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Effect of gonadotrophin treatment on circulating estradiol and progesterone profiles, growth and maturation of follicles and embryo collection in female camels for two successive superovulation trials

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Abstract

Gonadotrophic treatment of female camels for superovulation resulted in increased circulating concentration of Estradiol after day 6 of the initiation of gonadotrophic treatment followed by peak levels on days 7, 8 and 9, while the treatment did not show any increase in estradiol concentration for first 5 days after initiation of the treatment. The magnitude of estradiol peak varied individually between the two females receiving same sort of superovulation treatment, greater in one (69.79 pg/ml) than the other (40.81 pg/ml), and peak levels were also greater for the superovulated females (69.79 pg/ml, 40.81 pg/ml) as compared to females used as recipients (35 pg/ml and 41.35 pg/ml). This might have been due to higher dosages and combined use of two gonadotrophic preparation (Both PMSG and FSH plus LH Combined) used in donors as compared to low dosages and one gonadotrophic preparation (PMSG only) used in recipients. The rise in estradiol concentration is indicative of growth and maturation of multiple follicles in response to the treatment. Abnormal rise of progesterone was also observed in superovulated female camels due to treatment while there was no abnormal rise in female camels used as recipients due to treatment. Again this might be due to higher dosages and combined use of two type of gonadotrophin preparations (Both PMSG and FSH plus LH Combined) used in donors as compared to low dosages and one gonadotrophic preparation (PMSG only) in recipients. The rise subsided spontaneously and had no visible ill-effects, though theoretical inhibition of transport of gametes cannot be denied. Sonographic monitoring of ovaries of female camels under gonadotrophic treatment revealed growth and maturation of follicles in large numbers in females treated for superovulation than those synchronized as recipients. Non-surgical flushing of uterus yielded relatively good number of embryos in hatched blastocyst stages. The accidental observation of recovering majority of the embryos from wall of embryo filter after soaking it thoroughly in fluid in a bigger embryo searching dish towards the ending part of this study, gives rise to a thinking of tendency of embryos sticking to the walls of embryo filter and this gives an insight that performance are likely to be improved by learning for factors

affecting like that of sticking of embryos and improving upon these for higher embryo recovery. It is concluded that superovulation can be successfully induced in female camels as evident by the growth and maturation of follicles visible in sonography and the results on increased peripheral concentration of estradiol, the key points are that the days around 10 after initiation of gonadotrophic treatment are vital for evaluating females for maturity of follicles before breeding, and taking the day of breeding as day 0, collection on day 9 after breeding can yield good recovery of embryos in hatched blastocysts stages either in spherical shape or elongating. The abnormal rise of progesterone had no visible deleterious effect on embryo collection, the factors like sticking of embryos to filter and similar more can be learned with more exposure and improvement upon these may further improve the performance.

Keywords: Gonadotrophin, estradiol, progesterone, embryo, camels, superovulation

Introduction

Considerable progress has been made in the past decade or two for inducing multiple ovulation and embryo transfer in dromedary female camels, but the publications on this topic is still desired because numerous earlier published reports on this topic are misleading. There are several published reports on isolation of good quality of morula and successful impregnation and calving with transplantation of such embryos (Vyas *et al*., 1998; Vyas *et al*., 2004; Ahmed-Azizi Moghdam, 2010), while now it is well established that morula in camel is a non-transferable embryo, it indicates arrested development, the transferable embryos in camels have been hatched blastocyst stages (Annouasii and Tibary, 2013). Discrepancies and doubts are also prevailing on the time of collection of embryos, two type of criteria are used viz. Days after ovulation and Days after mating, which are not synonymous but creates confusion and attempt to collect embryos at wrong time under this confusion leads to failure. The time of breeding of female under superovulation is another important aspect, earlier breeding in numerous earlier publications seems to be the reason of failures. Camel has a different physiology, different protocols are now used for inducing multiple ovulation in camel for example combined use of eCG and FSH for superovulation, it requires relatively longer period for growth and maturation of follicles after initiation of gonadotrophic treatment, relatively delayed transportation of embryos from fallopian tube to uterus, relatively large amount of air used to inflate bulb of foley catheter for blocking uterine horns than that of cattle and advanced stages of embryos in hatched blastocyst stages being recovered during flushing, because of all these attributes it is always worth sharing the results and knowledge gained through experimentation on superovulation and collection of embryos in dromedary camels. The study was conducted to monitor effect of gonadotrophic treatment on circulating estradiol and progesterone profiles, growth and maturation of

follicles in the ovaries and embryo collection on two female camels which were given superovulation treatment of PMSG and FSH+LH Mixture combined either with or without progesterone pre-treatment in a repeated superovulation trial at an interval of 50 days between the trials.

Materials and Methods

Animals

The study was conducted on 4 pluriparous female camels which were divided into two groups of 2 each as Donor and recipients, respectively.

