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TRANSPORT OF PREIMPLANTATION EMBRYOS IN THE GENITALIA OF BUFFALO HEIFERS SUPEROVULATED WITH PFSH AND VARIABLE DOSES OF LH

(With 3 Tables)

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انتقال الأجنة قبل التثبيت فى الجهاز التناسلى لعزبات الجاموس فائق التبويض بهرمون الحاثة الجرابية وكميات مختلفة من الهرمون المنبه للجسم الأصفر

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تم فى هذا البحث دراسة معدل انتقال الاجنة قبل التثبيت فى الجهاز التناسلى لعزبات الجاموس وكذا محاولات لتحسين معدلات احداث التبويض الفائق لعدد ١٢ عزبه جاموسى يتراوح اعمارهم بين ٢١ -٢٥ شهر ويتراوح اوزانهم من ٣٢٥ -٣٨٥ ك . ولاحداث التبويض الفائق تم الحقن اثناء مدة وجود الجسم الاصفر حيث استخدم هرمون الحاثه الجرابية النقى (pFSH) المصنع فى كندا (شركة وبث أيرستر) بحقن كمية اجمالية ٦٥ وحدة (Super-ov NIH) مقسمة الى ستة اجزاء متساوية (١,٤ مللى كل ١٢ ساعة مع حقن ٢٥ مللى جرام ليوتاليز مع الحقنة الخامسة لهرمون الحاثة الجرابية ايماء لتوجيهات الشركة المصنعه) ولتحسين معدل التبويض تم فى هذا البحث اضافة حقن كميات مختلفة من الهرمون المنبه للجسم الاصفر LH : صفر، ٢، ٤، ٥، ٧، ١٠ الف وحده (USP) المصنع فى معامل سترز – فونكس – أريزونا صباح اليوم الرابع من بداية التجربة اثناء الشياع. تم استخدام ٢ عزبه جاموسى مع كل كمية من الهرمون المنبه للجسم الاصفر حيث تركت المجموعة الاولى ضابطة (اجمالى ٦ مجموعات) كما تركت هذه الاناث مع فحول جاموسى سليمة تناسليا للتلقيح الطبيعى اثناء فترة شياعها. بالنسبة لدراسة معدل انتقال الاجنة من خلال قناه فالوب الى قرن الرحم فقد تم تقسيم هذه الحيوانات الى ثلاثة مجاميع متساوية (ن = ٤) حيث تم ذبح كل مجموعة على فترات مختلفة من بداية الشياع (٧٢-٨٩، ١٠٠ -١٠٦،١١٨ -١٢٠ ساعة على التوالى). بعد الذبح مباشرة تم اخذ الجهاز التناسلى بالكامل الى المعمل فى مثلج حيث تم فحص المبايض وعد الاجسام الصفراء حديثة التكوين والحويصلات التى لم يحدث بها تبويض. بعد ذلك تم فصل قناه فالوب عن قرن المبيض فى كل جانب تشريحيا وبعد اجراء غاسول بمحلول الملح الفسيولوجى لكل عضو تم فحص وعد الاجنة المتواجده بالغاسول لكل من قناه فالوب وقرن الرحم كل على حده لمعرفة معدل وصول الاجنة الى هذه الاماكن. وبينت النتائج أن متوسطات فترة الشياع وعدد الاجسام الصفراء الحديثة

والحويصلات التى لم يحدث بها تبويض كان لكل الحيوانات ١١,٢ ± ٤١,٥ ساعة ، ١٠٣ ± ٣,١ ، ٧٦,٠ ± ١,١ على التوالى. أما معدل التبويض فكان ٧٢,٥ % ومعدل اكتشاف الاجنة فكان ٥٤%. ولوحظ ان اعلى معدلات للتبويض (٦٦-٨٠%) كان مع عزبات الجاموس التى حقنت ٤٠٠٠ وحده واكثر من الهرمون المنبه للجسم الاصفر أما أعلى معدل لاكتشاف الاجنة (٥٠-١٠٠%) كان مع الحيوانات التى حقنت ٤٠٠٠ -٧٠٠٠ وحدة. تبين من فحص غاسول قناه فالوب وقرن الرحم أن ٦ أجنة اكتشفت وجودهم فى قناه فالوب وجنين واحد في قرن الرحم عند الفترة الاولى من ٧٢-٨٩ ساعة من بداية ظهور الشياع أو ٤٨-٦٥ ساعة من أخر وثبه. كما لوحظ وجود بعض الحيوانات المنوية الغير متحركة فى هذه المجموعة. عند الفترة الثانية والثالثة بعد ١٠٠-١٠٦ ، ١١٨-١٢٠ ساعة من بداية ظهور الشياع لم يكن هناك اى جنين فى قناه فالوب وكان هناك ٧و٦ أجنة فى ارحام تلك المجموعتين على التوالى.

