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EVALUATON OF SUPEROVULATORY RESPONSE IN ANOESTROUS AND CYCLIC NATIVE COWS IN EGYPT WITH EMPHASIS ON HORMONAL AND BLOOD BIOCHEMICAL CHANGES

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SUMMARY

Ovarian status of donors at the time of commencing superovulation was a detrimental factor in superovulation regimen. The present study was carried out on 8 purebred native cows. Three superovulation regimens were conducted. Experiment1(n=8), non cyclic cows received norgestomet ear implants, 3500 i.u. eCG on day 7 post implantation and 250 µg GnRH analogue was injected during the induced oestrus. Experiment 2(n=6), cyclic cows received the same regimen with injection of 2500 i.u. of eCG. Experiment 3 (n=6), the same cyclic cows received the same treatment as in experiment 2, GnRH was replace with 2000 i.u. hCG. i.m during oestrus. Timing to oestrus and duration of oestrus were recorded. Ovarian response was determined on day 7 post oestrus using rectal palpation and ultrasonography. Blood samples were collected on days-11, -4, -2, 0 and +7 during reatments Clear plasma was separated and stored at -20°C until analysis of P4 and T4 (RIA), cholesterol, total lipids, triglycerides, total protein, albumin, glucose and alkaline phosphatase (Spectrophotometery). The analysis of data showed that anoestrous cows displayed oestrous behaviour at a significantly ($P<0.05$) longer time (52.00 ± 2.53, 50.40 ± 2.19 and 45.60 ± 2.40 for exp. 1, 2 and 3 resp.), and significantly higher plasma P4 levels at implant removal ($P<0.01$) and during oestrus ($P<0.05$). The number of CL and unovulated follicles did not differ significantly between anoestrous and cyclic cows (5.80 ± 0.80 and 6.00 ± 1.34 ; 7.60 ± 1.31 and 5.20 ± 2.13, 8.00 ± 1.34 and 3.40 ± 1.83 for exp.1, 2 and 3 resp). Plasma P4 levels reached their maximal values on day 7 post oestrus with a significant ($P<0.01$) lower valuc in exp. 1 compared with exp. 2 and 3. Plasma T4 level significantly ($P<0.01$) decreased in exp.1 following

treatment. Plasma P4 and glucose concentrations at eCG injection correlated significantly (P<0.01) with the subsequent ovulation rate. Plasma P4 correlated significantly (P<0.01) with the number of corpora lutea, glucose and cholesterol values. However, the correlation between other blood metabolites and either P4 or ovulation rate lack significanc. In conclusion, ovarian response to superovulation in native cows in Egypt is not affected by the ovarian status. Plasma P4 and glucose play a significant role in the subsequent ovulation rate. Other blood metabolites were of no value in the control of ovarian response to superovulation in native cows in Egypt. Ultrasonography is more accurate in predicting the superovulation response than rectal palpation.

Keywords: Reproductive status, anoestrum, superovulation, ultrasonography, hormones, blood metabolites.

INTRODUCTION

Increasing the reproductive performance of endogenous cattle breed remains an important challenge for developing countries aiming for increased self sufficiency in animal production. In the same time, the application of modern technology such as embryo transfer could be used as a tool for the production of large numbers of offspring that can be obtained from superior animals.

Superovulation protocols in cattle is still associated with variable and unpredictable ovulation rate and recovery of transferable embryos. This variability is influenced by breed and individual factors (Crister et al., 1980, Bindon et al., 1986) as well as dose and batch of gonadotrophin (Hill et al., 1984, Goulding et al., 1991). In addition, hCG can be used for control of oestrous expression and consequently ovulation and fertilization rates in superovulated animals (Uoc et al., 1997). Conflicting results appear in the literature regarding the effect of reproductive status as well as the level of blood metabolites on the out come of attempts for induction of superovulation in cattle. Some reports recorded that blood metabolites are not a good indicator for ovarian activity (Tegegne et al., 1993) or the number of corpora lutea after superovulation (Gehrken, 1986).

Ultrasonography scanning of the ovaries holds a promise for improving superovulation by increasing our understanding of the source of individual animal variability and offer a mean for accurately monitoring of physiological changes occurring in bovine ovaries when subjected to oestrous synchronization and superovulatory treatments (Armstrong, 1993, Sawyer et al., 1995 and Taneja et al., 1995).