Induction of Superovulation

Donor females were subjected to superovulation treatment (repeatedly) twice at an interval of 50 days between the two trials. The superovulation protocol during repeated trials differ in regards that progesterone pre-treatment was given before gonadotrophic treatment in first trial while in repeat trial corpus luteum was induced with GnRH treatment before gonadotrophic treatment.

The superovulation protocol used during 2nd trial is same as above except that progesterone pre-treatment was replaced by GnRH induced corpus luterum. Additionally Injection prostaglandin F 2 alpha was given on 6th day of gonadotrophic treatment.

The protocol used for superovulation during first trial is tabulated below.

S. No.	Hormone administered	Dosages	Route	Frequency and duration
1	Injection Progesterone (Primolut depot, Bayer Scherring Pharma)	0.5 ml (125 mg)	Intramuscular	Once daily for 15 days
2	Injection Folligon (Intervet)	2500 i.u.	Intramuscular	Once only on 15th day morning
3	Injection Pluset (Laboratorios Caliper, S.A., Spain)	3.0 ml	Intramuscular	Twice at 12 hr interval on day 15th
4	Injection Pluset	2.5 ml	Intramuscular	Twice at 12 hr interval on day 16th
5	Injection Pluset	2.5 ml	Intramuscular	Twice at 12 hr interval on day 17th
6	Injection Pluset	2.0 ml	Intramuscular	Twice at 12 hr interval on day 18th
7	Injection Pluset	1.5 ml	Intramuscular	Twice at 12 hr interval on day 19th
8	Injection Pluset	1.25 ml	Intramuscular	Twice at 12 hr interval on day 20th
9	Injection Pluset	1.0 ml	Intramuscular	Twice at 12 hr interval on day 21st

Synchronization of Recipients

The recipients were subjected to progesterone treatment-125 mg daily (Primolut depot, Bayer Schering Pharma) of 10 days to be initiated and finished in such a way that gonadotrophic treatment in the recipients in form of a single injection of PMSG (Folligon, Intervet) 1500 i. u. is administered at the end of 10 days progesterone treatment exactly 48 hour later to the initiation of gonadotrophic treatment of donors.

Sonographic Monitoring

The growth and maturation of follicles in the ovaries was monitored using 7.5 MHZ linear rectal probe of Exago sonography machine.

Endocrine Monitoring

Daily peripheral circulating concentration of Estradiol-17 Beta and Progesterone was monitored from -4 days before initiation of gonadotropic treatment to 20 days after in super-ovulated female camels and from -6 days before initiation of gonadotropic treatment to 20 days after in recipient female camels for the first trial only. The serum samples were harvested at appropriate times from these animals and preserved in -20 degree Celcius till analysis.

Analysis of Sera Samples

These hormones were estimated through analytical kits based on chemi-luminiscent assay with automated machine called COBAS.

Breeding

The super-ovulated females were mated at appropriate times by a virile male.

Flushing of Donor Females

Flushing of donor females was attempted on day 9 after mating considering the day of mating as Day 0. The camels were sedated using 20% Xylazine injection (Thiazine 100, Nature Vet, NSW)-1ml intra-venously and 2% Xylocaine injection (Vetoquinol, S. A., France) 5 ml epidurally, the external area around genitals were washed and disinfected, two way long Foley's catheter (Mini-tub) specially made for camels and flushing media (Camel flush, Mini-tub) also specially made for camels were used for flushing the uterine horns. The left and right horns of the uterus were flushed separately after blocking the individual horns by inflated bulb of the catheter, usually the left horn admitted 80 ml of air while right horn admitted 50 ml of air for blockage of their lumen. The flush was passed through an embryo filter (Mini-tub), which was then seen under microscope for isolation of embryo.

Statistical Analysis

The daily values of Estradiol-17 beta higher than Mean±2 S.D. was considered as significantly different (McDonald, 2009). Paired t test (McDonald, 2009) was applied on daily estradiol profiles from different female camels. The progesterone values during gonadotrophic treatment and prior to

mating in excess of 1 ng/ml were considered as abnormal progesterone rise.

Results

Estradiol concentration (Mean±S.E.) in Female camels before and after gonadotrophic treatment of donor for superovulation and recipient for synchronization:

The data on daily circulating concentration of estradiol in 4 female camels have been presented in Table 1, and Fig. 1-4, which showed that there have been individual animal variations in the Mean ± S.E. circulating concentration of estradiol (32.02 ± 2.95^{a}, 23.94 ± 1.49^{b}, 23.21 ± 1.20^{b}, 32.09 ± 0.83^{a}), the variations between animals have been statistically significant (Data with different superscript) ($P<0.05$) also. The range values for individual animals have been presented in Table 1. The peak values of circulating estradiol concentration in 4 female camels have been 69.79 pg/ml, 40.81 pg/ml, 35 pg/ml and 41.35 pg/ml. The data also showed that peak values were in general greater for the females receiving gonadotropic treatment for superovulation as donor (69.79 pg/ml and 40.81 pg/ml) than females receiving gonadotrophic treatment for synchronization as a recipient (35 pg/ml) to donor. The data also showed that estradiol peak was greater in one female camel(69.79 pg/ml) receiving gonadotropic treatment as donor as compared to another donor female camel(40.81 pg/ml) receiving the exactly similar gonadotrophic treatment.

