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VALIDITY OF GNRH AND MELATONIN INTERACTION ON SOME REPRODUCTIVE PERFORMANCE OF BARKI RAMS

(With 2 Tables and 4 Figures)

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استخدام الهرمون الحاث لإفراز الجونادوترفين مع الميلاتونين لرفع الكفاءة التناسلية للكباش البرقى

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استخدم لهذه الدراسة عدد ١٢ كبشاً برقيا بالغا حيث تم تقسيمهم إلى أربعة مجموعات (٣ كباش في كل مجموعة) وتم حقن المجموعة الأولى بـ ٥ر١ جم من الميلاتونين تحت الجلد وحقنت المجموعة الثانية عضلياً بـ ١٢٥ ميكروجرام من الهرمون الحاث لإفراز الجونادوتروفين. أما المجموعة الثالثة فحقنت بالميلاتونين في اليوم الأول والثالث ثم بالهرمون الحاث لإفراز الجونادوتروفين في اليوم السابع والعاشر بنفس الجرعة والطريقة في المجموعتين السابقين. بالنسبة للمجموعة الرابعة تم حقن الهرمون الحاث لإفراز الجونادوتروفين أولاً ثم الميلاتونين بنفس طريقة الحقن في المجموعة الثالثة. تم تجميع عينات دم وسائل منوي قبل وأثناء فترة الحقن ثم استمر التجميع حتى ٩ أسابيع. قيمت عينات السائل المنوي من حيث حجم القذفة ، تركيز الحيامن نسبة الحركة الأمامية ، نسبة الحيامن الحية، التشوهات الشكلية للحيوان المنوى، محيط غشاء الصفن وزمن الوثبة، كما تم قياس تركيزات الهرمون الحاث لحويصلات جراف وهرمون الأباضة وهرمون الذكورة. أسفرت النتائج على أن حقن الميلاتونين فى المجموعة الأولى كان له تأثيراً معنوياً حيث زادت نسبة الحيامن الحية وتركيز الحيامن ومحيط غشاء الصفن وزمن الوثبة والتشوهات الشكلية للحيوان المنوى مع زيادة فى تركيزات الهرمونات الثلاثة بالنسبة لقبل المعالجة. أما فى المجموعة الثانية فقد قل حجم القذفة وزمن الوثبة وتركيز الحيوانات المنوية معنوياً أما نسبة الحيامن الحية والتشوهات الشكلية وهرمون الذكورة فقد ازدادت معنوياً عن قبل الحقن. بالنسبة للمجموعة الثالثة اظهرت النتائج انخفاضاً معنوياً فى نسبة الحركة الأمامية وهرمون الذكورة بينما نتائج المجموعة الرابعة فقد اظهرت زيادة معنوية فى نسبة الحيامن الحية

والهرمون الحاث لحويصلات جراف وهرمون الذكورة وقلة فى هرمون الاباضة بالنسبة لقبل المعالجة وقد رجع زمن الوثبة إلى معدله الطبيعى بعد أن زاد أثناء التجربة. نستخلص مـــن هذه الدراسة أن حقن الميلاتونين بعد الهرمون الحاث لإفراز الجونادوتروفين صالحـــاً لرفـــع الكفاءة التناسلية فى الكباش البرقي.

SUMMARY

12 Sexually, mature Barki rams were used. They were divided into four comparable groups (3 rams/each), Group (1) was injected S/C twice daily by 1.5 mg melatonin, then injections were repeated at 3rd and 7th day. Group (2) was injected by 125 µg GnRH I/M at the same regimen of group 1. Group (3) was injected by melatonin at the 1st and 3rd day than followed by GnRH injection as in the first two groups. Group (4) was injected by GnRH followed by melatonin as the third group. Blood and semen samples were collected before and all over the period of treatment and persist till 9 weeks as post treated period. Semen samples were evaluated for semen volume, sperm concentration, percentages of live spermatozoa individual motility and total sperm abnormalities. Scrotal circumference and reaction time were measured. Plasma FSH, LH and testosterone were also determined. The obtained results revealed that melatonin injection (Group 1) was significantly increased the percentages of live spermatozoa, sperm concentration, scrotal circumference, reaction time and total sperm abnormalities. Also the levels of FSH, LH and testosterone were increased. While GnRH injection (group 2) decreased significantly the semen volume, reaction time and sperm concentration but with a significant increase in percentage of line spermatozoa, total sperm abnormalities sperm concentration and testosterone secretion. Injection of melatonin followed by GnRH (group 3) showed a significant decrease in percentage of individual motility and testosterone. While injection of GnRH followed by melatonin (group 4) revealed a significant increase in percentage of live spermatozoa, FSH and testosterone with decrease in LH level. The reaction time increased then return back to the normal. It was concluded that the use of melatonin after GnRH treatment are validated for reproductive improvement of Barki rams.