The current study was undertaken to evaluate: (1) the effect of ovarian status and different superovulation regimens on the ovulation rate in native cows in Egypt , (2) changes and relationship between the levels of P4, T4 and some blood me

tabolites and the ovarian response to superovulation.

MATERIALS AND METHODS

Animals and Husbandry.

Eight non lactating purebred pluriparous native cows (4-6 years old and weighing 350-450 kg) were used in this study. Cows were stall fed and maintained at the National Research Center Experimental Farm (Abou Rawash, Giza, Egypt). The study was conducted between October 1997 and June, 1998.Each cow was fed on 5 kg commercial concentrate mixture and rice straw (ad libitum). Barseem (*Trifolium alexandrinum*) was provided from December to May (ad libitum).

In experiment 1, eight cows were used, however, in experiment 2, and 3, six cows were used (2 cows were excluded). The superovulation regimen was repeated every 2-3 month.

Hormonal preparations.

The synthetic progestogen used is a silicon ear implant containing norgestomet (17 α-acetoxy-11 B-methyl-19-nor-preg-4-en,3,20-dione)which was available as a commercial synchronizing treatment, Crestar® (Intervet, Boxmeer, The Netherlands). Each implant containing 3 mg norgestomet. Two ml injection solution containing 3 mg norgestomet and 5 mg oestradiol valerate was given at implant insertion.

The same batch of Folligon® (Freeze dried 1000 i.u of equine chorionic gonadotrophin, eCG + 5 ml sterile phosphate buffered saline), Fertagile® (GnRH analogue, 100 µg/ml) and Prosolvin® (Luprostiol, prostaglandin F2α, 7.5 mg/ml) were purchased from Intervet Egypt. Pregnyl® (5000 i.u of freeze dried human chorionic gonadotrophin, hCG, + 1 ml solvent was purchased from Nile Pharmaceutical Co. Egypt.

Ovarian ultrasonography.

Trans-rectal ultrasonography of ovaries was carried out using Pie Medical 480 (Masstricht, The Netherlands) with a rectal 5.0 MHZ probe and provided with a printer. Each ovary was scanned on day 7 post oestrus. The image was frozen on the screen and printed out.

Blood analysis.

Blood samples were collected from all cows before progestogen (D-11), at gonadotrphin treatment (D-4), progestogen withdrawal (D-2), day of superovulatory oestrus (D 0) and on day 7 post-oestrus (D 7). Samples were collected into heparinized tubes by direct Jugular vein puncture, centrifuged (x 1500g for 15 minutes) and the harvested plasma was frozen at-20°C until P4 and T4 levels were analyzed using commercial RIA kits (Coated A. Count, Diagnostic Product Corp., Los Angles, California, USA) according to Abraham (1981) and Albertine and Ekins (1982) for P4 and T4 resp. The sensitivity of the assays were 0.02 ng/ml and 0.25 µg/ml for P4 and T4 resp. The intra-and-inter-assay coefficient of variations

were 4.65, 5.15 and 3.15 and, 8.18 % for P4 and T4 resp.

Cholesterol, total lipids, triglycerides, total protein, albumin, glucose, and alkaline phosphatase concentrations were chemically determined using specific kits and spectrophotometery (Wilding and Kennedy, 1977). Globulin levels were determined by subtracting albumin from total protein values.

Experimental design.

Experiment 1, was carried out on eight anoestrous cows, that received norgestomet ear implants for 9 days. On day 7 after implant application, 3500 i.u. eCG was injected i.m. followed by 48h later by implant removal and injection of 15 mg $PGF_2\alpha$. During oestrus, cows were injected with 250 µg GnRH analogue.

Experiment 2, was carried out on six cyclic cows from the previous experiment, using the same protocol except for injection of 2500 i.u. eCG instead of 3500 i.u.

Experiment 3, the same six cyclic cows were treated as in experiment 2. However each cow was injected with 2000 i.u. hCG i.m. during oestrus instead of GnRH.

In all experiments. cows were monitored for oestrus by visual observations twice a day (at 9.00 a.m. and 18.00 p.m) using intact bull. Timing to oestrus and duration of oestrus were recorded. The number of corpora lutea and unovulated follicles were determined on day 7 post oestrus using rectal palpation and ultrasonography. On day 8 post oestrus, cows were injected with 15 mg $PGF_2\alpha$.