Daily estradiol profiles (Fig. 1) of a female camel under superovulation treatment revealed that there has been no change in peripheral circulating concentration of Estradiol (range from 21.94 to 34.37 pg/ml) on days 1, 2, 3, 4, and 5 after initiation of gonadotrophic treatment from the circulating concentration (range from 24.32 pg/ml to 27.71 pg/ml) on days 0, -1,-2, -3 and -4 prior to initiation of gonadotrophic treatment but thereafter the circulating concentration of estradiol increased on day 6 (51.17 pg/ml), 7 (65.71 pg/ml), 8 (69.69 pg/ml) and 9 (59.89 pg/ml) after gonadotropic treatment, followed by decline and no rise till day 20 for which the concentration was monitored. The values of circulating estradiol on days 6, 7, 8 and 9 after initiation of gonadotrophic treatment were found statistically significant ($P<0.05$).

Daily estradiol profiles (Fig. 2) of another female camel under similar superovulation treatment revealed that there also has been no change in peripheral circulating concentration of Estradiol (ranging from 21.69 pg/ml to 28.68 pg/ml) on day 1, 2, 3, 4 and 5 after initiation of gonadotrophic treatment from the circulating concentration (ranging from 20.93 pg/ml to 30.41 pg/ml) on days -4, -3, -2, -1 and 0 before initiation of gonadotrophic treatment but thereafter

Table 1: Circulating Estradiol profiles (Mean ± S. E., Range and significant peak values) of 4 female camels (Donor (n=2) and recipient (n=2)) subjected to super-ovulation treatment and synchronization.

S. No.	Number and status of the female Camel	Mean ± S.E. (pg/ml)	Range (Pg/ml)	S. D.	Mean ± 2 S. D.	Significant ($P< 0.05$) Peak Values and day in relation to start of gonadotrophin treatment
1	1, DONOR	32.02 ± 2.95^{a}	17.1- 69.79	14.18	60.38	65.71 (8^{th} day), 69.79 (9^{th} day), 59.68 (10^{th} day)
2	2, DONOR	23.94 ± 1.49^{b}	14.86-40.81	7.16	38.26	40.81(8^{th} day), 38.85(9^{th} day)
3	3, RECIPIENT	23.21 ± 1.20^{b}	16.37-35	5.79	34.79	35 (8^{th} day), 34.92 (9^{th} day)
4	4, RECIPIENT	32.09 ± 0.83^{a}	25.02-41.35	3.99	40.07	41.35 (20^{th} day*)* Not related to the treatment

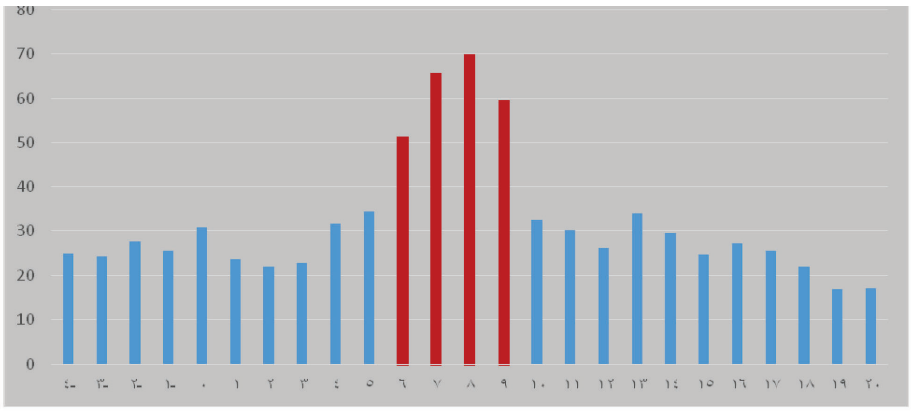

Fig. 1: Daily Estradiol Profiles of a female camel under superovulation treatment (X-axis-Days in relation to gonadotropic treatment Y-Axis estrodial (pg/ml)