Key words: *Gnrh and Melatonin Interaction on Reproduction of Barki Rams.*

INTRODUCTION

Sheep is a photoperiodic species with tendency to the short day length for stimulating their reproductive cycle (Lincoln and Kelly 1989). This tendency is created by the pineal gland which modulate photoperiodic information to the hypo thalamohypophyseal, gonadal axis (Bhagat et al.,1994; Finley, et al., 1995) resulting in rhythmic secretion of melatonin with affinity to darkness (Rasmussen, 1993; Malpaux et al., 1996) Studies on the effect of either photpheriod (Schanbacher et al., 1979; Ravault, et al., 1986) or melatonin implants (Chemineau et al., 1988; Fitzgerald and Stellflug, 1991) were seemed to be of practical value in sheep management. Melatonin clearly affects the activity of the reproductive axis in many diverse species, but whether melatonin acts directly on gonadal tissue to either activate or inhibit function, or at the level of the hypothalamus, is still not clear (Kennaway and Hugel, 1992). Several studies by (Valenti et al., 1995) reported the inhibitory effect of Melatonin on testosterone secretion by purified rat Leydig cells. Thus, a relationship was supposed to present between melatonin and GnRH in their action on the reproductive cycle of rams (Kelly et al., 1994).

So, the present study was designed to assess the effects of Melatonin and/or GnRH in a synchronizing manner on the semen characteristics, scrotal circumference, reaction time and the associated hormonal changes (FSH, LH and testosterone) in Barki rams.

MATERIALS and METHODS

12 sexually, mature healthy Barki rams aged 3-5 years old were used. The rams were bred at the experimental farm of Animal Reproduction Research Institute (ARRI) Giza, Egypt. They were housed in an open yard under natural light condition. They were fed on farm diet which consists of concentrate mixture contains 14% crude protein and 11% crude fiber, 4 kg green corn (Darawa). Water and mineral blocks were offered ad. libtum. At the start of the experiment (Summer season) rams were divided into four comparable groups (3 rams/each), Group (1) was injected S/C twice daily (9 am"m". and 9 pm"e".) by 1.5 mg melatonin (Memphis Co. for Pharm. & Chemical Ind.Cairo Egypt), injections were repeated at 3rd and 7th day.

Group (2) was injected I/M by 125 µg GnRH (Fertagyl) Gonadorelin, Intervet Boxmeer Holland). At the same regimen of melatonin group.

Group (3) was injected by melatonin at the 1st and 3rd day followed by GnRH injection at the 7th and 10th day by the same route and dose as in the first two groups.

Group (4) was injected by GnRH at the 1st and 3rd day followed by Melatonin at the 7th and 10th day as the third group.

Heparinaized blood samples were collected from all rams 3 times before injection and served as control. After injection Heparinaized samples were taken twice daily at the 1st, the 3rd, 7th day and every 15 days for 9 weeks, and in group 3 and 4 exceeded by samples taken at the 10th days. The blood samples were centrifuged for 15 minutes at 3000 rpm then plasma were separated and stored at - 20°C for hormonal assay (FSH, LH and testosterone).

FSH and LH concentrations were determined by RAI (Radioimmunoassay) following the technique described by (Zia and Coombes, 1981) Testosterone was measured by the double antibody radioimmunoassay (Yen & Jaffe, 1978) using kits obtained from DPC (Diagnostic Production Corporation, Loss Angeles CA, USA).