Statistical analysis.

Data were statistically analyzed using ANOVA and correlation analysis (← Snedecor and Cochran,1980).

RESULTS

Ovarian response.

Data presented in Table (1) depict the response of native cows to the different superovulation regimens. Occurrence of oestrous behavior after PGF2α injection differed significantly among treatments. Cows in experiment 1 displayed oestrous behavior at a significant ($P<0.05$) longer time. However, the duration of oestrus was more or less the same in all experiments.

The number of CL detected by rectal palpation and confirmed by ultrasonography (Fig. 1) on day 7 post oestrus did not differ among experiments, the highest ovulation rate was recorded in experiment 3. In the mean time, ultrasound examination illustrated the presence of CL of different sizes (0.89-1.86 cm, Fig. 1), with a high number of unovulated follicles in response to superovulation treatments. The highest number of unovulated fol-

Table 1 : Response of anoestrous and cyclic native cows in Egypt to superovulation regimens (Mean + S. E.).

Exp	Animals (No.)	Resp. (%)	Timing to oestrus (h)	Duration of oestrus (h)	Ovarian response	
					CL	Unovulated follicles
I	8	100	52.00 ± 2.53(b)	~24	5.80 ± 0.80	6.00 ± 1.34
II	6	100	50.40 ± 2.19	~24	7.60 ± 1.31	5.20 ± 2.13
II	6	100	45.60 ± 2.40(a)	~24	8.00 ± 1.34	3.40 ± 1.83

Columns with different superscripts differ at $P < 0.05$.

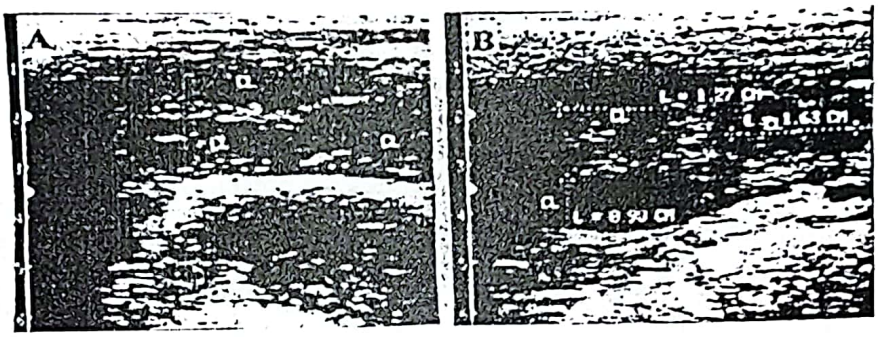

Fig. 1 : Ultrasonographic picture of ovaries in native cows in Egypt during superovulation (a) ovarian response (b) different sized CL

lides was detected in experiment 1.

Blood Analysis

Hormonal levels.

Plasma P4 and T4 levels are presented in Fig. 2. In exp.1, plasma P4 level was low ($P<0.01$) in anoestrous cows before norgestomet application and levels were nearly similar in all experiments at eCG administration. However, P4 level was significantly higher in cows in experiment 1 at norgestomet ear implant withdrawal ($P<0.01$) and during oestrus ($P<0.05$). On day 7 post oestrus, P4 level reached its maximal value in all experiments. Moreover, cows in experiments 2 and 3 had a significant ($P<0.01$) higher P4 levels when compared with cows in experiment 1(Fig. 2).

Analysis of data indicated that plasma P4 level on

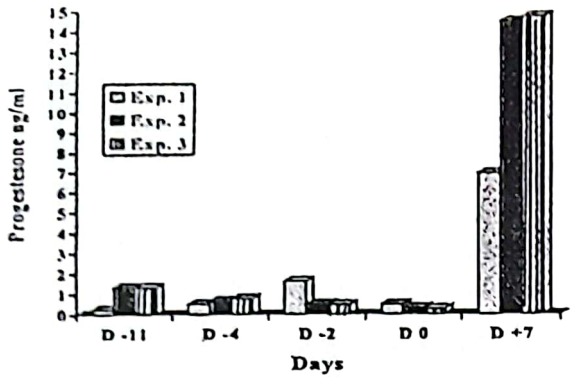

Fig. 2 : Progesterone profile during superovulation treatments in native cows in Egypt.