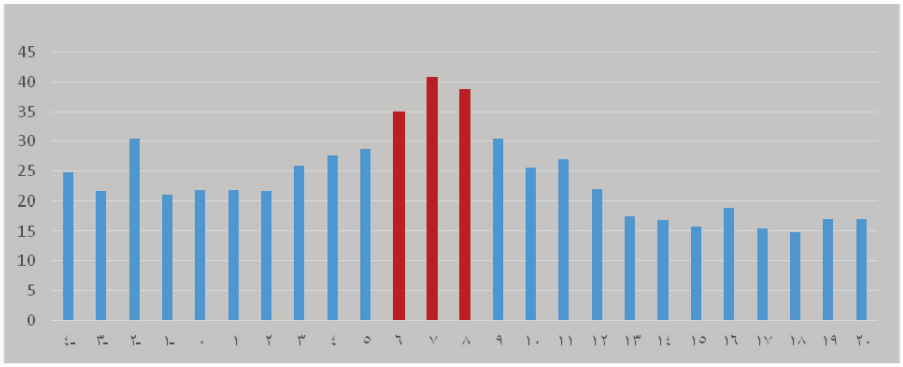

Fig. 2: Daily Estradiol profiles of a female camel under superovulation treatment (X-axis-Days in relation to gonadotropic treatment, Y-Axis-Estrodiol (pg/ml)

the circulating concentration of estradiol increased on days 6 (34.98 pg/ml), 7 (40.81 pg/ml) and 8 (38.85 pg/ml) followed by decline and no rise till day 20 for which the concentration was monitored. The values of circulating estradiol on days 7 and 8 after initiation of gonadotrophic treatment were found statistically significant ($P<0.05$).

Daily estradiol profiles of yet another female camel (Fig. 3) in relation to gonadotrophic treatment for synchronization as recipient with the donor female camel revealed that there was no change in circulating concentration of estradiol (ranging from 21.35 pg/ml to 29.23 pg/ml) on days 1, 2, 3, 4 and 5 after gonadotropic treatment from that of circulating concentrations (ranging from 17.62 pg/ml to 24.97 pg/ml) on days -6, -5,- 4, -3,- 2, -1 and 0 before gonadotrophic treatment but it had slightly higher levels

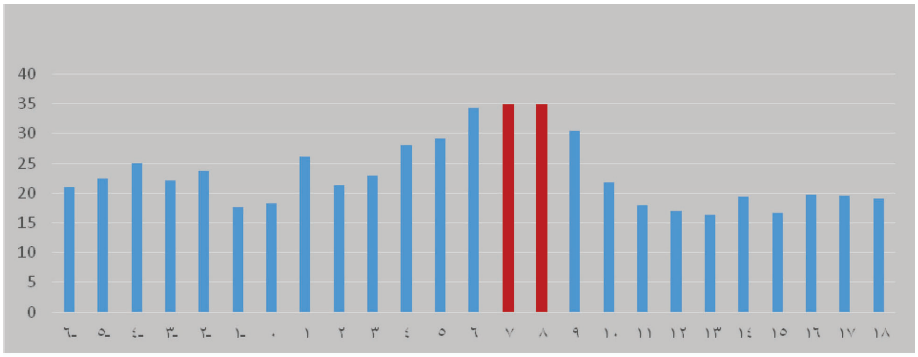

Fig. 3: Daily Estradiol profile of a female camel under gonadotropic treatment for synchronization as recipient with the donor. (X-axis-days in relation to gonadotropic treatment, Y-Axis-Estrodiol (pg/ml)

on days 6 (34.34 pg/ml), 7 (35 pg/ml) and 8 (34.92 pg/ml) after gonadotrophic treatment of which the values recorded on days 7 and 8 were found statistically significant ($P< 0.05$).

Daily estradiol profiles of yet another female camel (Fig. 4) in relation to gonadotrophic treatment for synchronization as recipient with the donor female camel revealed that there was no change in circulating concentration of estradiol (ranging from 27.06 pg/ml to 37.19 pg/ml) on days 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 after gonadotropic treatment from that of circulating concentrations (ranging from 25.02 pg/ml to 35.32 pg/ml) on days -6,-5,-4,-3,-2,-1 and 0 before gonadotrophic treatment.

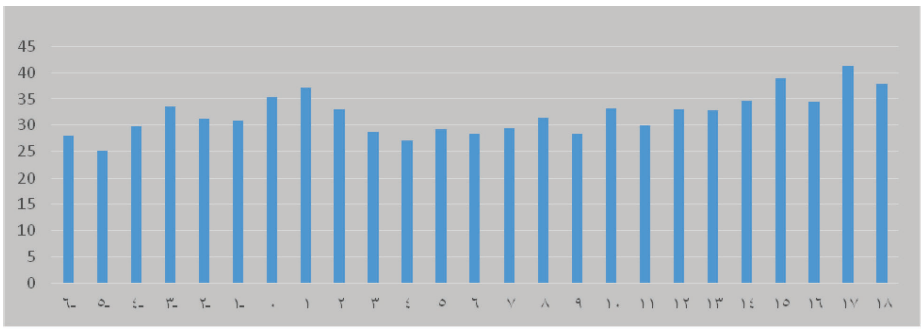

Fig. 4: Daily Estrodiol profile of a femal camel under gonadotropic treatment synchronization as recipient with the donor (X-axis-Days in relation to gonadotropic treat, Y-Axis-Estradiol (pg/ml)

Abnormal rise of Progesterone due to gonadotrophic treatment for superovulation:

Progesterone profiles of a female camel receiving gonadotrophic treatment for superovulation have been presented in Fig. 5, which showed that there have been an abnormal rise in peripheral progesterone concentration from day 4 onward after initiation of gonadotrophic treatment, increasing steeply on day 5, 6, 7, 8 and 9 followed by declining on day 10 and 11 and subsiding thereafter. The abnormal rise in progesterone in this particular animal exceeded 10 ng /ml.