Semen samples were collected using artificial vagina where first and second ejaculates were pooled from each ram.

Morning semen collections from control and treated rams were done weekly in a parallel time to blood collection.

Immediately after collection semen samples were evaluated (Salisbury et al., 1978) for semen volume (ml), sperm concentration ($X10^6$ sperm/ml), Percentages of individual motility, live spermatozoa and abnormal spermatozoa (Bloom, 1983). Scrotal circumference (mm) and reaction time (Sec.) also was measured.

All date were statistically analyzed using Costat computer Program, version 3.03 Copy right (1986) Cottort Software.

RESULTS

It was clear from the present results (Table 1) that melatonin injection significantly increase the percentage of live spermatozoa (treated and post treated periods), sperm concentration, scrotal

circumference, reaction time (treated period) and the percentage of total sperm abnormalities at the post treated period only. Neither semen volume nor percentage of individual motility affected by melatonin injection. Concerning the hormonal levels, melatonin injection was resulted in a significant increase in FSH, LH at the 3rd day and testosterone at the 1st day of the treated period.

At the same time, GnRH injection (Table 1) revealed a significant decrease in semen volume, reaction time (treated and post treated periods) and sperm concentration (Post treated period). While, a significant increase was observed in percentages of live spermatozoa (treated and post treated periods), total sperm abnormalities (post treated period) and sperm concentration (treated period). On the other hand, percentage of individual motility and scrotal circumference not affected by GnRH injection. Hormonal secretion under the effect of GnRH injection showed a significant increase in testosterone level at the last injection followed by a decrease in the 2nd week of treated period. No detectable change in FSH and LH concentrations.

Injection of melatonin in group 3 (Table 2) at the 1st and 3rd day showed an increased in percentage of individual motility and sperm concentration without any change in the mean values of hormonal concentrations. The following injection of GnRH at the 7th and the 10th day resulted in a significant decrease in percentages of sperm motility, live spermatozoa and testosterone level without any effect on the other parameters measured. The semen and blood samples collected after treatment showed a significant decrease in percentage of individual motility and testosterone level with a significant increase in percentagof individual motility and FSH level.

On the other hand, injection of GnRH in group 4 (Table 2) for two alternative days resulted in a significant decrease in percentage of individual motility and sperm concentration with a significant increase in FSH and testosterone concentration. The following injection of melatonin at the 7th and the 10th day revealed a significant increase in percentages of live spermatozoa, total sperm abnormalities, FSH and testosterone secretion. The post injection samples showed increase in percentage of live spermatozoa, FSH and testosterone with decrease in LH level. The reaction time increased then return back to the normal.

Fig. 1, 2, 3 and 4 showed a hormonal level of FSH, LH and testosterone under the effect of melatonin and/or GnRH injection.

DISCUSSION

Many trials have been adopted to synchronize the reproductive cycle of sheep as photoperiodic species (Lincoln and Ebling, 1985; Lincoln and Kelly 1989 and Malpaux et al., 1996). Most of these trials depended mainly on striking the hypothalamic-hypophyseal gonadal axis to evoke the sexual and testicular activities. This mechanism is controlled by some factors among them is the pineal melatonin secretion (Binkley, 1983; Bittman et al., 1983 and Finley et al., 1995).

The use of melatonin as a sexual promotor in sheep were reported by Morgan et al. (1992) and Robinson et al. (1993). The mechanism by which melatonin increased reproduction were through GnRH (Rasmussen, 1993), Sperm survival (Anwar et al., 1996), testicular LH concentration (Amador et al., 1986), testicular testosterone secretion (Boulakoud and Goldsmith, 1995) and sperm concentration, motile spermatozoa (Asher et al., 1987) on the other hand, many authors reported that melatonin decrease reproductive performance through decrease LH (Christopher et al., 1991), testosterone secretion (Anwar, 1987), testis width, Score count of spermatogenesis (Steinlechner Niklowitz, 1992 and Xiao et al., 1994).