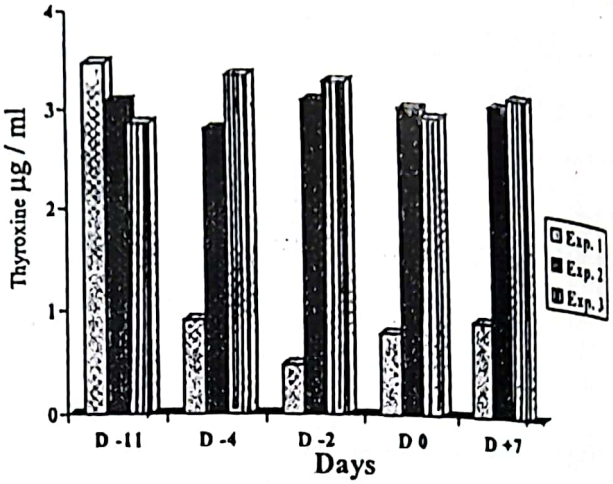

Fig. 3 : Plamsa T4 levels during superovulation in native cows in Egypt.

day of eCG injection was correlated significantly (r=0.844, P<0.01) with the subsequent ovulation rate. In addition, significant correlation (r=0.789, P<0.01) was detected between P4 level on day 7 post oestrus and the number of CL.

It is evident that T4 levels (Fig. 3) showed marked variations (P<0.01) between anoestrous and cyclic native cows among the different periods of the study. Plasma T4 level was significantly (P<0.05) higher in anoestrous cows before starting of treatment when compared with cows in exp. 2 and 3. However, during treatment, T4 levels markedly (P<0.01) decreased in cows in exp. 1 until day 7 post- oestrus.

Non significant correlations were detected between T4 and P4 levels or the ovarian response following superovulation.

Blood metabolites :

Concerning the biochemical analysis of plasma in anoestrous and cyclic superovulated cows, mean values are presented in Table 2. Among the three experiments and during the different periods, total lipids, cholesterol, total protein, globulin, glucose and alkaline phosphatase values revealed marked variations. Furthermore, anoestrous cows had lower energy related metabolits values when compared with cyclic cows.

During the different periods of the same experiment, most of the obvious changes were recorded in cholesterol, triglycerides, total proteins, albumin, globulin and glucose (Table 2). Correlation analysis indicated a significant relationship between P4 values and each of cholesterol (r=0.567, P<0.01) and glucose levels (r = 0.909, P<0.01) in all experiments. Glucose concentration at eCG injection correlated significantly with the subsequent ovulation rate (r = 0.764, P<0.01)

Table (2): Changes in some blood metabolite values in superovulated anoestrous and cyclic native cows in Egypt during different days of the experiment (Mean ± S. E.).