Progesterone profiles of another female camel receiving similar gonadotrophic treatment for superovulation have been presented in Fig. 6, which also showed exactly similar pattern of abnormal rise in peripheral progesterone concentration from day 4 onward after initiation of gonadotrophic treatment, similar steep rises on days 5, 6, 7, 8 and 9 followed by declining on day 10 and 11 and subsiding thereafter though the magnitude of rise was low in this particular animal and it exceeded 3 ng/ml only.

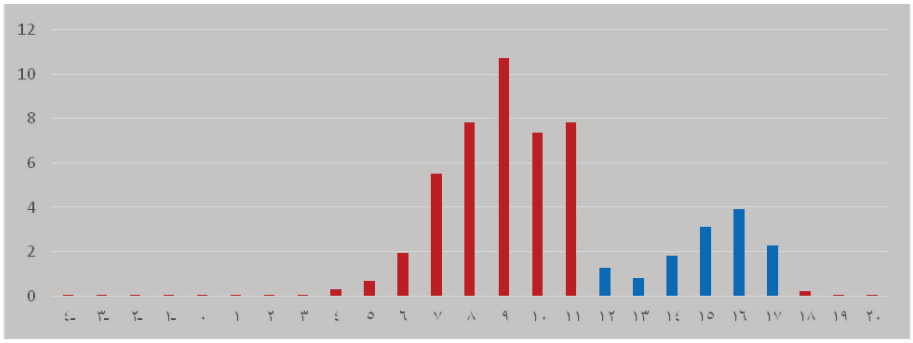

Fig. 5: Abnormal Progesterone rise due to gonad tropic treatment of donor female camel (x-axis-Days in relation to gonad tropic treatment Y-Axis-Progesterone (ng/ml)

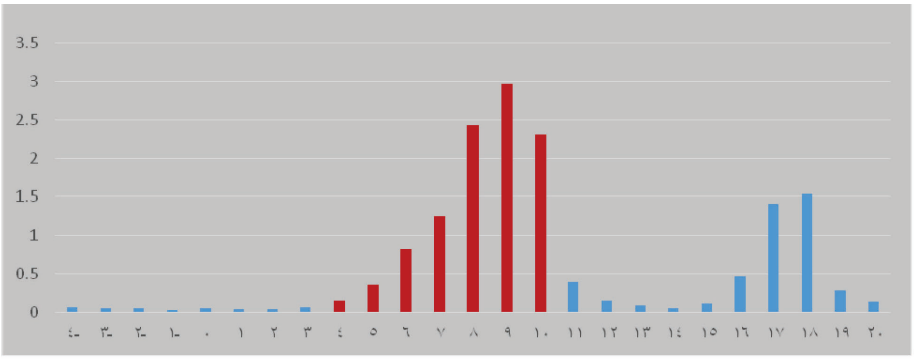

Fig. 6: Abnormal Progres terone rise in a female camel under gonadotrophic treatment for superovulation as a donor (X-axis-Days in relation to gonadotropic treatment, Y-Axis-Progesterone (ng/mg)

Progesterone profiles of two other females receiving gonadotrophic treatment for synchronization as recipients to donor have been presented in Fig (s) 7 and 8, which showed no abnormal rise in progesterone concentration in these females.

Growth and Maturation of Graffian Follicles

Growth and Maturation of follicles could be monitored through sonography as depicted in figures 9, 10, 11 and 12.

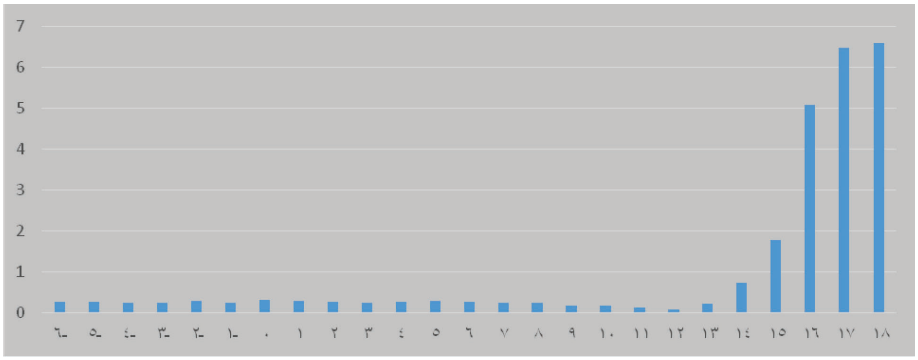

Fig. 7: No abnormal rise of progesterone in a female camel under tropic treatment for synchronization as recipient to the donor. (X-axiz-Days in relation to gonadotropic treatment, Y-Axis Progesterone (ng/ml)