SUMMARY

Twelve buffalo heifers of similar age (21-25 month) and body weight (325-385 kg) were superovulated during mid luteal phase using pFSH (total 65 NIH unit Super-Ov divided into 6 equal dose, 1.4 ml each, for 3 consecutive days) and lutalyse (25mg injected with the 5th injection). To improve the ovarian response to the variable doses of LH (0, 2, 4, 5, 7 and 10 thousands USP Unit, Steris, Lab. Inc Phoenix, Arizona) were injected at the morning of the 4th day of the treatment in 6 trials (n=2). Fertile bulls were allowed to mount heifers frequently after 24 hour (h) from onset of estrus. Heifers were classified into 3 equal groups (2 trials/each) which slaughtered at various time intervals from the onset of estrus: 72-89, 100-106 and 118-120 (h). After slaughter, the intact genitalia were dissected free and transported to the lab in a thermos container at 4C°. The number of newly formed corpora lutea (CL) and unovulated large follicles (UF) in both ovaries were done. Flushings of the oviduct and uterine horn were performed separately using phosphate buffer saline to identify the numbers and locations of embryos. The duration of estrus(h),numbers of CL and UF were 41.5± 11.2, 3.1±1.3 and 1.1±0.76 respectively. The overall ovulation and embryo recovery rates were 72.5 and 54% respectively.Group without LH gave low response. The higher ovulation rate(66-100%) were recorded for heifers supplemented with 4000 and more USP unit LH while the higher embryo recovery rates (50-100%) were associated with the doses of 4000-7000 unit. At 72-89h postestrus (48-65h post-insemination) 6 embryos were collected from the oviducts and one embryo from the uterus. Some non-motile spermatozoa were observed in the oviduct. At 100-106 and 118-120 h postestrus (76-82 and 94-96 h post-

insemination) 7 and 6 embryos were recovered respectively from the uterus. The rate of embryo transport in the oviduct of superovulated buffalo heifers appeared to be 30 h and more faster than in buffalo or bovine cows.

Key words: *Superovulation, preimplantation embryos, FSH, LH.*

INTRODUCTION

Field application of recent technology in cattle reproduction still facing a lot of problems in buffaloes. Embryo transfer technique which had been well refined in cattle breeding required more scientific researches to be favourably used in buffalo.

The rate of embryo transport in the oviduct is a matter of vital significance to optimise the day of non surgical collection of embryos from the uterus. In superovulated cows, Hackett *et al.* (1993) cited that embryos were found in the oviduct upto 6 days from insemination. In superovulated buffalo cows, Drost (1991) reported that embryos reached the uterus arround days 4 to 5 after the onset of estrus. Misra et al., (1998) found that ova/embryos reached the uterus about 134 (h) after the onset of superovulatory estrus in buffalo cows.

The available literature lack similar informations on superovulated buffalo-heifers. Within the scope of this topic, Desaulniers *et al.* (1995) reported poor response to superovulation in mature cows when compared to heifers less than 2 years old.

Ismail *et al.* (1993) reported that administration of LH with FSH improved ovarian response in superovulated buffalo cows. Osman *et al.* (2001) found that addition of 2000 unit LH improved significantly the ovulation rate in superovulated buffalo cows in comparison to the control while buffalo heifers showed the lowest level of ovarian response.

Donaldson *et al.* (1986) cited that excessive LH during treatment to induce superovulation resulted in low rates of fertilization in cow. Thus, optimisation of LH dose might be benefit to get better response.