		Cholesterol mg/dl	T.lipid mg/dl	Triglycerides mg/dl	T. Protein g/dl	Albumin g/dl	Globulin g/dl	Glucose mg/dl	Alkaline Phosphatase u/l
D-11	1	127.833 ± 6.405^{c,f}	206.223 ± 25.064^{b}	97.725 ± 4.633^{e}	6.720 ± 0.306^{b,f}	3.050 ± 0.137	3.683 ± 0.285^{b,e}	75.343 ± 6.399	29.395 ± 1.806
	2	157.277 ± 9.050^{a,e}	273.187± 11.557^{a}	106.043 ± 6.438^{f}	7.268 ± 0.311	3.115 ± 0.050	4.138 ± 0.338	65.938 ± 3.475	27.880 ± 2.679
	3	175.000 ± 13.708^{a}	267.778 ± 28.822^{a}	112.475 ± 6.652	7.350± 0.1718^{a}	3.010 ± 0.11	4.340 ± 0.169^{a}	66.530 ± 1.425^{f}	28.270 ± 1.625
D-4	1	141.125 ± 11.065^{e}	247.780 ± 29.586	110.625 ± 10.982	7.525± 0.356	3.330 ± 0.092	4.195± 0.340	67.323± 5.185^{e}	30.595 ± 2.502
	2	147.500 ± 14.563^{e}	275.778 ± 20.977	106.408± 6.998^{f}	7.213± 0.244	3.153 ± 0.132	4.056± 0.289	72.307± 6.589	29.890± 2.522
	3	160.002 ± 11.053	287.644 ± 27.321	119.592 ± 7.294	7.170 ± 0.058	3.180 ± 0.067	3.989 ± 0.122^{e}	85.865 ± 2.382^{a,d}	33.712 ± 2.449
D-2	1	133.560 ± 11.630^{f}	270.370 ± 29.045	129.850 ± 13.226^{d}	7.823 ± 0.245^{a,b}	3.363 ± 0.186	4.460 ± 0.216^{a,d}	65.797 ± 4.179	27.517 ± 1.390^{e}
	2	149.315 ± 14.191^{e}	275.713 ± 14.042	127.310± 1.730^{d}	6.765 ± 0.355^{b}	3.155 ± 0.124	3.610 ± 0.301	67.983 ± 0.738	31.808± 3.757
	3	155.000 ± 6.292^{e}	280.050 ± 20.573	130.360 ± 3.178	7.296 ± 0.194^{b}	2.982± 0.036^{e}	4.314± 0.188^{a}	60.748± 7.419^{f}	37.075± 1.300^{a,d}
D-0	1	142.50 ± 7.808^{f,b}	251.110 ± 6.868^{b}	108.348 ± 4.833	7.284± 0.119^{e}	3.036 ± 0.153	4.248 ± 0.158^{a}	77.388 ± 6.731	32.848 ± 2.120
	2	161.063 ± 5.644^{a,e}	279.613± 20.127	120.560± 9.727	7.028 ± 0.205^{b}	3.345 ± 0.165	3.683 ± 0.228^{b}	74.960± 3.797	37.512± 5.347
	3	165.250 ± 6.680^{a}	282.268 ± 15.745^{a}	110.625 ± 6.287	7.484 ± 0.144^{a}	3.376 ± 0.191^{d}	4.114 ± 0.234	70.036± 4.291^{f}	36.433± 2.453
D+7	1	167.500 ± 4.528^{d}	271.388 ± 21.631	110.370 ± 6.421	7.178± 0.136^{e}	3.028 ± 0.181	4.150 ± 0.213	76.463 ± 5.506	33.330 ± 3.490
	2	175.373 ± 6.608^{d}	297.816 ± 20.725	124.430± 3.957	6.998 ± 0.233^{d}	3.400 ± 0.137	3.598 ± 0.097^{c}	73.805± 3.549	30.308± 3.252
	3	175.240 ± 7.401^{d}	303.504 ± 23.587	122.343 ± 5.264	7.456 ± 0.122^{a}	2.992 ± 0.040^{e}	4.464 ± 0.119^{a,d}	72.400 ± 4.696^{f}	30.948 ± 2.419^{e}

* a, b $P < 0.05$ among different expeiments.
* a, c $P < 0.0$ among different expeiments.
* d, e $P < 0.05$ among different days within the same expeiments.
* d, f $P < 0.0$ among different days within the same expeiments.

Correlations between the other biochemical parameter and P4 level or the ovulation rate throughout the experiments were non significant.

DISCUSSION

In the present study anoestrous cows were treated with exogenous progestogen which allows ovarian function to be regulated through LH secretion rather than by direct action on the ovaries (Kafi and McGowan, 1997). The first trial for induction of superovulation in anoestrous cows was achieved by using norgestomet ear implant together with a high dose of eCG and injection of GnRH during oestrus. In this experiment, timing from implant removal to the onset of oestrus was significantly ($P<0.05$) longer and associated with higher plasma P4 levels at implant removal ($P<0.01$) and during oestrus ($P<0.05$). The higher P4 levels during the preovulatory period may be ascribed to a higher possibility of the presence of a dominant follicle capable of ovulation at the time of commencing gonadotrophin treatment which results in failure of expression of oestrous behavior and/or failure of LH surge (Kafi and McGowan. 1997). In the mean time, this higher P4 levels during the preovulatory period may be responsible for the presence of high number of unovulated follicles as obtained in this experiment. In addition, the ovarian response to superovulation treatment revealed lack of significance between anoestrous and cyclic cows. This finding was in complete agreement with Uoc et al. (1997) who found that ovarian activity is not the essential factor determining the response to superovulation treatments in buffaloes. On the other hand, Monniauex et al. (1983) suggested that the ovarian status of the donor at the time of commencing superovulation is a factor determining the superovulatory response in cattle. Moreover, in the present study there is a significant positive correlation between P4 levels at the time of gonadotropin treatments ($r=0.844$, $P<0.01$), on day 7 post oestrus ($r=0.789$, $P<0.01$) and the number of corpora lutea. Similar results were reported by Ullah et al. (1992) in buffaloes, Lubbaodeh and AL-Nimer (1995) and Samartzi et al. (1995) in cattle. On he other hand, different reults were given by Saumonde (1980), Robertson et al. (1993) and Kanuya et al. (1997) those authors did not find any relationship between progesterone level after commencement of superovulatory treatment and the subsequent number of ovulations. Furthermore, plasma P4 levels were significantly ($P<0.01$) higher in experiments 2 and 3 when compared with experiment 1. These higher values were a reflection of the superstimulation in these groups in response to superovulation.