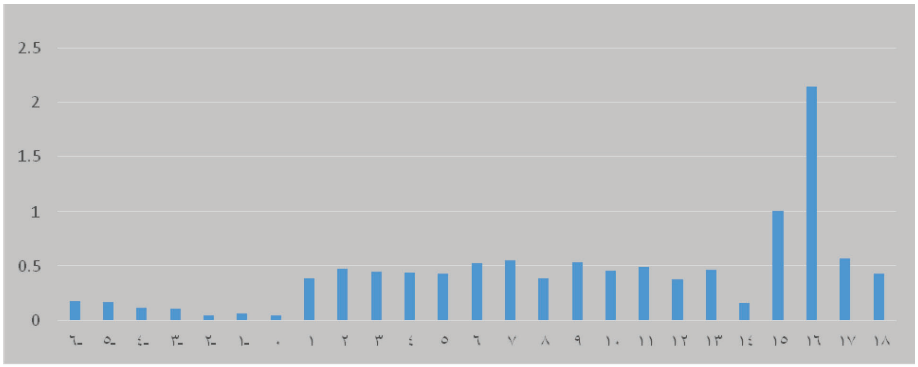

Fig. 8: No Abnormal rise of Progesterone due to gonadotropic treatment of a female camel for synchronization as recipient to donor. (X axis-Days in relation to gonadotropic treatment, Y, Axis-Progesterone (ng/ml)

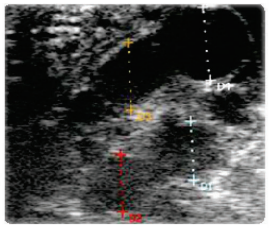

Fig. 9: Sonography scanning of mature follicles in the ovaries of superovulated camels.

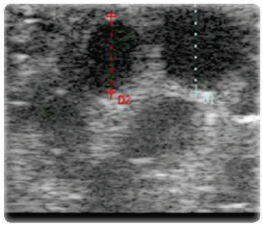

Fig. 10: Sonography scanning of mature follicles in female camel.

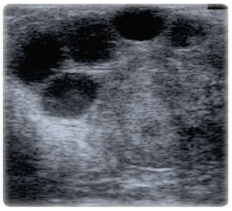

Fig.11: Sonography scanning of female camel for mature follicles.

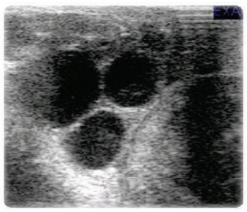

Fig. 12: Sonography scanning of female camel for mature follicles.

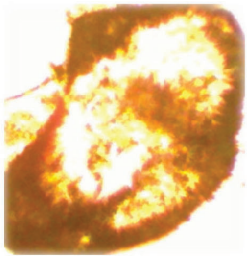

Fig. 13

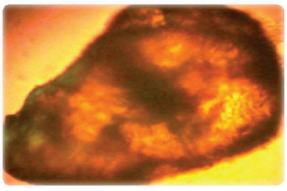

Fig. 14

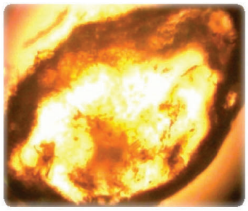

Fig. 15

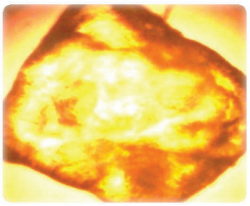

Fig. 16

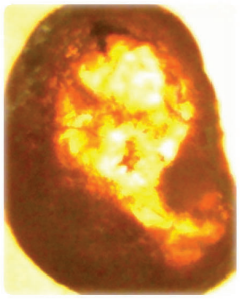

Fig. 17

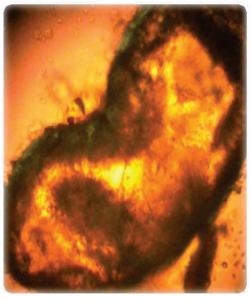

Fig. 18

Embryo Collection

Non-surgical flushing of 2 donor female camels on day 9 after ovulation yielded 6 hatched blastocysts from one female and none from other female. The hatched blastocysts recovered from one female camel have been presented in Fig. 13-18.

In repeat trial 4 and 8 embryos were recovered from these females, respectively and these have been documented as Fig. 19-30.

