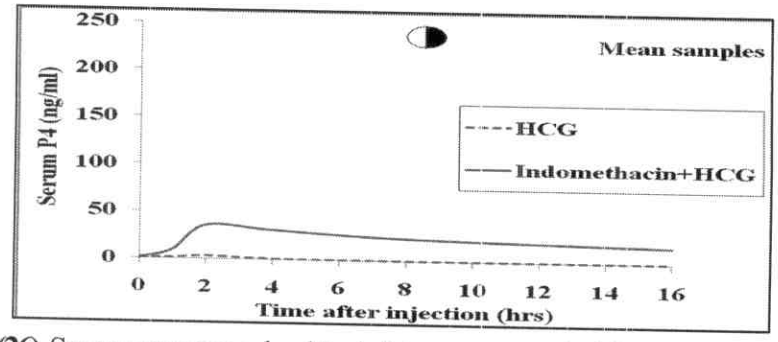


Figure (26) Serum progesterone level (ng/ml) in response to administration of indomethacin +HCG vs HCG at equal dark / light photoperiod (12D/12L).

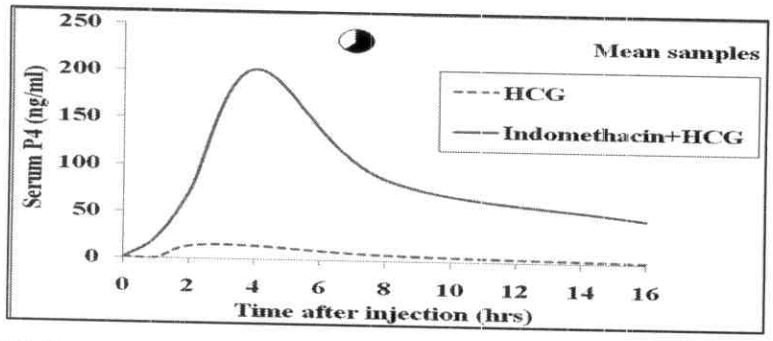
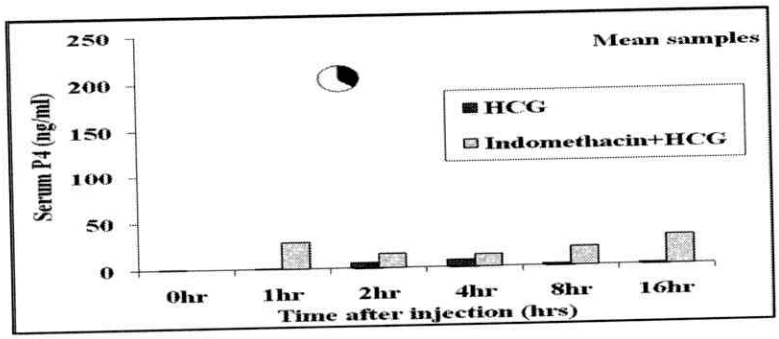
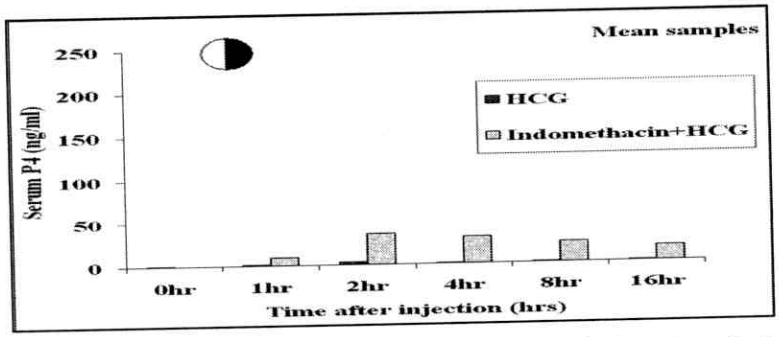


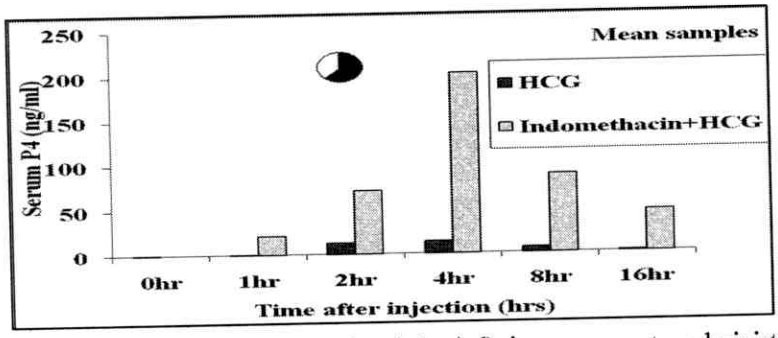
Figure (27) Serum progesterone level (ng/ml) in response to administration of indomethacin +HCG vs HCG at long darkness photoperiod (16D/8L).



Histogram (25) Serum progesterone level (ng/ml) in response to administration of indomethacin +HCG vs HCG at short darkness photoperiod (8D/16L).



Histogram (26) Serum progesterone level (ng/ml) in response to administration of indomethacin +HCG vs HCG at equal dark / light photoperiod (12D/12L).



Histogram (27) Serum progesterone level (ng/ml) in response to administration of indomethacin +HCG vs HCG at long darkness photoperiod (16D/8L).