In all experiments, one of the important feature was the presence of a high number of unovulated follicles in cows in response to superovulation. This finding was confirmed by using ultrasonography on day 7 post oestrus. Kafi and McGowan (1997) attributed the condition to the LH content or LH like activity of some batches of FSH or

CG. Although, a notable finding of this study as the comparision of accurracy for assessing e ovarian response to superovulation treatment hen using manual rectal palpation and ultra-und examination. The discripency between the o methods is clearly evident particularly in the esence of visible follicles or small cystic struc-es duc to the effect of overstimulation. The mber of corpora lutea can be assessed more ac-rately using ultrasonography than by rectal pal-tion. Similar results were recorded by Robert-n et al. (1993).

ie second goal of this study was to gain insights the relationship between superovulation treat-ents and blood metabolites. Blood analysis dur-g the different periods of superovulatory regi-en indicated a markedly lower concentration of ergy related metabolites (T4, total lipids and olesterol) in anoestrous compared with cyclic ows before treatments and during oestrus. This nding is parallel with that reported by Hawkins al. (1995), Roche et al. (1995) and Ahmed et al. 998). Those authors reported that energy status bviously affecte follicular development in cattle d consequently cessation of ovarian function as detected in cows in negative energy balance. loreover, positive correlations were reported be-ween energy status and each of LH levels Houghton et al., 1990) and ovarian activity Sinclair et al., 1994). Meanwhile, in the present tudy glucose concentrations on day-4 (eCG in-ection) were correlated (r=0.764, P<0.01) with the subsequent ovulation rate and plasma P4 (r =0.909, P<0.01). These findings were in line with Randel (1990) who reported that glucose affects hypothalamo-pituitary-ovarian axis as a bi-ological mediator for GnRH pulse frequency and consequently LH secretion and follicles matura-tion. In addition, in experiment 1, there was a sud-den drop in plasma T4 from d-4 to d+7 when compared with experiment 2 and 3, this may be attributed to the high ambient temperature (Lubbaodeh and Al-Nime, 1995). Plasma T4 showed no significant correlation with superovu-lation response or with plasma P4 levels and not affected by superovulation treatments and further studies still need on this point.

In the current study, the significant (P< 0.05) de-crease in cholesterol and total lipid concentra-tions in experiment 1 during oestrus was simi-lar to that reported by Schafer et al. (1990) who recorded that cows with low concentration of cholesterol produced a significantly below aver-age number of transferable embryos. Also, the significant correlation between P4 and cholesterol levels in the present study was in parallel with the results of Hawkins et al. (1995) who found that serum P4 levels correlated with total steroidogen-ic area occupied by lipid and serum cholesterol concentrations.

The lack of significant correlation between the other studied blood metabolites and the ovulation rate or plasma P4 levels is confirmative to the

previous results of Tegegne et al. (1993) who reported that blood metabolite concentrations are not a good indicator of ovarian activity.

In conclusion :

Ovarian response to superovulation in native cows in Egypt was not affected by the ovarian status. Plasma P4 level and glucose concentration played a singificant role in the subsequent ovulation rate. Ultrasonography is more accurate in predicting the superovulation response than rectal palpation. Other blood metabolites were of no value in the control of ovarian response to superovulation in such cows.

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