Accidental Observation

The filter used in line for collection was immersed in collection fluid filled in a big embryo searching dish and towards the last part of the study as many as 4 embryos were recovered from this dish which gives an insight that embryos probably get stick to filter and the flushing of filter with syringe as advised by firm was not sufficient to dislodge the sticking embryos.

Amount of air insufflated in bulb of Foley's Catheter while blocking the individual horns: It accommodated 80 ml and 50 ml air, respectively in the left and right horns to block the horns adequately for collection of flushed fluid in the uterine horns.

Discussion

Circulating Estradiol Concentration

The basal levels of circulating estradiol profiles have been relatively higher in camels (around 20-25 pg/ml) than other species of domestic animals, this was apparent in the present study and study conducted by first author previously at National Research Centre on Camel, Bikaner, India and many other studies (Skidmore, 1994; Homeida *et al*, 1988**;** Khalil, 1989; Basiouni, 2007). The levels of estradiol also differ in individual females as depicted in the present study.

Effect of Gonadotrophic treatment on circulating estradiol concentration

The results indicated that for the first five days of the gonadotrophic treatment the follicles were not mature enough to secrete significant amount of estradiol but thereafter the circulating estradiol concentration showed a statistically significant rise on days 6, 7, 8 and 9 of treatment in one donor female and on days 7 and 8 in other donor female camel. Similar significant rises in the other animals treated with lower dosage of eCG for synchronization as recipients were not observed. The rise in estradiol concentration in superovulated females and peak levels of estradiol (around 70 pg/ml) in one (around 70 pg/ml) of the two superovulated female camels can be ascribed to growth and maturation of multiple number of follicles on the ovaries as a result of the gonadotrophic treatment. These results on rise in estradiol concentration from day 6 of treatment followed by highly significant peak of around 70 pg /ml on days 7, 8 and 9 are the kind of results which one should have expected as a result of treatment but there are no published

Fig. 19

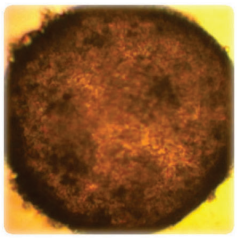

Fig. 20

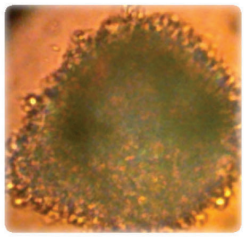

Fig. 21

Fig. 22

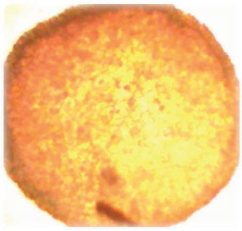

Fig. 23

Fig. 24

Fig. 25

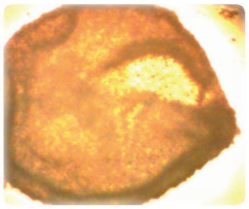

Fig. 26

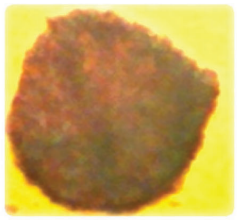

Fig. 27

Fig. 28

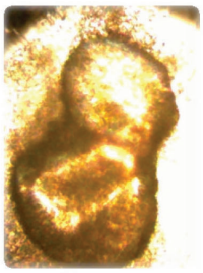

Fig. 29

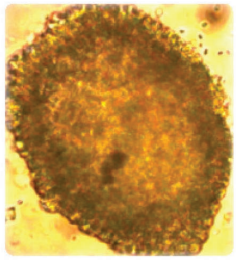

Fig. 30

results like this on this particular aspect, the earlier study from Skidmore, 1994 *report*ed a rise from basal levels of 25.0 ± 0.4 pg/ml to 39.0 ± 1.8 pg/ml as the follicle reach a diameter of 1.7 ± 0.1 cm., the peak values reported is much lower than observed in present study in one animal. The results showed that around days 10 are required for estradiol peaks in female camels under this type of treatment. These results gives an insight for further investigations on this hormone profile in female camels to develop a monitoring tool for the optimum timing of breeding of the superovulated female camels.

Abnormal Progesterone rise

The results showed that the gonadotropic treatment used for induction of superovulation in female camels has resulted in statistically significant abnormal rise in peripheral concentration of plasma progesterone on days 4 to 11 after initiation of gonadotropic treatment. The abnormal rise subsided after day 11 of the treatment. The only known reason that can be ascribed to the abnormal rise has been from leutinization of the growing follicles due to LH activity of the gonadotrophins used for induction of superovulation. But that peripheral concentration of progesterone subsided spontaneously and embryos have been successfully collected from the animals, gives rise to a thinking that the abnormal rise should have little deleterious effect if any on embryo production though the theoretical deleterious effects on inhibition of sperm transport and abnormal transport of embryos to the uterus cannot be denied. There are no published reports on this aspect in camels.