The data obtained showed that melatonin increased semen picture through percentage of live spermatozoa and sperm concentration as reported by Asher et al., (1987). The increased effect on the percentage of live spermatozoa due to the antioxidant effect of melatonin (Poeggeler et al., 1993). Also, melatonin increased FSH, LH and early testosterone which may be resulted in orgasmic reaction of testosterone on the concentration of sperm.

The activating effect of melatonin injection on hormonal level was confirmed by Morgan and Williams (1989) they proved the activating effect of melatonin on the hypothalamic receptors, enhancing the GnRH release (Rasmussen, 1993) and consequently increase gonadotropins as well as testosterone level (Amador et al., 1986 and Boulakoud & Goldsmith 1995).

The use of GnRH in the reproduction was commonly accepted.

The previous reports showed an increase in testicular size (Lincoln, 1979), LH and testosterone (Jenweiz and Jhonson, 1983), Spermatogenesis (Courot and Ortavant, 1981) and improve semen production and Libido (Rao, 1990 and El-Azab et al., 1996).

The present data agree with the previous results in improvement of semen picture and decreased in the reaction time. The prolongation of testosterone as a result of GnRH was previously reported with Jenweiz and Johnson (1983).

One of the most common problems in the use of GnRH in the field of reproduction is the desensitizing effect as reported by Change et al. (1990) and Parinaud et al. (1992).

This desensitizing effect of GnRH may be due to the loss of its receptors by higher or prolonged doses (Davis et al., 1978 and Hertel and Perkins, 1984).

The use of melatonin pre and post GnRH treatment to decline its desensitizing effect and improve semen picture and reproductive performance were discussed in the present results.

Firstly, melatonin pre treated GnRH were resulted in decrease in percentage of individual motility and testosterone secretion with increase total sperm abnormalities accompanied by higher increasein FSH. On the other hand, melatonin post GnRH treatment resulted in improvement spermatogenesis in the form of percentage of live spermatozoa and improve the sperm concentration and semen volume as compared to GnRH injection alone. Moreover the increase in testosterone during the length of experimental periods were obtained. The third benefit is the decrease in the LH level due to its utilization by testes for sustained testosterone level. FSH also increased by combination of both treatments for spermatocyto genesis.

Finally, the use of meltonin after GnRH treatment are validated for reproductive improvement of Barki rams.

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Table (1): Effect of Melatonin / GnHR on Some Reproductive Performance (Mean ± S.E)