The aim of the present experiment is to investigate the transport of preimplantation embryos in the oviduct and uterus of superovulated buffalo heifers. Trials were also performed during the experiment to improve the ovulation and embryo recovery rates using variable doses of LH injected as an additive to the superovulatory regieme.

MATERIALS and METHODS

Twelve buffalo heifers of similar age (21-25 month) and body weight (325-385 kg) were used for the present experiment.

These animals were healthy, non pregnant,cycling and selected from a local governamental buffalo farm at El-Hawatka Station, Assiut.

All animals were kept in the farm under the same feeding and management systems. The buffalo heifers were assigned to be at mid luteal phas before starting the superovulatory treatment.

A dose of 1.4 ml Super-Ov contained 12.5 NIH pFSH (Mfd.W.S. Montreal Inc. Canada) was injected i.m. morning and evening for 3 successive days. At the morning of the 3ed day, 5 ml lutalyse contained 25 mg $PGF_{2\alpha}$ was injected at the time of the 5^{th} FSH injection.

As a trial to improve ovarian response and embryo recovery rate variable doses of LH (0, 2, 4, 5, 7 and 10 thousands USP unit, Steris, Lab, Inc Phoenix, Arizona) were injected at the morning of the 4^{th} day of the treatment in 6 trials each with 2 heifers.

Fertile buffalo bulls were allowed to mount heifers frequently after 24 hours (h) from the onset of estrus.

The superovulated buffaloes were kept untied in open yard. The onset and duration of heat were recorded through close observation by 2 herdsmen in the farm.

Heifers were classified into 3 equal groups (2 trials/each) which slaughtered at variable time intervals from the onset of estrus: 72-80, 100-106 and 118-120 (h).

After slaughter, the intact genitalia were dissected free and transported to the lab in a Thermos container at 4C°. The number of follicles and corpora lutea in both ovaries were taken. Flushings of the oviduct and uterine horn were performed separately using phosphate buffer saline to identify the numbers and locations of embryos.

Data obtained were expressed as Mean ± St.Dev. and analyzed statistically using Costat Computer program (1986).

RESULTS

The overall duration of estrus (h) was 41.5 ± 11.2 while the overall numbers of CL and UF were 3.1 ± 1.3 and 1.1 ± 0.76 respectively (Table 1). The onset of estrus began within 24 (h) from

the last pFSH injection. The results of this experiment were distributed according to the time of slaughter in Table 2 .

At 72-89h postestrus (48-65h post-insemination) 6 embryos were collected from the oviducts and one embryo from the uterus. Meaning that 14% of embryos could reached the uterus at this early period. Some non-motile spermatozoa were observed in the oviduct. The case in which one embryo could be recovered from the uterus at this early period, received 7000 USP unit LH.

After this period all embryos located the uterus. At 100-106 and 118-120 h postestrus (76-82 and 94-96 h postinsemination) 7 and 6 embryos were recovered respectively from the uterus.

Both ovulation and embryo recovery rates were nearly simillar among the different periods of slaughter.

Table 3 showed the results distributed according to the variable doses of LH additives. The higher ovulation rates (66-100%) were recorded for heifers supplemented with 4000 and more USP unit LH while the higher embryo recovery rates (50-100%) were associated with the doses of 4000-7000 unit.

Table 1: Durations of estrus (h) and numbers of corpora lutea (CL) and unovulated large follicles (UF) in superovulated buffalo heifers

Buffalo Heifers	Doses of LH (USP Units)	Durations of Estrus (h)	Numbers of CL	Numbers of UF (>8 mm)
1	00	46	2	2
2	00	46	1	1
3	2000	30	2	2
4	2000	52	2	2
5	4000	36	4	1
6	4000	34	5	0
7	5000	40	5	1
8	5000	33	4	0
9	7000	54	2	1
10	7000	67	4	2
11	10000	30	2	0
12	10000	30	4	1
Mean ± St.Dev.		41.5 ± 11.2	3.1 ± 1.3	1.1 ± 0.76

Table 2: Results distributed according to the time of slaughter from onset of estrus in superovulated buffalo heifers