Sonographic monitoring of growth and maturation of follicles:

The sonographic images of the ovaries have shown that the growth and maturation of follicles in female camels can be easily monitored and depending upon the size of majority of the follicles present measuring around 15 mm, the animals can be bred on around 10 days after initiation of gonadotrophic treatment. Earlier attempt by first author (Aminu Deen and Sahani, 2006) at National Research Centre on Camel, Bikaner, India for induction of superovulation in female camels with progestagen ear implant and PMSG did not bring fruitful results, probably due to breeding of females much earlier in relation to the gonadotrophic treatment so that the follicles ere not mature enough to have provided a superovulation response. The examination of protocol published by Vyas *et al.* (1998) and Vyas *et al.* (2004) also suggested that these authors have attempted breeding of females too early (day 6 in one group and day 7 in another group) in relation to gonadotrophic treatment and that should have been one of the several factors for poor performance. The present study data and many other workers (Tinson and Kuhad, 1998; Skidmore and Billah, 2005; Skidmore and Billah, 2011) have

revealed that growth and maturation of follicles require around 10 days period in the camels.

Embryo Collection

Embryo collection can be conveniently accomplished 7-8 days after ovulation or on day 9 after mating, considering the day of mating as day 0. The time of collection of embryos have been described in publications in regards to either ovulation or mating and one should be very careful of the fact that days after ovulation and days after mating are not the same terminologies. There is a big difference of 36-48 hours between these two. One should also be careful that because of the delayed transport of embryos from fallopian tubes to uterus as compared to that of cattle, collection of embryos at stages parallel to that of cattle may yield with poor embryo recovery and that is what has happened in many of the initial studies. The protocols described by Vyas et al, (1998; 2004) and Abdul Azizi Moghdam (2010) were examined by the author and it was apparent in all these cases to have attempted collection at early stages and it is felt that this is the reason for poor results in these cases. The protocol described by Ismael *et al* from Al-Ahsa of Saudi Arabia also appears to have the same reason of have attempted collection at early stages for low embryo recovery. Tinson and Kuhad (1998) reported of collection of embryos on day 9 after mating. Tibary and Anouassi (2013) and Skidmore opined to collect embryos on day 7.5 after ovulation. The time of collection of embryos as 7.5 days after ovulation is equivalent to that of 9 days after mating. Skidmore and Billah (2005; 2011) are consistently having good recovery of embryos. In light of these facts, the recommendations of McKinnon et al (1994) published for higher recovery rate of embryos on day 7-7.5 after mating appears to have been erroneous or published by oversight as evident by the fact that one of the co-author has published higher recovery on day 9 after mating (Tinson and Kuhad, 1998).

Stage of embryo

The embryos recovered are in hatched blastocoel stages. Majority of the workers are now agreed in this regard that embryos are recovered in camels at hatched blastocoel stages. In light of these developments, the results published by Vyas *et al.* (1998; 2004), Ahmed Azizi Moghdam (2010) of unfertilized ova, 2 cell stages and morula are now erroneous. Tibari and Anouassi (2013) and other groups are now agreed on a point that only hatched blastocoels are transferable, anything like morula in camels is a non-transferable stage, however beautiful it may look like, these have been regarded as arrested development. In light of these facts, the results reported by Vyas *et al* (1998; 2004) and Ahmed Azizi Mogdham (2010) of attempting transplantations with morula and obtaining pregnancies and live births seem to be erroneous.

Amount of Air to block the horns

The amount of air accommodated into the bulb of Foley's Catheter to block the lumen of individual uterine horns has been 80 and 50 ml, respectively in left and right uterine horns and it has been more than any other publication reported on camel, authors have noted it that air lower than this do not block the horns adequately in camels, authors have observed that there has been no bleeding or endometrial splitting in camels in accommodating the said volume of air. The published literature in this aspect do not match with recommendations of the present study.

Sticking embryo on to embryo filter wall:

The accidental observation in the ending part of present study regarding recovery of the majority of the embryos sticking to the filter walls which were recovered after long time bathing of the filter in fluid in a separate big sized embryo searching dish accommodating whole of the filter for bathing in the fluid presents a possibility of significant improvement in results over present study by picking up and improving upon the factors/ mistakes committed during process.

Conclusion

It is concluded that superovulation can be successfully induced in female camels, the growth and maturation of follicles resulted into increased peripheral concentration of estradiol from day 6 after gonadotrphic treatment and continue to rise and peaked on day 7, 8 and 9, the abnormal rise of progesterone had no visible deleterious effect on embryo collection, the key points are that the days around 10 after initiation of gonadotrophic treatment are vital for evaluating females for maturity of follicles before breeding, and taking the day of breeding as day 0, collection on day 9 after breeding can yield good recovery of embryos in hatched blastocysts stages either in spherical shape or elongating. The factors like sticking of embryos to filter and similar more can be learned with more exposure and improvement upon these may further improve the performance.

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