Treated Period	Volume ml		Indiv / Motil %		Live Sperm (%)		Concentration (x 100mil)		Abnormality %		Scr. Circ (mm)		Reaction Time (sec)		FSH		LH		Testosteron	
	G1	G2	G1	G2	G1	G2	G1	G2	G1	G2	G1	G2	G1	G2	G1	G2	G1	G2	G1	G2
Control	1.78 ± 0.12	1.87 ± 0.11	85.00 ± 2.88	88.89 ± 1.66	77.00 ± 4.00	81.07 ± 1.2	2850.0 ± 142.2	2743.3 ± 76.2	4.00 ± 1.53	4.00 ± 1.00	30.33 ± 0.32	30.30 ± 1.32	51.67 ± 10.42	63.03 ± 13.45	46.96 ± 13.00	41.61 ± 8.32	38.50 ± 5.33	43.48 ± 18.17	2.73 ± 0.73	3.38 ± 0.74
1st	1.50 ± 0.21	0.93** ± 0.08	88.33 ± 1.66	90 ± 0.0	72.67 ± 2.83	85.6 ± 5.89	3266.7 ± 756.6	3453.3 ± 633.7	3.67 ± 0.88	3.33 ± 0.33	30.83 ± 0.53	30.70 ± 1.67	64.33* ± 3.33	30.0* ± 1.50	48.41 ± 16.22	24.48 ± 3.46	26.97 ± 8.23	18.0 ± 2.50	7.78** ± 0.64	3.88 ± 0.85
3rd	1.33 ± 0.34	1.13** ± 0.12	86.67 ± 3.33	90 ± 0.0	90.33* ± 1.45	80.67 ± 1.2	4566.7** ± 118.3	2553.3 ± 300.2	5.33 ± 2.18	5.00 ± 2.08	30.00 ± 2.08	29.3 ± 1.44	48.33 ± 4.40	98.7 ± 37.7	83.13* ± 7.00	32.51 ± 8.08	80.65* ± 15.3	81.12* ± 1.95	5.86* ± 1.09	2.02 ± 0.71
7th	1.67 ± 0.66	1.40 ± 0.38	81.67 ± 8.33	86.33 ± 1.66	87.33* ± 2.49	91.00* ± 2.07	5146.7** ± 836.8	3813.3* ± 352.9	4.33 ± 0.33	2.67 ± 0.33	32.27** ± 0.14	32.00 ± 1.15	116.67** ± 8.0	26.00 ± 8.63	39.97 ± 24.26	72.45 ± 15.51	40.08 ± 15.09	50.95 ± 17.53	1.94 ± 0.83	8.42* ± 1.22
10th	1.83 ± 0.32	1.73 ± 0.41	90.00 ± 2.88	90 ± 0.0	89.67* ± 2.84	89.33** ± 0.67	2853.3 ± 311.8	2453.3 ± 240.1	2.67 ± 0.88	4.00 ± 1.00	31.83 ± 0.92	31.3 ± 1.04	63.33 ± 4.40	84.70 ± 14.60	47.55 ± 3.88	54.49 ± 12.88	44.81 ± 24.25	71.03 ± 8.88	5.24 ± 1.12	1.15* ± 0.20
Post treated period	1.49 ± 0.14	1.46 ± 0.12	88.67 ± 1.59	82.62 ± 2.09	85.95* ± 1.33	85.19 ± 1.64	2908.7 ± 281.6	1640.9** ± 117.66	8.95 ± 1.38	7.57* ± 1.06	31.14 ± 0.40	30.52 ± 0.53	63.71 ± 8.09	37.48* ± 4.07	54.18 ± 5.40	59.74 ± 8.98	51.93 ± 5.27	44.18 ± 8.97	3.49 ± 0.59	3.31 ± 0.56
Over all	1.48 ± 0.35	1.43 ± 0.30	88.28 ± 4.01	86.03 ± 2.97	85.03 ± 4.08	85.28 ± 3.85	3259.7 ± 317.9	2387.8 ± 460.8	6.89 ± 0.17	6.00 ± 0.72	31.15 ± 1.00	30.60 ± 1.38	63.53 ± 15.15	46.00 ± 16.16	52.16 ± 14.25	51.13 ± 13.80	44.41 ± 16.98	50.75 ± 15.67	4.45 ± 1.25	3.13 ± 1.38

G1: Group (1) Melatonin treatment

G2: Group (2) GnRH treatment

* P < 0.005

** P < 0.01

Compared with control sample

Table (2): Effect of (Melatonin + GnRH) and (GnHR + Melatonin) on Some Reproductive Performance (Mean ± S.E)