Time of slaughter from Onset of Estrus (hour)	Number of New CL	Number of UF		Ovulation Rate (%)	Number of Recovered Embryos		Embryo Recovery Rate (%)
		4-8 mm	>8 mm		Oviduct	Uterus	
72-89	3.0 ± 1.0	5.5 ± 2.8	1.0 ± 0.7	78.0 ± 13.92	1.5 ± 1.1	0.5 ± 0.3	50.0 ± 35.35
(48-65) ♣	(2-4)	(3-10)	(1-2)	(66-100)	(1-3)	(0-1)	(0-100)
100-106	3.0 ± 1.58	4.0 ± 1.58	1.0 ± 0.7	70.0 ± 21.21		1.75 ± 1.78	38.75 ± 38.79
(76-82) ♣	(1-5)	(2-6)	(1-2)	(50-100)	-	(0-4)	(0-80)
118-120	3.25 ±1.2	4.5 ± 1.5	1.25 ± 0.82	70.8 ± 21.6		1.5 ± 1.11	40.0 ±23.45
(94-96) ♣	(2-5)	(3-6)	(0-2)	(50-100)	-	(0-2)	(0-60)

Mean ± St.Dev. CL= Corpora lutea UF = Unovulated follicles
(-) ♣ Postinsemination periods n= 4/each group ( ) Range

Table 3: Results distributed according to the different dosses of LH additives in superovulated buffalo heifers

Doses of LH (U S P Unit)	Number of New CL/animal		Number of UF/animal				Ovulation Rate (%)		Number of Recovered Embryos		Embryo Recovery Rate (%)	
			4-8 mm		>8 mm							
	Case 1	Case 2	Case 1	Case 2	Case 1	Case 2	Case 1	Case 2	Case 1	Case 2	Case 1	Case 2
00	2	1	5	6	2	1	50	50	0	0	0	0
2000	2	2	6	6	2	2	50	50	1	0	50	0
4000	4	5	2	3	1	0	80	100	3	4	75	80
5000	5	4	3	3	1	0	83	100	3	2	60	50
7000	2	4	3	3	1	2	66	66	1	4	50	100
10000	2	4	10	6	0	1	100	80	0	2	0	50

CL = Corpora lutea UF = Unovulated follicle

DISCUSSION

The recovery of single preimplantion embryo in the uterus of superovulated heifer as early as 72-89 h postestrus or 48-65 h post insemination denoted a faster rate of transport by at least 30 (h) and more than those reported in cattle and buffalo by Newcomb *et al.* (1976) and Misra *et al.* (1998) respectively. The low embryo recovery rates reported in the present study are in comparable to those reported in superovulated cows and buffaloes by Donaldson (1985) and Baruselli *et al.* (1994) respectively. The last authors declared that low embryo recoveries in buffalo may be explained by a failure of oocytes to enter the oviduct after superstimulation of follicular growth. Moreover, Osman *et al.* (2001) focused about the transfer of some unovulated follicles to cysts with consequent reduction in the number of oocytes entering the oviduct.

The overwhelming majority of our data in this experiment suports the conclusion that nonsurgical embryo transfer in buffalo heifers can be carried out successfully as early as the 4^{th} or 5^{th} day from insemination to avoid rapid growth and hatching of the morula. There are conflicting reports regarding the hatching of blastocyst on day 5 (Karaivanov *et al.*, 1987) or day 6 to 7 (Alexiev *et al.*, 1988) in buffalo. In this work all embryos located the uterus at 76-82 (h) post insemination (about 3 to 3.5 day).

It might be of interest in relation to LH additives, that all heifers received LH gave higher ovarian response than the control group without LH. Moreover, a dose ranged between 4000 and 7000 USP unit LH appeared much appropriate to induce the best ovulation and embryo recovery rates in buffalo heifers. Conflicting results were recorded in cow and buffalo cows with authors used lower doses of LH (1500-3000 IU LH) as cited by Sugie *et al.* (1980), Ismail *et al.* (1993) and Osman et al., (2001). The argument developed in this aspect could be traced from the review of Mapletoft and Pierson (1993) who suggest that maximum acceptable level of LH contamination to an FSH preparation is between 15-20% of the original LH content of the extract. It seems possible that buffalo heifers might need such high dose of LH in addition to pFSH to express favourable response with superovulation and embryo recovery.

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