Treated Period	Volume ml		Indiv / Motil %		Live Sperm (%)		Concentration (x 100Mil)		Abnormality %		Scr. Circ (mm)		Reaction Time (sec)		FSH		LH		Testosteron	
	G3	G4	G3	G4	G3	G4	G3	G4	G3	G4	G3	G4	G3	G4	G3	G4	G3	G4	G3	G4
Control	1.80 ± 0.3	1.50 ± 0.12	86.00 ± 1.66	86.66 ± 1.66	85.67 ± 0.88	80.66 ± 0.32	2033.3 ± 168.2	2730.0 ± 219.6	3.33 ± 1.76	6.33 ± 1.33	30.0 ± 0.57	30.0 ± 1.99	54.67 ± 35.38	68.0 ± 9.53	39.14 ± 5.43	16.03 ± 1.22	37.17 ± 19.38	78.39 ± 8.12	6.57 ± 1.73	1.31 ± 0.38
1st	1.43 ± 0.48	1.60 ± 0.32	90.0* ± 0.0	88.33 ± 1.66	87.0 ± 2.08	81.33 ± 2.02	2040.0 ± 208.8	2746.0 ± 484.1	5.00 ± 1.53	8.67 ± 0.67	30.33 ± 1.83	31.10 ± 0.16	8.00 ± 3.05	54.66 ± 22.67	48.73 ± 24.89	59.17** ± 9.79	64.78 ± 9.49	26.15** ± 2.73	3.84 ± 2.07	0.19** ± 0.01
3rd	0.93 ± 0.21	1.10 ± 0.26	85.0 ± 0.00	65.0* ± 7.63	98.00 ± 2.31	79.0* ± 0.65	3113.3** ± 184.1	2333.3* ± 509.2	7.33 ± 1.86	7.33 ± 0.33	30.33 ± 1.32	31.0 ± 1.73	24.33 ± 11.91	93.0 ± 30.04	39.29 ± 11.67	57.58 ± 15.75	61.34 ± 18.35	43.16 ± 18.82	5.71 ± 1.37	3.48** ± 0.17
7th	2.03 ± 0.6	1.63 ± 0.53	83.33* ± 8.67	88.33 ± 6.66	65.67** ± 2.96	84.66* ± 1.44	1680.0 ± 200.3	2546.67 ± 429.4	3.33 ± 0.33	18.00** ± 2.08	30.33 ± 1.87	31.70 ± 0.36	40.67 ± 22.25	68.33 ± 14.52	60.87 ± 9.65	35.38** ± 2.25	42.5 ± 18.51	83.38 ± 0.55	5.90 ± 2.08	3.41** ± 0.3
10th	1.83 ± 0.49	1.40 ± 0.55	86.67** ± 4.4	86.66 ± 3.33	83.33** ± 3.33	83.33 ± 2.96	2280.0 ± 334.8	2600* ± 421.5	10.67 ± 5.46	16.33** ± 0.88	31.33 ± 1.20	32.0 ± 0.57	57.33 ± 1.33	68.0 ± 15.28	60.86 ± 13.63	88.29* ± 0.52	31.80 ± 7.84	56.38 ± 10.74	2.41* ± 0.38	8.58** ± 0.95
2nd w	1.23 ± 0.38	1.53 ± 0.54	83.33 ± 8.66	90 ± 0.0	83.33 ± 8.89	83.0 ± 1.52	2660.0 ± 514.7	3568.7 ± 237.1	6.00 ± 1.73	7.0 ± 1.0	30.05 ± 0.50	31.0 ± 0.5	55.00 ± 12.49	130.33** ± 9.57	61.24** ± 6.87	86.21** ± 0.8	10.96 ± 3.42	77.78 ± 12.34	1.23* ± 0.50	4.29 ± 1.78
Post treated Period	1.45 ± 0.14	1.46 ± 0.15	78.67** ± 2.0	87.38 ± 0.74	85.05 ± 0.74	85.05** ± 0.74	2068.8 ± 110.9	3720.9± 57.64	8.90* ± 1.50	8.00* 1.19	30.08 ± 0.64	31.00 ± 0.26	64.78 ± 7.24	85.19 ± 10.37	60.99 ± 7.88	47.25** ± 10.22	57.66 ± 11.83	47.08 ± 8.54	3.69* ± 0.61	3.18* ± 0.84
Over all	1.48 ± 0.38	1.48 ± 0.38	79.11 ± 5.91	85.51 ± 4.57	81.74 ± 5.14	83.64 ± 2.45	2180.0 ± 313.8	33515.5 ± 402.0	7.54 ± 0.96	9.05 ± 0.82	30.38 ± 1.30	31.22 ± 0.83	64.18 ± 24.27	84.51 ± 29.09	60.82 ± 12.77	49.42 ± 17.82	47.21 ± 16.05	47.92 ± 15.02	5.05 ± 0.58	3.54 ± 1.48

G3: Group (3) Melatonin followed by GnRH

G3: Group (4) GnRH followed by Melatonin

* P < 0.005

** P < 0.01

Compared with control sample